# MARKET AND PRODUCT ASSESSMENT OF PROBIOTICS AND PREBIOTICS AND PROBIOTIC STRAINS FOR COMMERCIAL USE

by

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Thesis presented for the partial fulfilment of the requirements for the degree of Master of Nutrition Science at the University of Stellenbosch

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April 2004

## Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

## Summary

Probiotics (live microbes) and prebiotics (non-digestible food-ingredients) are rapidly gaining interest worldwide as supplements and functional food ingredients but little South African information in this regard is available. Furthermore, the availability of South African produced probiotic concentrates for commercial use is also very limited. The aims of this study therefore were to complete a market and product assessment of probiotic and prebiotic containing products in South Africa and to evaluate probiotic strains for commercial use in South Africa.

For the purposes of market and product assessment probiotic and/or prebiotic containing products manufactured in South Africa were identified. The scientific and legal correctness of health and content claims made on the labels of the products were assessed. An exploratory survey was conducted to determine the awareness of South African consumers of probiotics and prebiotics. For the evaluation of probiotic strains for potential commercial use in South Africa, a panel of twelve lactic acid bacteria (LAB) were screened for inhibitory activity against two porcine pathogens and indicator strains from the LMG-panel isolated from the faeces of patients diagnosed with AIDS. The five LAB with the best inhibitory activity were tested for growth in soymilk-base and for the effect of lyophilization on the inhibitory activity thereof. The effect of prebiotics on the growth and inhibitory activity of the strains was tested *in vitro*.

A range of products containing probiotics and prebiotics available on the South African market was identified. Irregulatories concerning health claims on the labels were found, but content claims seemed to be less of a problem. The results also indicate that the proposed South African regulations for the labelling of probiotic and prebiotic containing products need to be revised to include the probiotic and prebiotic related health claims for which sufficient scientific evidence is available. The probiotic strains with potential for commercial use in South Africa that were identified, include *Lactobacillus plantarum* 423, *Lactobacillus casei* LHS, *Lactobacillus salivarius* 241, *Lactobacillus curvatus* DF38 and *Pediococcus pentosaceus* 34. These strains were grown successfully in soymilk-base and lyophilization did not have a negative effective on the inhibitory activity thereof. The growth and inhibitory activity of the five LAB were promoted when combined with 1% (w/v) Raftilose®Synergy1.

It is concluded that although a variety of probiotic and prebiotic containing products are available on the South African market, the scientific and legislative correctness of especially health related claims is not satisfactory and that South African consumer awareness of these products is low. It is also concluded that a combination of at least three of the five identified LAB and 1% (w/v) Raftilose®Synergy1 can be used by South African manufacturers for the production of probiotic and prebiotic containing supplements.

# Opsomming

Die belangstelling in probiotika (lewendige mikrobe) en prebiotika (onverteerbare voedselbestanddele) as supplemente en funksionele voedselbestanddele is besig om wêreldwyd toe te neem, alhoewel weinig Suid-Arikaanse inligting in hierdie verband beskikbaar is. Die beskikbaarheid van Suid-Afrikaans geproduseerde probiotika konsentrate vir kommersïele gebruik is ook baie beperk. Die doelwitte van hierdie studie was dus om 'n mark- en produkevaluering van probiotika- en prebiotika-bevattende produkte in Suid-Afrika uit te voer en om probiotiese stamme te evalueer vir uiteindelike kommersiële gebruik in Suid-Afrika.

Vir die doel van die mark- en produkevaluering is probiotika- en prebiotika-bevattende produkte wat in Suid-Afrika vervaardig word geïdentifiseer. Die wetenskaplike en wetlike korrektheid van die gesondheids- en inhoudsaansprake op die etikette van die produkte is evalueer. 'n Markopname is uitgevoer om die bewustheid van Suid-Afrikaanse verbruikers van probiotika en prebiotika vas te stel. Vir die evaluering van probiotiese stamme vir potensïele kommersiële gebruik in Suid-Afrika is 'n paneel van twaalf melksuurbakteriëe getoets vir inhibitoriese aktiwiteit teen twee patogene geïsoleer uit varke asook teen indikator stamme van die LMG-paneel. Die vyf melksuurbakteriëe met die beste inhibitoriese aktiwiteit is getoets vir groei in sojamelk-basis en ook vir die effek van vriesdroging op die groei en inhibitoriese aktiwiteit van die stamme daarvan. Die effek van prebiotika op die groei en inhibitoriese aktiwiteit van die stamme is *in vitro* getoets.

'n Reeks van probiotika- en prebiotika-bevattende produkte wat beskikbaar is op die Suid-Afrikaanse mark, is geidentifiseer. Ongeruimdhede met die gesondheidsaansprake op die etikette is gevind, maar inhoudsaansprake was minder problematies. Die resultate dui ook daarop dat die voorgestelde Suid-Afrikaanse regulasies vir die etikettering van probiotika- en prebiotika-bevattende produkte hersien moet word om al die probiotika- en prebiotika-verwante gesondheidsaansprake waarvoor voldoende wetenskaplike bewyse beskikbaar is in te sluit. Die probiotiese stamme met potensiaal vir kommersiële gebruik in Suid-Afrika sluit die volgende in: *Lactobacillus plantarum* 423, *Lactobacillus casei* LHS, *Lactobacillus salivarius* 241, *Lactobacillus curvatus* DF 38 en *Pediococcus pentosaceus* 34. Hierdie stamme is suksesvol gekweek in sojamelk-basis en vriesdroging het nie 'n negatiewe effek op die groei en inhibitoriese aktiwiteit daarvan gehad nie. Die kombinasie van die vyf melksuurbakteriëe met 1% Raftilose®Synergy het die groei en inhibitoriese aktiwiteit daarvan bevorder.

Die gevolgtrekking wat gemaak word is dat alhoewel 'n variasie van probiotika- en prebiotika-bevattende produkte beskikbaar is op die Suid-Afrikaanse mark, die wetenskaplike en wetlike korrektheid van spesifiek die gesondheids-verwante aansprake op die etikette daarvan nie bevredigend is nie en dat die bewustheid van die Suid-Afrikaanse verbruikers van hierdie produkte laag is. Die

gevolgtrekking kan ook gemaak word dat 'n kombinasie van ten minste drie van die vyf geïdentifiseerde melksuurbakteriëe en 1% Raftilose®Synergy deur Suid-Afrikaanse vervaardigers gebruik kan word vir die vervaardiging van produkte wat probiotika en prebiotika bevat.

## Acknowledgements

My gratitude and appreciation goes to the following people:

My Heavenly Father for power and strength

My collegues and friends in the laboratory for support and advice especially Louise, Taryn and Kate

My parents and Cobus for all their love and support

Marjanne Senekal for all her input, enthusiasm and critical comments

Prof. L.M.T. Dicks for his advice and guidance

# CONGRESS PRESENTATIONS

BRINK, M., SENEKAL, M., MARé, L. & DICKS, L.M.T. *In vitro* testing of probiotics combined with prebiotics for a formula to optimize the gastrointestinal ecosystem in children. 7TH BIENNIAL CONGRESS OF THE ASSOCIATION FOR DIETETICS IN SOUTH AFRICA (19TH BIENNAL CONGRESS OF THE NUTRITION SOCIETY OF SOUTH AFRICA) at the CAMPUS OF THE POTCHEFSTROOM UNIVERSITY FOR CHE, NOVEMBER 2002.

BRINK, M., FRASER,T., TODOROV, S.D., VAZ-VELHO,M., SENEKAL, M. and DICKS, L.M.T. A combined use of probiotics and prebiotics in a soymilk-based food supplement aimed at improving the gastro-intestinal flora of children with HIV AIDS. I INTERNATIONAL CONGRESS "ATLANTIC DIET", VIANA DO CASTELO, PORTUGAL, JULY, 2003.

BRINK, M., SENEKAL, M and DICKS, L.M.T. A combination of probiotics and prebiotics as a supplement in baby food. THE 17TH SAAFoST INTERNATIONAL CONGRESS, PRETORIA, SOUTH AFRICA, SEPTEMBER, 2003.

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# CHAPTER 1

# INTRODUCTION

# Introduction

## 1. Introduction and problem identification

There is an increasing awareness that the health of any individual is linked to the activity of bacteria resident in the gastrointestinal tract (Buddington *et al.*, 2002). Gut microflora consist of a complex collection of micro-organisms that form an integral and biologically important component of the body. These microflora interact with one another and their host and are largely involved in the breakdown of substrates (Cummings & Macfarlane, 1991).

The mature gastrointestinal ecosystem is resistant to colonization by invading species when in balance with its host (Buddington *et al.*, 2002). However, factors such as diet, medication, stress and environmental factors could cause disturbances of the gastrointestinal tract bacteria (Fuller & Gibson, 1997), leading to a microbial imbalance.

The composition of the gut microflora can be manipulated through dietary supplementation of specific compounds, known as probiotics (Collins & Gibson, 1999). In Greek the word probiotic means "for life" and is defined by Fuller (1989) as "a live microbial food supplement that beneficially affects the host by improving its intestinal microbial balance". Probiotic strains are found in various fermented products and are commercially available in various forms including powders, tablets and suspensions. These products may contain monocultures or mixed species (as many as nine), mostly members of the genera *Lactobacillus, Bifidobacterium* and *Enterococcus.*

The potential effects of probiotics were first studied by Metchnikoff (1907) at the beginning of the century. He concluded that the healthier condition of Bulgarian peasants was due to the consumption of large quantities of fermented milk. Metchnikoff developed the theory that ingestion of sour milk modifies the activity of colonic microflora. He isolated the bacteria and studied them in human feeding trials. After his death his colleagues and many others continued with similar research and the field has subsequently developed to the current knowledge and application of probiotics in improving the health of individuals (Gibson & Fuller, 2000).

The potential health benefits of probiotics have been investigated in several studies. Results indicate that probiotics could alleviate lactose intolerance, enhance immunity, reduce colon cancer, and reduce gastrointestinal disorders such as diarrhea (Fuller & Gibson, 1995; Kopp-Hoolihan, 2001; Saavedra *et al.*, 1995; Salminen *et al.*, 1998).

Mechanisms involved in the establishment of these beneficial effects include the following: competition with pathogens for available nutrients and other growth factors (Rolfe, 2000),

antagonising pathogens directly through production of antimicrobial and antibacterial compounds such as bacteriocins and butyric acid (De Vuyst & Vandamme, 1994; Dodd & Gasson, 1994; Kailasapathy & Chin, 2000), reduction of gut pH by stimulating the growth of lactic acid bacteria (Langhendries *et al.*, 1995), competition for binding at receptor sites that pathogens may occupy (Fujiwara *et al.*, 1997; Kailasapathy & Chin, 2000), improvement of immune function and stimulation of immunomodulatory cells (Isolauri *et al.*, 1991; Isolauri *et al.*, 1995; Rolfe, 2000), and production of lactase which aids lactose digestion (Rolfe, 2000).

However, a probiotic is only effective if it does actually lead to one or more of the above mentioned benefits, is safe, non-pathogenic, non-invasive, non-carcinogenic, contains more than $10^6$ viable cells, survives in the gut, remains viable during storage as part of food-items or supplements and has good sensory properties (Fooks *et al.*, 1999; Fuller, 1989; Fuller & Gibson, 1997; Saavedra, 1995).

A number of factors influence the viability (growth and inhibitory activity) of beneficial gut microflora, e.g. gastric acidity and the action of bile salts in the small intestine and the presence of competing micro-organisms for nutrients (Bezkorovainy, 2001; Mattilla-Sandholm *et al.*, 2002). One very important factor is the availability of fermentable substrates in the gastrointestinal tract such as oligosaccharides and polisaccharides (Roberfroid, 2001). The supplementation of such substrates, also known as prebiotics, to optimise the balance of gastrointestinal tract microflora, is under investigation (Fooks *et al.*, 1999). A prebiotic is defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity, or both of one or a limited number of bacterial species already resident in the colon" (Gibson & Roberfroid, 1995). Prebiotics are found in foods such as onion, garlic, banana, chicory, asparagus, leek and Jerusalem artichoke. Prebiotics can also be synthesised from simple sugars such as sucrose or lactose, or may be formed by controlled hydrolysis of starch or other polysaccharides (Gibson, 2001; Van Loo *et al.*, 1995).

A true prebiotic has to comply with the following criteria: it has to reach the colon without being hydrolysed or absorbed in the upper part of the gastro-intestinal tract, be selectively fermented by potentially beneficial microorganisms in the colon such as lactic acid bacteria, balance the gut microflora by changing the composition of the colonic microflora towards a healthier composition, and benefit host health (Fooks *et al.*, 1999). Currently the only food-ingredients with convincing prebiotic effects are non-digestible oligosaccharides which contain fructose, xylose, raffinose, stachyose, galactose, glucose and mannose (Roberfroid, 2001). It is important to note that prebiotics have also been found to have health benefits unrelated to the effect on the gut microflora. These benefits include increased mineral absorption and improved bone health, a decrease in cholesterol and constipation relief (Roberfroid, 2000).

The effect of a combination of probiotics and prebiotics, referred to as synbiotics, on the balance of gastrointestinal tract microflora has also been investigated. A higher survival rate of the probiotic strain was found than when the probiotic was supplemented on its own (Gibson & Roberfroid, 1995). These findings indicate that combining probiotics with prebiotics should be seriously considered in the case where probiotic supplements are recommended.

The perceived benefits of probiotic and prebiotic containing products have led to an expansion in the probiotic and prebiotic market over the past five years (Daly & Davis, 1998; Temmerman *et al.*, 2002,). In Europe, consumer awareness with regard to probiotics is quite high and the probiotic market, especially dairy products such as yoghurts and fermented milks, has experienced rapid growth. France represents the largest market in Europe followed by Germany where the availability of probiotic containing yoghurts has increased by 150% from 1996 to 1997. During the same time the market in the United Kingdom grew only by 26%. In the United States probiotic containing products are almost exclusively dairy products such as fluid milk, yoghurt and kefir (Heller & Weir, 2001). However, consumer awareness of probiotic containing products in the United Stated is not that high as probiotics ranked only in the 5$^{th}$ or lowest level of marketing for the trimester ending July 2001 (STS, 2002). The Japanese also use probiotics as a significant and functional ingredient in many of their fermented and/or functional drinks (Stanton, 2001). In South Africa probiotic and prebiotic containing products seem to be available, although no detailed information regarding these products or consumer awareness thereof could be traced. Furthermore, imported probiotic strains are apparantly mostly used in the production of probiotic containing products in South Africa (Personal communication, 2002, Prof. L.M.T. Dicks, Department of Microbiology, University of Stellenbosch).

The growing use of probiotic and prebiotic containing products necessitates effective commercial production and marketing. Prebiotics can easily be used in any matrix, in almost any food, whereas the application of probiotics is much more complicated (Saavedra *et al.*, 2002). When commercialising probiotic cultures, factors such as the following should be considered: safe probiotic strains, the functional and technological characteristics of the strains, as well as the use of growth mediums and appropriate food vehicles that are safe for human consumption (Saarela *et al.*, 2000; Salminen *et al.*, 1996a).

Safety aspects of probiotic bacteria involve GRAS[1] status, specifications such as origin (healthy human gastrointestinal tract), non-pathogenicity and antibiotic resistance (Mattilla-Sandholm *et al.*, 2002). The functional characteristics that need to be considered are viability and persistence in the gastrointestinal tract, immunomodulation, antagonistic and antimutagenic properties. Essential technological characteristics include good sensory properties, viability during processing as well as

[1] GRAS = generally recognised as safe by the Food and Drug Administration of the USA

stability during manufacturing and storage (Mattilla-Sandholm *et al.*, 2002; Saarela *et al.*, 2000). The food vehicles appropriate for human consumption should ensure probiotic stability. Fermented dairy products (e.g. yoghurt), soymilk and other food vehicles such as baby foods, cereals, drinks and confectioneries have been found to be successful in this regard (Andersen, 1998; Kamaly, 1997; Svensson, 1999). Yoghurt has a long history as an appropriate carrier for probiotic bacteria and in recent years there has been a significant increase in the popularity of yoghurt (Lourens-Hattingh & Viljoen, 2001). Soymilk has also gained interest as a medium for growth and biochemical activities of probiotic bacteria and has been known to be successfully in a number of studies (Kamaly, 1997). Soymilk is more affordable than MRS[2] medium (De Man *et al.*, 1961), is safe for human and animal consumption and can be used as an alternative to dairy products for lactose-intolerant people (Kamaly, 1997). The stability of probiotic bacteria in other food vehicles might be more challenging as the probiotic bacteria do not multiply in the food matrix (Mattilla-Sandholm *et al.*, 2002).

The packaging materials used and the conditions under which the products are stored are also important for the quality of products containing probiotic bacteria. Probiotic cultures are freeze-dried or spray-dried when used in special formulations such as capsules or tablets. These culture concentrates are also supplied to manufactures for food fortification or the production of fermented food products (Saarela *et al.*, 2000). Freeze-drying is the most popular method as many probiotic bacteria cannot tolerate the relatively high temperatures that are used in spray-drying (Mattilla-Sandholm *et al.*, 2002). Micro-encapsulation (protective coating of probiotic bacteria) and added oligosaccharides in probiotic containing products have also been found successful in increasing the survival of the probiotic bacteria (Kailasapathy & Rybka, 1997). Concentrated probiotic cultures supplied to manufacturers should be filled in gas and light proof containers to protect the cultures against light and humidity. Aluminium-foil coated cartons and humidity are most often used (Honer, 1995). The storage temperatures of probiotic containing products should be 3-4°C for fermented dairy and soymilk products and room temperature for baby foods and cereals (Kailasapathy and Rybka, 1997).

The final step in commercialisation involves the labelling of products. The information printed on the labels of probiotic and prebiotic containing products has to be correct regarding probiotic species-identification, number of viable cells and prebiotic type and concentration (Temmerman *et al.*, 2002). In general, probiotic and prebiotic containing products only claim general health benefits and specific content. In Europe no specific legislation regarding the labelling of probiotic and prebiotic containing products exists (Richardson, 1996). However in the United States the FDA strictly regulates the labelling and marketing of conventional foods containing probiotic bacteria and therefore no statements regarding health benefits for probiotics can be stated on the labels of the products. In South

[2] MRS = De Man, Rogosa and Sharpe growth medium for LAB which is used in the laboratory

Africa permissible information regarding labelling of health and content claims of probiotic and prebiotic containing products are included in the proposed South African regulations governing the labelling and advertising of Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act no. 54 of 1972; http://www.doh.gov.za, 2003/05/02 accessed). Permissible health claims include improvement of the health and functioning of the digestive tract, manufacturing of B vitamins, inhibition of the growth of harmful pathogens, and may, when ingested on a regular basis as part of a prudent, balanced diet, assist in improving the immune status, the digestion of lactose and may help reduce the risk of colon cancer. The content claims include permissible probiotic strains, viable cell numbers, prebiotic type and concentration.

Most of the probiotics and prebiotics are available as over-the-counter products in outlets such as pharmacies and food and health stores (Reid, 2001). Good marketing is essential to raise consumer awareness and subsequently ensure continued growth of the probiotic and prebiotic market (Menrad, 2003). Marketing involves the identification of the target market i.e. health conscious consumers, determining the communication strategy for example advertisements and brochures, price positioning and distributing the products to the target market (Du Plessis, 1999).

Although a number of probiotic and prebiotic containing products seem to be available on the South African market, very little scientific information has been published in this regard with specific reference to the full range of products available on the market, the legal correctness of health and content claims as well as consumer awareness of these products. Furthermore, a need seems to exist for the identification of viable strains of probiotics that can be cultured in South Africa for use in the manufacturing of probiotic containing products in the country.

## 2. Aims and objectives

The aims of this study were to complete a market and product assessment of probiotic and prebiotic containing products in South Africa and to evaluate probiotic strains for commercial use in South Africa.

The following objectives were formulated to attain these aims:

Market and product assessment:

- To identify probiotic and/or prebiotic containing supplements and food-items available on the South African market
- To evaluate the health and cell count related claims stated on the labels of the identified products
- To investigate South African consumer awareness of probiotic/prebiotic containing supplements and food-items

Evaluation of probiotic strains:

- To identify probiotic strains for potential commercial use in South Africa
- To determine the effectiveness of soymilk-base as a growth medium
- To determine the effect of lyophilization on the growth and inhibitory activity act of probiotics grown in soymilk.
- To determine the effect of selected prebiotics on the inhibitory activity of selected probiotic strains
- To formulate recommendations regarding the composition of a probiotic and prebiotic containing formula based on the identified probiotics.

## 3. Outline of the thesis

In Chapter 2 an overview of the relevant literature concerning probiotics, prebiotics as well as related market and marketing aspects is presented. The market and product assessment of probiotic and prebiotic containing products is presented in the first article in Chapter 3. The second article which reports on the investigation of awareness of South African consumers of probiotic and prebiotic containing products is presented in Chapter 4. The influence of prebiotics on the inhibitory activity of lactic acid bacteria and their viability in soymilk-base for commercial production is presented in the third article in Chapter 5. A general discussion of all three articles and final conclusions are presented in Chapter 6. The journals to which the three manuscripts included in this thesis will be submitted were not yet finally identified at the time of the finalization of the thesis. The referencing system applied is therefore based on the system prescribed by the Department of Food Science, University of Stellenbosch.

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# CHAPTER 2

# LITERATURE OVERVIEW

# Literature review

This chapter provides an overview of the literature on taxonomy, properties and health benefits and culturing of probiotics as well as products containing probiotics. Compounds with convincing prebiotic effects and the physiological and metabolic effects and manufacturing of prebiotics as well as prebiotic containing products are also discussed. The concept of synbiotics is also alluded to. Finally the market, consumer awareness, and marketing of probiotic/prebiotic containing products are reviewed.

## 1. Probiotics

### 1.1 Definition of probiotics

Lilly and Stillwell (1965) introduced the term probiotics as substances produced by one microorganism that stimulates the growth of other microorganisms. Sperti (1971) extended this definition to microorganisms or tissue extracts, which improves microbial growth. Parker (1974) first used the word probiotic in the context of animal feed supplementation and defined it as, "organisms and substances that contribute to intestinal microbial balance". The most common definition currently used is that of Fuller (1989), i.e "Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance." This definition was expanded by Havenaar and Huis in't Veld (1992) to include food and non-food use and the use of mono and mixed cultures.

### 1.2 Taxonomy of probiotic bacteria

In the past lactic acid bacteria were classified on the basis of phenotypic properties, e.g., morphology, mode of glucose fermentation, growth at different temperatures, lactic acid configuration and fermentation of different carbohydrates. However, studies based on comparative 16S ribosomal RNA sequencing analysis showed that some taxa generated on the basis of phenotypic characteristics do not correspond with the suggested phylogenetic groupings. Some species in the *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus paracasei* groups, and certain bifidobacteria, are not readily distinguishable. Modern molecular techniques, including polymerase chain reaction, combined with other genotyping methods, are therefore important for species identification. The classification and identification of a probiotic strain may give a good indication of its typical habitat and origin. The species and genus name may also indicate the strain's safety and technical applicability for use in probiotic products (Holzapfel *et al.*, 1998).

#### 1.2.1 Overview

Lactic acid bacteria (LAB) is a diverse group that consists of Gram-positive, non-spore-forming bacteria that occur as cocci or rods. They grow in complex media, with fermentable carbohydrates as energy source (Axelsson, 1998). Food sources of LAB include dairy products, fermented meat,

sourdough, fermented vegetables, silage and beverages. They are also found on plants, sewage and the genital, intestinal and respiratory tracts of man and animals (Wood & Holzapfel, 1995). These bacteria are non-motile, do not form spores and are not capsulated.

On the basis of hexose catabolism, LAB can be divided into two groups, namely homofermentative bacteria that use the Embden-Meyerhof-Parnas pathway and heterofermentative bacteria that use the 6-phosphogluconate pathway (Orla-Jensen, 1919). The Embden-Meyerhof pathway involves the degradation of glucose to pyruvate in the presence or absence of oxygen. The 6-phosphogluconate pathway operates aerobically and anaerobically and is important in biosynthesis and catabolism (Prescott *et al.*, 1999). Homofermentative bacteria, such as the genera *Lactococcus, Pediococcus, Streptococcus,* and *Lactobacillus* spp. degrade hexoses to lactate or to lactate and additional products such as acetate, ethanol, $CO_2$ and formate. Heterofermentatives, which include the bifidobacteria and some *Lactobacillus* spp. produce acetate and lactate at a molar ratio of 3:2 (Kandler, 1983).

The phylogenetic relatedness of microorganisms is determined by comparing the sequences of ribosomal RNA (rRNA). On the basis of the available information on 16S or 23S rDNA sequences, phylogenetic trees or dendograms are constructed. Gram-positive bacteria cluster in two of the seventeen eubacterial phyla, which coincide with their DNA base composition (Schleifer & Ludwig, 1995a, 1995b). Lactic acid bacteria that can be used in probiotic foods or food supplements include *Lactobacillus acidophilus*, *Lactobacillus johnsonii, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium bifidum, Bifidobacterium infantis, Enterococcus faecalis* and *Enterococcus faecium* (Gibson & Fuller, 2000; German *et al.*, 1999). Gram-positive bacteria with a DNA composition of 55 to 67mol% guanine plus cytosine (G+C), such as bifidobacteria, belong to the so-called *Actinomycetes* branch. The *Clostridium* branch comprises organisms of G+C content higher than 55%. The phylogenetic relation of the different genera of the *Clostridium* branch is shown in Fig. 2.1 and is based on the comparison of 16S rRNA sequences. *Carnobacterium*, *Enterococcus, Vagococcus, Aerococcus, Tetragenococcus* and *Lactosphaera* are more closely related to each other than the other LAB. *Lactococcus* and *Streptococcus* are relatively closely related, whereas the genus *Lactobacillus* is phylogentically diverse (Schleifer & Ludwig, 1995a; 1995b). According to 16S rRNA sequencing data, the genera *Lactobacillus* and *Pediococcus* are phylogenetically intermixed as five species of a *Pediococcus* cluster with 32 homo- and heterofermentative *Lactobacillus* spp. in the *Casei* and *Pediococcus* group (Collins *et al.*, 1991). Only LAB from the genera *Lactobacillus*, *Enterococcus* and *Pediococcus* were used in this study and will therefore be described in more detail.

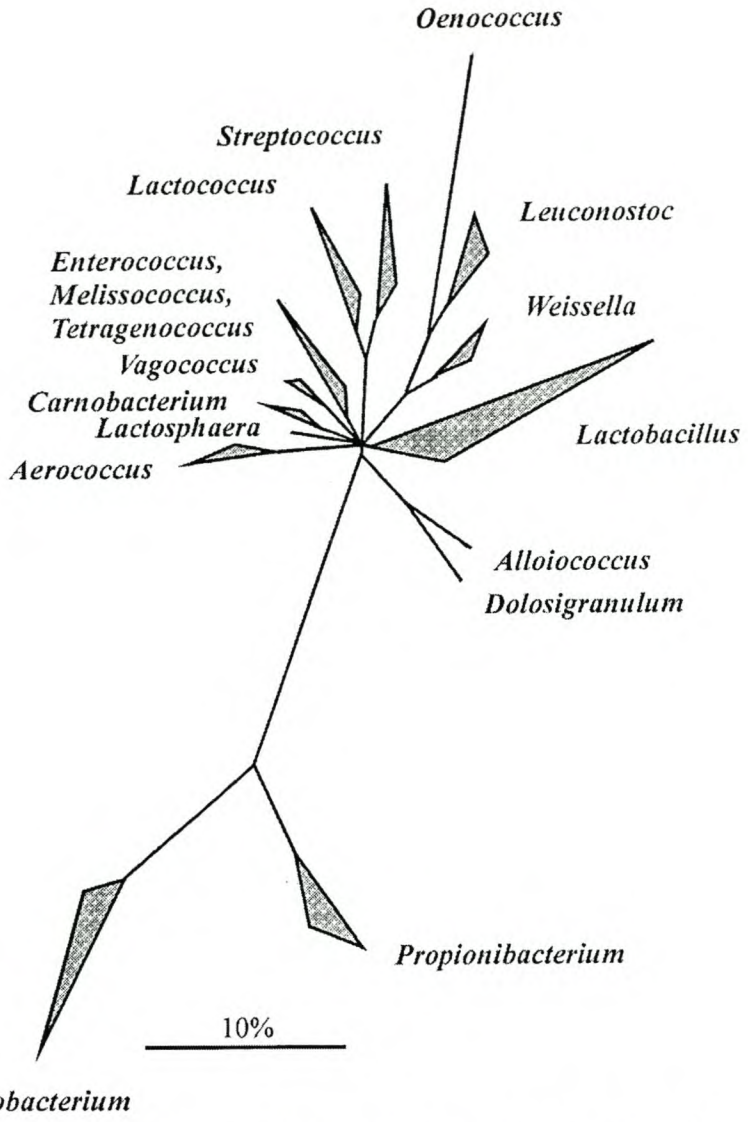


**FIGURE 2.1** Consensus tree, based on comparative sequence analysis of 16S rRNA, showing major phylogenetic groups of lactic acid bacteria with low mol% guanine plus cytosine in the DNA and the nonrelated Gram-positive genera *Bifidobacterium* and *Propionibacterium* (Scheifer & Ludwig, 1995a; 1995b).

#### 1.2.1.1 Genus *Lactobacillus*

Members of the genus *Lactobacillus* are found in a variety of habitats with rich, carbohydrate-containing substrates, such as mucosal membranes of vertebrates (oral cavity, intestine and vagina), on plants or material of plant origin, in manure and man-made habitats such as sewage and fermented or spoiled food (Kandler & Weiss, 1986). Lactobacilli are non-spore-forming rods or coccibacilli with a G + C content of DNA usually between 33 and 55 mol%. Lactobacilli are aero-tolerant or anaerobic, acidophilic and have complex nutritional requirements regarding needs for carbohydrates, amino acids, peptides, fatty acids esters, salts, nucleic acid derivatives and vitamins (Kandler & Weiss, 1986). Lactobacilli may metabolise various compounds, e.g. citrate, malate, tartrate, quinolate, nitrate and nitrite and use these as an energy source (e.g. via building a proton motive force) or electron

acceptors (Kandler & Weiss, 1986). The major divisions of lactobacilli are based on their fermentative characteristics. The divisions include obligately homofermentative, facultative heterofermentive and obligately heterofermentative (Stiles & Holzhapfel, 1997). Obligately homofermentative lactobacilli ferment glucose exclusively to lactic acid and do not ferment pentoses or gluconate (Stiles & Holzhapfel, 1997). It represents Orla-Jensen's thermobacteria and the important food-associated species include *L. acidophilus, L. delbrueckii, L. helveticus, L. farciminis* and *L. kefiranofaciens* (Hammes & Vogel, 1995; Pot *et al.*, 1994; Vandamme *et al.*, 1996). Facultative heterofermentive lactobacilli ferment hexoses to lactic acid and may produce gas from gluconate but not from glucose. They also ferment pentoses by an inducible phosphoketolase to produce lactic and acetic acids (Stiles & Holzhapfel, 1997). Important food-associated species include *L. casei, L. plantarum, L. pentosus, L. curvatus* and *L. sakei* (Hammes & Vogel, 1995; Pot *et al.*, 1994; Vandamme *et al.*, 1996). Obligately heterofermentative lactobacilli ferment hexoses to lactic acid, acetic acid and/or ethanol and carbon dioxide. The production of gas from glucose is a characteristic feature of these bacteria. The most important food-associated species is *L. sanfrancisco* which converts maltose to lactic acid and acetic acids and various flavour compounds in sourdough bread (Stiles & Holzhapfel, 1997). Other species include *L. fermentum, L. kefir, L. reuteri, L. bifermentans, L. fructivorans, L. brevis, L. buchneri* and *L. viridescens* (now *Weissella viridescens*) (Hammes & Vogel, 1995; Pot *et al.*, 1994; Vandamme *et al.*, 1996). The heterofermentative lactobacilli that lack aspartic acid or diaminopimelic acid in their peptidoglycan appear phylogenetically different from other lactobacilli and were grouped with *Leuconostoc paramesenteroides* into a new genus *Weissella* (Collins *et al.*, 1993).

#### 1.2.1.2 Genus *Pediococcus*

Members of the genus *Pediococcus* are most often found in low numbers, together with leuconostocs and lactobacilli, on plant materials including barley, malt, hops, dried leaves, hay, citrus fruits, apples and strawberries, in various foods and as spoilage agents in alcoholic beverages such as beer (Wood & Holzapfel, 1995).

Pediococci are the only lactic acid bacteria that divide alternately in two perpendicular directions to form tetrads although pairs of cells may also occur (Gunther, 1959). The colonies are typically 1-3 mm in diameter, generally smooth-edged and invariably not pigmented when grown in a rich medium, such as De Man, Rogosa and Sharpe (MRS) agar (Garvie, 1986). Pediococci contain between 35 and 39 mol% G + C, is facultatively anaerobic and the growth temperature is between 39 and 45 °C (Garvie, 1968). Lactose in milk is not a readily available carbohydrate, which makes milk a poor growth medium (Garvie, 1968). Fermentation of glucose follows the Embden Meyerhof pathway with $_{DL-}$ or $_{L-}$(+) - lactate as the major end product under optimal conditions. Pyrovate can be diverted to other end products and diacetyl acetoin is often produced by *P. damnosus*, while *P. pentosaceus*

produces equimolar amounts of lactate and acetate from pentoses (Fukui *et al.*, 1957). Amino acid requirements are not known and vitamin requirements are similar for most species (Jensen and Seeley, 1954). Species of *Pediococcus* and related tetrad-forming bacteria include *P. damnosus, P. dextrinicus, P. parvulus, P. inopinatus, P. pentosaceus, P. acidilactici, Aerococcus urinae-equi* (previously *P. urinae-equi*) and *Tetragenococcus halophilus* (previously *P. halophilus*) (Sneath *et al.*, 1986).

#### 1.2.1.3 Genus *Enterococcus*

The Genus *Enterococcus* is associated with the gastro-intestinal tract of man and animals and serves as indicators of faecal contamination of water and food. The taxonomy of Enterococci has been vague. There are no phenotypic characteristics that separate the genus from the other genera of gram-positive, catalase-negative cocci. The enterococci have been described by Sherman (1937) as those organisms that grow between at 10 and 45°C, in 6.5% NaCl and at pH 9.6 and survive heating at 60°C for 30 minutes. Several species and strains of the genus *Enterococcus* do not meet all of these criteria (De Vriese *et al.*, 1993). Enterococci are ovoid and occur single, in pairs or in short chains and are non-motile. They are facultatively anaerobic and chemo-organotrophs and their G + C content ranges from 37 to 40 mol%. They have a homofermentative lactic acid fermentation and the predominant end product of glucose fermentation is $_{L}$ (+)-lactic acid. They have a PEP PTS system for uptake of lactose and other carbohydrates, including gluconate (Bernsmann *et al.*, 1982). Their nutritional requirements include vitamins, biotin, pyridoxine, riboflavin, nicotinate and panthothenate and several amino acids (DeVriese & Pot, 1995). The species included in the genus *Enterococcus* are *E. faecalis, E. faecium, E. avium, E. casseliflavus, E. cecorum, E. columbae, E. dispar, E. durans, E. fallox, E. flavescens, E. gallinarum, E. hirae, E. mundtii, E. malodoratus, E. pseudoarium, E. raffinosus, E. saccharolyticus, E. sertolicida, E. solitarius* and *E. sultureus.*

## 1.3. Properties and health benefits of probiotic bacteria

### 1.3.1 Overview

Probiotic bacteria have the ability to survive in the gastrointestinal tract and reach the colon to colonize and consequently benefit the host (Roberfroid, 2000). This contributes to the production of short-chain fatty acids and associated health benefits, including inhibition of pathogen colonization, reduced colon cancer, cholesterol and constipation and an increased immune stimulation (Crittenden, 1999). The properties of probiotic bacteria that lead to these benefits include acid tolerance, tolerance to human gastric juice and bile, adhesion to epithelial surfaces, increased immune stimulation, antimutagenic and anticarcinogenic properties, antagonistic activity against pathogens and production of the enzyme ß-galactosidase (lactase).

### 1.3.2 Bile- and acid resistance

The bacterial numbers and populations in the gastrointestinal tract vary between the stomach, small intestine and colon. The total bacterial count in gastric contents is usually below $10^3$ per gram while it ranges between $10^4$ per ml in the small intestine to about $10^6$ – $10^7$ per ml in the terminal ileum (Gorbach *et al.*, 1967). The colon hosts a stable ecosystem that consists of $10^{11}$ – $10^{12}$ different microorganisms per gram contents (Cummings & Macfarlane, 1991).

The ecosystem of the small intestine is less stable and more susceptible to modifications than that of the colon (Brassart & Schiffrin, 1997). It is therefore more difficult to modify the microflora in the colon by administrated probiotic bacteria than in the small intestine. To be effective in this regard, probiotic bacteria should survive passage through the stomach and the small intestine to reach the colon in an active form so that it can influence the microbial ecosystem of the colon. The main obstacles in this process are gastric acidity and the action of bile salts in the small intestine (Bezkorovainy, 2001). The pH-value found in the stomach of humans after food consumption changes from pH 5.0 at initiation of acid secretion to pH 1.8 after 80 minutes (Marteau *et al.*, 1997). In the small bowel the pH is 6.5 while the pH in the right or proximal colon ranges from 5.5 and 6.0 and from 6.5 and 7.0 in the left or distal area of the colon (Fooks *et al.*, 1999; Marteau *et al.*, 1997). The bile salt concentration in the duodenum is 15 mmol/L after a meal which progressively decreases to 5 mmol/L and eventually in the ileum the concentration is below 4 mmol/L (Marteau *et al.*, 1997).

The effect of pH levels on the viability of probiotic bacteria is illustrated by the following *in vitro* study: Six *L. acidophilus* and nine *Bifidobacterium* strains were maintained at a pH of 1.5 - 3.0 for up to three hours. Probiotic viability depended on the species and strains used and the length of exposure to acid and the pH. The best results were obtained with three *L. acidophilus* strains, *B. longum* and *B. pseudolongum* that survived a pH of 1.5 – 3.0 (Lankaputhra *et al.*, 1995).

Bile salt hydrolases present in probiotic bacteria deconjugate bile salts and these acids are better bacterial lysing agents of pathogenic bacteria than conjugated bile acids (Gopal *et al.*, 1996). To determine the effect of bile salts on the viability of probiotic bacteria, *in vitro* studies focus on the assessment of the growth and survival of probiotic bacteria in the presence of bile acids. In a study done by Lankaputhra *et al.* (1995), *Lactobacillus* and *Bifidobacterium* strains were maintained at bile concentrations of 0 – 1.5% for up to three hours. Colonies were counted after the bacterial suspensions were plated. Results indicated that the survival of the strains varied and depended on bile concentration and exposure times. Gopal *et al.* (1996) found that *L. acidophilus*, *B. infantis* and *Bifidobacterium adolescentis* were the most resistant in the presence of 0.3% oxgall. These strains deconjugated maximum concentration taurocholic acid (bile acid) after 12h of growth, but no correlation between extent of deconjugation and growth inhibition was observed. Ibrahim and Bezkorovainy (1993) grew several species from the American Type Culture Collection for 24h in the

presence of 0.6 – 3.0g glycocholic acid/L. The cultures were transferred twice to fresh media containing no bile salts. After the second transfer, the growth resumed to maximal extent and their bile salt hydrolases were recovered.

The effect of acid and bile acid on the survival of probiotics was investigated using an artificial model of the gastrointestinal tract. This model comprised four serial compartments simulating the stomach, duodenum, jejenum and ileum, which were connected to computer-controlled valve pumps. The organisms most resistant to stomach acid were *B. bifidum* and *L. bulgaricus* which survived for 140 minutes, whereas *Streptococcus thermophilus* and *L. acidophilus* only survived for 40 min. The delivery of *B. bifidum* and *L. acidophilus* to the cecum in the presence of low bile salt concentrations (2mmol/L) was significantly higher than in the presence of physiologic bile salt concentrations. These values were comparable with those that have been observed *in vivo* (Marteau *et al.*, 1997).

Results from these experiments indicate that variables which determine the degree of probiotic survival through the gastrointestinal tract include the level of stomach acidity, length of exposure to acid, concentration of and length of exposure to bile salts and the level of bile salt hydrolase activity. Many probiotics do survive these conditions and enter the colon in viable state in sufficient amounts to affect the microbial ecosystem in the colon (Bezkorovainy, 2001).

### 1.3.3 Adhesion properties and immune effects

Adhesion refers to the ability of a microbe to attach to intestinal mucosal cells, permanently establish in the host's intestine and ensure beneficial effects. Adhesion processes involve the interaction between receptor molecules on epithelial cells and adhesion molecules on the surfaces of adherent bacteria (Salminen *et al.*, 1996). Probiotic bacteria have definite adhesion properties (Salminen *et al.*, 1996a) which contribute to the ability to establish permenantly in the host's intestine. The adhesion ability of these strains ensures that they persist longer than non-adhering strains and consequently contribute to the following physiological effects: exclusion of pathogens on the intestinal epithelium by competition from adherent probiotic bacteria (Salminen *et al.*, 1996a) and mediation of local and systemic immune effects through interaction with the mucosal surface that facilitates contact with gut associated lymphoid tissue (Salminen *et al.*, 1996a).

To illustrate the exclusion of pathogens by adherent probiotic bacteria, Hudault *et al.* (1997) conducted an *in vivo* study using tissue samples from the colon of humans. The results indicated that probiotic bacteria did interfere with the adherence of pathogens such as *Salmonella typhimurium* to Caco-2 cells (Hudault *et al.*, 1997). In a similar *in vitro* study, both living and heat-killed *L. acidophilus* cells have been effective in adhering to the colon tissue and inhibition of pathogens (Coconnier *et al.*, 1993a).

The mediation of local and systemic immune effects by probiotic bacteria is illustrated by human studies done on the antagonism between pathogens and probiotic bacteria adhered to the gut associated lymphoid tissue. The results indicated that the antagonism enhances immune response by stimulating nonspecific host resistance to microbial pathogens as well as modulating the host's immune responses to potentially harmful antigens (Haschke *et al.*, 2001; Isolauri, 1998; Isolauri *et al.*, 2001).

Immune defences affected by probiotics can be specific- and non-specific. The most important specific immune defence mechanism that is affected involves the production of IgA immunoglobulins by B lymphocytes (Pfeifer & Rosat, 1999). The secretory IgA's play an important role in agglutination and capture of intraluminal antigens, immunglobulin-dependent cytotoxity after attachment of bacteria, inhibition of pathogen adhesion to the mucosa, antitoxin action and antiviral activity (Pfeifer & Rosat, 1999). A number of studies have reported that supplementation with probiotics increases IgA levels (Table 2.1).

**TABLE 2.1:** The effect of probiotic supplementation on IgA levels

| **Study** | **Effect on IgA levels** | **Author(s)** |
|---|---|---|
| Infants received supplements of a strain of *L. casei* for 5 days | The concentrations of circulating IgA were increased and rotavirus-induced diarrhea was reduced. | Kaila *et al.*, 1992 |
| Children with acute diarrhea received supplements of *L. rhamnosus* GG for 7 days. | An increase in specific antibody-secreting cells in the IgA class was evident when compared to control subjects. | Kaila *et al.*, 1992 |
| Volunteers were immunized with attenuated *Salmonella typhi* Ty21a to determine if fermented milk containing *L. johnsonni* LJ-1 ($5 \times 10^9$ CFU per day for 3 weeks) and bifidobacteria could have immunomodulatory effects. | The fermented milk and bifidobacteria group showed a 4.08-fold increases in serum IgA antibody titers and the control group only a 2.48-fold increase. | Link-Amster *et al.*, 1994 |
| Infants received a viable preparation of *Lactobacillus* GG for 7 days during acute rotavirus gastroenteritis. | A significant rotavirus specific IgA response during recovery was indicated. | Salminen *et al.*, 1996 |
| Subjects with milk-hypersensitive symptoms received a supplement of *L. rhamnosus* GG for 7 days. | The milk-induced inflammatory response was down-regulated. It had an immunostimulatory effect in healthy subjects and promoted IgA immune response in Crohn's disease patients. | Pelto *et al.*, 1998 |

Nonspecific immune defense systems affected by probiotic bacteria involve phagocytosis by cells of myeloid origin such as monocytes, macrophages or polymorphonuclear cells (Pfeifer & Rosat, 1999). Phagocytosis is responsible for early activation of the inflammatory response before antibody production. When foreign bodies such as bacteria appear in the blood stream, phagocytes will be activated to release toxic agents such as reactive oxygen intermediates and lytic enzymes that will

destroy these foreign bodies (Isolauri *et al.*, 2001; Pfeifer & Rosat, 1999). The role of oral introduction of probiotic bacteria in the enhancement of non-specific host defence against microbial pathogens is illustrated by the work of Schiffrin *et al.* (1995) who found that the ingestion of *L. johnsonni* LJ-1 from fermented milk products for three weeks increased the blood phagocytic capacity in humans. Similar results were found with the consumption of *L. acidophilus* Lal (Schiffrin *et al.*, 1995). Schiffrin *et al.* (1995) also reported that oral introduction of *L. casei* and *L. bulgaricus* activated the production of macrophages and that the oral introduction of *L. casei* and *L. acidophilus* activated phagocytosis in mice.

Research indicates that the effect of probiotic bacteria on the adhesion of pathogens to the intestinal mucosal cells and immune response is specific relevant in the treatment of conditions such as acute diarrhea, radiation enteritis and intestinal inflammatory conditions such as Crohn's disease and ulcerative colitis (Salminen *et al.*, 1988; Isolauri *et al.*, 1991).

### 1.3.4 Antimutagenic/anticarcinogenic activity

The strongest link between cancer and probiotic intake has been found for colon cancer, although links with bladder cancer are also evident (Gibson & Macfarlane, 1994; Morotomi *et al.*, 1990). Researchers believe that microflora in the colon play an important role in the development of colon cancer (Rowland, 1988). Several species of bacteria found in the colon produce carcinogens and tumour promoters from food components that reach the colon. Many of these microorganisms also synthesise enzymes that generate toxic products (Table 2.2).

**TABLE 2.2:** Colonic bacteria that produce toxic products (compiled from Rastall & Gibson 2002)

| Enzyme | Substrate |
|---|---|
| β-glucosidase | Plant glycosides, e.g. rutin, cycasin |
| Azoreductase | Azo compoundes, e.g. benzidines |
| Nitroreductase | Nitro-compounds, e.g. nitrochrysene |
| β -glucuronidase | Bilary glucuronides, e.g. benzidine |
| IQ hydratse-dehydrogenase | 2-amino-3-methyl-3H-imidazo-4,5-*f*.quinolineIQ |
| Nitrate/nitrite reductase | Nitrate, nitrite |

Antimutagenic mechanisms that have been proposed for probiotics include the following: binding and degradation of micro-organisms that produce (pro) carcinogens, production of antimutagenic compounds by viable probiotic cells, modulation of procarcinogenic enzymes in the gut, alteration of colonic transit time to remove faecal mutagens more effectively, reduction of the intestinal pH, thereby altering microflora activity and bile solubility and suppression of tumours by enhancing defence immune mechanism (Hirayama & Rafter, 1999; McIntosh, 1996).

Evidence for the possible role of probiotics in the prevention/treatment of colon cancer has come from *in vitro*, animal and human studies. *In vitro* assessment of two LAB isolated from human faeces

showed that the strains have an anti-proliferate effect on a tumour cell line (Zabala *et al.*, 2001). The parenteral and oral administration of *L. casei* strain Shirota, *Lactobacillus* GG and *L. acidophilus* to rats resulted in immune stimulatory and antitumour effects against experimentally implanted tumours (Morotomi, 1996). *L. rhamnosus* GG and *L. casei* strain Shirota supplementation reduced fecal β-glucuronidase, nitroreductase and glycoeholic acid hydrolase activities in humans (Lidbeck *et al.*, 1992). The dietary administration of lyophilized cultures of *B. longum* strongly suppressed colon and mammary tumour development in humans (Reddy, 1999). This effect was associated with a decrease in the colonic mucosal cell proliferation. The consumption of *L. rhamnosus* GG and *L. gasseri* strain ADH also resulted in reduced fecal and urinary mutagenicity in humans (Lidbeck *et al.*, 1992b; Aso and Akazan, 1992). Two independent studies on the treatment of human urinary bladder cancer using *L. casei*, indicated that probiotic bacteria could suppress urinary mutagenicity (Grill *et al.*, 1995).

### 1.3.5 Antagonistic activity against pathogens

Antagonism by probiotic bacteria against pathogenic bacteria is important for the maintenance of a normal intestinal balance. Antagonism is mediated by the production of antimicrobial substances such as bacteriocins (proteins or protein complexes), organic acids (acetic and lactic acids) and low molecular weight metabolites (diacetyl, $CO_2$ and acetaldehyde) by probiotic strains (Saarela *et al.*, 2000).

The main fermentation products of LAB are organic acids (lactate and acetate) that lead to a lowering of pH and inhibition of the growth of Gram-negative pathogens, moulds and yeasts (Gililand, 1989; Silva *et al.*, 1987). These acids are more effective in their undissociated form whereby they penetrate the microbial cell, reduce the intercellular pH and interfere with essential cell functions (Silva *et al.*, 1987).

Bacteriocin production contributes to probiotic competitiveness, colonisation and growth in the gastrointestinal tract and has been reported for most of the LAB group (De Vuyst & Vandamme, 1994). Bacteriocins are proteins or protein complexes with a bactericidal or bacteriostatic mode of action affecting closely related species (Tagg *et al.*, 1976). Four distinct classes of bacteriocins have been identified on the basis of biochemical and genetic characterization including (I) antibiotics, (II) small heat-stable, non-lanthionine peptides, (IIa) Listeria-active peptides, (IIb) poration complexes consisting of two pepeides for activity, (IIc) thiol-activated peptides, (III) large heat-labile proteins, and (IV) complex bacteriocins (Klaenhammer, 1993). An overview of bacteriocins produced by LAB is presented in Table 2.3.

**TABLE 2.3:** Bacteriocin (Like) substances produced by LAB

| Producing microorganism | Bacteriocin (like) compound | Original reference |
|---|---|---|
| *L. casei* | | |
| LHS | caseicin LHS | Dicks *et al.* (1992) |
| *L. curvatus* | | |
| LTH 1174 | curvacin A | Tichaczek *et al.* (1992) |
| *L. plantarum* | | |
| A2 | lactolin | Kodama (1952) |
| SIK-83 | plantaricin SIK-83 | Andersson (1986) |
| NCDO 1193 | plantacin B | West & Werner (1988) |
| C-11 | plantaricin A | Daeschel *et al.* (1990) |
| BN | plantacin BN | Lewus *et al.* (1991) |
| LPCO-10 | plantaricin S | Jimenez-Diaz *et al.* (1993) |
| M1406 | plantaricin 406 | Larsen *et al.* (1993) |
| 423 | plantaricin 423 | Van Reenen *et al.* (1998) |
| *P. pentosaceus* | | |
| FBB-61, L-7230 | pediocin A | Etchells *et al.* (1964) |
| N5p | pediocin N5p | Strasser de Saad & Manca de Nadra (1993) |
| *E. faecalis* | | |
| S-48 | bacteriocin Bc-48 | Lopez-Lara *et al.* (1991) |
| 226 | enterocin 226NWC | Villiani *et al.* (1993) |
| *Enterococcus* spp. | Enterococcins (1-V) | Brock *et al.* (1963) |

Diacetyl, $CO_2$ and acetaldehyde, produced by probiotic bacteria are typical aroma compounds of fermented food products and beverages and are only antagonistic when present at high concentrations. The production of $CO_2$ is inhibitory mainly against aerobic microorganisms (Bernet-Camard *et al.*, 1997; Silva *et al.*, 1987). High concentrations of these low molecular weight metabolites are produced by *L. rhamnosus* strain GG, *L. acidophilus* strain LB and *L. johnsonni.* Gram positive and –negative pathogens and other microorganisms that seem to be inhibited by these metabolites include *Clostridium* and *Bacteriodes* spp., *Staphylococcus aureus, Listeria monocytogenes, S. typhhimurium, Shigella flexneri, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Pseudomonas aeruginosa,* and *Enterobacter* spp. (Bernet-Camard *et al.*, 1997; Coconnier *et al.*, 1997; Silva *et al.*, 1987). Microorganisms that were not found to be inhibited by low molecular metabolites include *Lactobacillus* or *Bifidobacterium* strains (Bernet-Camard *et al.*, 1997). It is therefore evident that supplementation with selected probiotic bacteria can inhibit the activity of a range of pathogen strains.

### 1.3.6 Production of β-galactosidase (lactase)

The enzyme, beta-galactosidase (lactase), hydrolyses lactose to glucose and galactose (Savaiano and Levitt, 1987). The enzyme is produced in the gastrointestinal tract for the first years of life. However, in specific ethnic groups, including blacks, Asians and South Americans, the levels of this enzyme decreases with age, resulting in lactose intolerance (inability to hydrolyse lactose) (Andersson *et al.*,

2001). Lactose intolerance can also develop secondary to an infection of the small intestine or destruction of mucosal cells due to other causes or other infections or conditions, such as diarrhea, AIDS or giardiasis, especially in children. Small bowel surgery or prolonged disuse of the gastrointestinal tract may also affect lactase activity (Tyus, 1994).

The symptoms of lactose intolerance include abdominal pain, flatulence, or diarrhea because the lactose behaves like an osmotic and non-digestible carbohydrate (Pfeifer and Rosat, 1999; Roberfroid, 2000). Probiotic bacteria used to produce fermented milk or yoghurt products, produce bacterial β-galactosidase in the intestine and stomach where lactose is degraded (Kopp-Hoolihan, 2001). Although lower concentrates are produced in the gastrointestinal tract than in the yoghurt starter cultures, *L. bulgaricus* and *S. thermophilus*, the resulting β-galactosidase levels are present in the gastrointestinal tract for longer time periods, facilitating lactose hydrolysation (Sanders, 1993). The role of probiotics in the improvement of lactose hydrolysation in those who are lactose intolerant, is illustrated by research that indicates that lactose from yoghurt, or milk containing the probiotic *L. acidophilus* is better absorbed by subjects with low β-galactosidase activity than milk that does not contain *L. acidophilus*. The symptoms of lactose intolerance in the subjects were fewer and bacterial fermentation of undigested lactose was also evident in breath hydrogen concentrations (Montes *et al.*, 1995).

Dairy products or other products containing the mentioned probiotic bacteria can therefore be included in the diet of people suffering from lactose intolerance to prevent/reduce the symptoms.

### 1.3.7 Summary of potential health effects of probiotics

The potential health effects of probiotics can be summarized as follows:

- Prevention/treatment of rotavirus diarrhea in children, traveler's diarrhea and inflammatory bowel disease conditions by production of antimicrobial substances, attachment to intestinal mucosal cells and stimulation of the immune response.
- Prevention of cancer by inactivation of dietary and intestinally generated mutagenic compounds and reduction of fecal bacterial enzymes.
- Alleviation of lactose intolerance by elevating ß-galactosidase (lactase) levels for long time periods.

## 1.4 Culturing of probiotics

For the commercialization of probiotics effective industrial production is essential, after which the survival of the microorganisms and retention of functionality during storage must be ensured before they can be delivered to manufacturers of products containing probiotic bacteria. Factors such as

storage form, packaging materials used and storage conditions play an important role in the delivery of a quality product (Saarela *et al.*, 2000).

### 1.4.1 Growth needs of probiotic bacteria

The acidophilic nature, complex nutritional requirements and the typical habitats of probiotic bacteria need to be taken into account in the development of specific growth media for these microorganisms (McCann *et al.*, 1996). The nutritional requirements of *Lactobacillus* species are met when the medium contains fermentable carbohydrates, peptones and meat and yeast extracts. Supplementation with tomato juice, magnesium, manganese, polysorbate, acetate and oleic acid esters, especially Tween 80, have been found to promote the growth of probiotic bacteria and might even be essential for most species. Mediums containing these supplements exhibit a low degree of selectively as *Pediococcus* and *Leuconostoc* species and other secondary bacteria may grow on this medium (De Man *et al.*, 1960; Kandler & Weiss, 1986).

In *in vitro* laboratory studies the most commonly used growth medium(s) which contains all the mentioned nutrients, are based on the MRS medium (De Man *et al.*, 1960). For commercial production, probiotic bacteria can also be grown in heat-treated milk and milk-based media. More recently whey-base, a byproduct from cheese manufacture, has been used successfully (Gilliland, 1985; Svensson, 1999). Milk is nutritious and contains carbohydrates, fat, casein protein, vitamins and minerals (Marshall & Tamime, 1997). To avoid too slow growth and the associated development of off-flavours when a milk-base is used, casein and whey protein hydrolysates, yeast extract, glucose, prebiotics, vitamins and minerals can be added to stimulate the growth and survival of the strains and enhance the texture of the products (Hunger & Peitersen, 1992; Mizota, 1996). Furthermore, the buffer capacity of fermented milk may be increased by the addition of protein. Probiotic strains will then survive better as the pH of the medium is decreased and further pH changes during storage are prevented (Kailasapathy & Supriadi, 1996). Lactobacilli can tolerate pH values in the range of 3.5 to 3.8, whereas the growth of bifidobacteria is retarded below pH 5.0 (Bergey's Manual, 1974). The growth of probiotic bacteria may also be influenced by antimicrobial substances such as cleansing agents, disinfectants and bacteriophages in milk (Svensson, 1999). Because the optimum growth temperature for most probiotic bacteria is 37°C, production temperatures of 37°C will favour the probiotic organism (Kneifel *et al.*, 1993). As vegetarianism is increasing, appropriate vegetarian probiotic products should also be developed (Beardsworth & Keil, 1991; 1992). For these purposes the probiotic inoculum should therefore be cultured in a growth medium free of animal-derived ingredients such as medium containing 25g/L soy peptone, glucose monohydrate and yeast extract (Heenan *et al.*, 2002).

### 1.4.2 Production of commercially available probiotic preperations

Manufacturers of commercially available probiotic preperations are currently selecting lactic acid bacteria with probiotic characteristics for these preparations (Mogensen & Friis, 1997). These commercially available probiotic preperations may consist of a single strain or a mixture of several strains (German *et al.*, 1999).

The probiotic preparations for distribution should contain a large number of viable cells. In the past, liquid and frozen preparations were used for distribution purposes. Currently freeze-drying and spray-driying and micro-encapsulation are used for these purposes (Champagne *et al.*, 1991; Gölker, 1993). Although spray-drying is more economical (Gölker, 1993; Johnson & Etzel, 1993), freeze-drying is the most popular method in the production of dried LAB preparations, as many LAB cannot tolerate the relatively high temperatures that are used in spray-drying (Porubcan & Sellars, 1979). However, protectants can be added to the cultures to be dried in order to prevent cell injury during drying and subsequent storage (Champagne *et al.*, 1991; Souzu, 1992). The most common protectants used at industrial scale are lactose or sucrose, monosodium glutamate (MSG), and ascorbate in milk or in a water base (Mäyrä-Mäkinen & Bigret, 1998). Micro-encapsulation (protective coating of probiotic bacteria) and added oligosaccharides in probiotic containing products have also been found successful in increasing the survival of the probiotic bacteria, although further research is needed to develop this technique (Kailasapathy & Rybka, 1997).

The commercial probiotic preparations are mostly supplied to the manufacturers of probiotic containing products in a highly concentrated form and constructed for DVS (direct vat set) application (Honer, 1995). DVS cultures are highly concentrated cultures that are prepared as mixtures of defined strains in predetermined proportions. They are supplied in frozen- or freeze-dried form and their high concentration allows for their ability to be inoculated directly into the carrier food or capsules (Honer, 1995). Deep frozen cultures usually contain more than $10^{10}$ cfu $g^{-1}$, whereas freeze-dried cultures contain more than $10^{11}$ cfu $g^{-1}$ (Oberman & Libudzisz, 1998). The cell concentration per gram of product varies with the culture and the type of organism (Mattilla-Sandholm *et al.*, 2002). Gas and light proofed containers are used to package these probiotic preparations to protect them against light and humidity. Aluminium-foil coated cartons and pouches are most often used (Honer, 1995).

## 1.5 Probiotic containing products

Probiotic bacteria may be incorporated in special formulations like capsules or tablets, or can be added to food to contribute specific probiotic or functional properties (Mattilla-Sandholm *et al.*, 2002). The viability and stability of probiotic bacteria in these products has been a marketing and technological challenge for industrial producers (Mattilla-Sandholm *et al.*, 2002).

### 1.5.1 Supplements

A dietary supplement is defined as a product intended for ingestion as a supplement to the diet. Supplements may contain one or more of the following ingredients: vitamins, minerals, herbs, botanicals, or other plant-derived substances; amino acids, enzymes, concentrates and extracts. Dietary supplements can be manufactured as pills, tablets, capsules, gelcaps, liquids and powders (Smolin & Grosvenor, 2000: 306). The production of supplements containing probiotic bacteria involves the lyophilization (freeze/spray dried) and micro-encapsulation of probiotic bacteria for fully protection in the gastrointestinal tract. Materials used for encapsulation include gelatin, shellac and amylose (http://www.danoneinstitute.org/, 2003/08/09 accessed).

### 1.5.2 Functional foods containing probiotics

Food products fortified with probiotic bacteria form part of a class of food items referred to as functional foods. Functional foods can be defined as physiologically active foods which provide health benefits beyond basic nutrition by affecting one or a limited number of functions in the body in a targeted way (Clydesdale, 1999; Roberfroid, 1999). "The component that makes the food 'functional' can be either an essential macronutrient with specific physiological effects such as resistant starch or omega-3 fatty acids or an essential micronutrient in intakes over and above the daily recommendations" (Roberfoid, 1999). It also includes food components that have some nutritive value, but are not classified as 'essential', such as oligosaccharides or food components with no nutritive value, such as live microorganisms (e.g. probiotics) or plant chemicals (Roberfoid, 1999).

Different types of products are proposed as vehicle foods for probiotic bacteria by which large amounts of probiotic cells can be consumed for therapeutic effect. These include fermented diary, soymilk products and non-dairy products such as cereals (Andersen, 1998; Honer, 1995; Lourens-Hattingh & Viljoen, 2001).

#### 1.5.2.1 Fermented dairy products

Probiotic strains used in the manufacturing of fermented dairy products are selected on the base of rheological (flow and deformation) and organoleptic (appearance, taste and smell) and potential health associated properties. The ability to grow and survive in milk and resulting shelf life also need to be considered (Kailasapathy & Rybka, 1997). The most important species used in fermented dairy products include *Lactobacillus* and *Bifidobacterium* species (Lourens-Hattingh & Viljoen, 2001).

Probiotic bacteria are often mixed with starter cultures to achieve the desired flavour and texture. Products fermented with *L. delbruekii* for example might be too acidic with a too heavy acetaldehyde flavour. Because of this, *S. thermophilus* and *L. delbrueckii* or ABT cultures of *L. acidophilus, Bifidobacterium* and *S. thermophilus* are used as starter cultures to flavour the products in which they

are used (Driessen & Loones, 1992). These cultures are also used in combination with other probiotic bacteria to hasten the acidification process to minimise the risk of contamination by pathogens at 37°C and ensure an acceptable production cost (Driessen & Loones, 1992).

The number of viable microbial cells that should be present in a fermented dairy product up to expiration date is between $10^6$ and $10^8$ CFU/ml (Svensson, 1999). It is essential that products sold with any health claims meet this criterion (Playne, 1994).

Factors that influence the viability of probiotics in dairy products include pH, storage temperature less than 4°C, and presence of competing micro-organisms and inhibitors (e.g. NaCl) and oxygen tension (Kneifel *et al.*, 1993; Mattilla-Sandholm *et al.*, 2002). The quantity of acid products, hydrogen peroxide produced and oxygen used by the different strains may effect the growth and survival of each strain, as the growth and survival properties differs between bacterial strains (Dave & Shah, 1997). Therefore, the combination of probiotic strains is also important, and must be tested for compatibility before being used in a product (Dave & Shah, 1997). The survival of probiotic bacteria can be improved by de-aerating milk in the dairy plant and by packing strains in oxygen-impermeable packages (Dave & Shah, 1997). The use of ascorbic acid as oxygen scavenger has also been successful to improve the viability of probiotic bacteria (Dave & Shah, 1997).

### 1.5.2.2 Fermented soymilk products

Soymilk is a water extract of soybean that provides an adequate and inexpensive supply of protein and energy for human consumption. Although it is a traditional oriental food beverage, the presence of the indigestible oligosaccharides, stachyose and raffinose and the raw bean flavour limits the wider consumption of soymilk and other soybean products (Wang *et al.*, 2002). However, because of the nutritional properties of soymilk it has been used successfully as a substrate for growth and biochemical activities of LAB to produce stable fermented soy-milk products which contain more readily digestible compounds (Kamaly, 1997), as is illustrated by the following examples:

- In a study done by Beasley *et al.* (2003) a monoculture of *Lactococcus lactis* LL3 strain isolated from human milk was used to ferment soymilk into a sour milk-type product. Results indicated that soymilk supported the growth of *L. lactis* and produced a microbiologically stable and flavourable sour milk-like product (Beasley *et al.*, 2003).
- Wang *et al.* (2002) described the behaviours of the bifidobacteria *B. infantis* and *B. longum* and of the lactic acid bacteria *L. acidophilus*, *L. bulgaricus* and *S. thermophilus* during the fermentation and storage of cultured soymilk drinks. Results indicated that soymilk supported the simultaneous growth of bifidobacteria and *L. acidophilus* or *S. thermophilus*. However *B. infantis* and *B. longum* had a deleterious effect on the growth of *L. bulgaricus* in soymilk. The numbers of

bifidobacteria and lactic acid bacteria remained stable in drinks with or without sucrose during the storage for 10 days at 5 °C.

- Wang *et al.* (2003) investigated the sugar and acid content of soymilk fermented with lactic acid bacteria alone or in combination with bifidobacteria. Results indicated that *L. acidophilus* and *S. thermophilus* metabolized the oligosaccharides, stachyose and raffinose present in the soymilk. During the 24 – 32h fermentation the content of fructose, glucose and galactose in soymilk increased and the pH decreased.

Probiotics can therefore be used in the manufacturing of acceptable soymilk fermented products. However, the pH, storage temperature at 4°C, and presence of competing micro-organisms and inhibitors (e.g. NaCl) and oxygen tension described in Section 1.6.1 should also be considered in the manufacturing of these products.

### 1.5.2.3 Other food vehicles for probiotic bacteria

Other possible food vehicles for the incorporation of probiotic bacteria include baby food, cereals and confectionary. It is important that the activity and viability of the probiotic bacteria is maintained in these products for extended periods of time as the probiotic bacteria are added as additives and do not multiply. (Mattilla-Sandholm *et al.*, 2002) Therefore the factors described in Section 1.5.2.1 regarding the viability of probiotic bacteria should also be considered in the production of these products. Storage at room temperature, which is common for many types of non-dairy products such as cereal products, drinks and confectionery can create stability problems for probiotic bacteria (Kailasapathy & Rybka, 1997). Recently, research on new probiotic formulations and micro-encapsulation technologies exploiting biological carrier and barrier materials and systems for enteric release, provided promising results in this regard. These technologies have been used to develop stable probiotic-containing baby food formulations and confectioneries currently on the market (Langhendries *et al.*, 1995; Fukushima *et al.*, 1997). The challenge for future probiotic processing and formulation technologies is focussed on the maintenance of low production and thus product costs (Mattilla-Sandholm *et al.*, 2002).

## 1.5.3 Safety criteria

The use of probiotic bacteria has a long history and this provides evidence that these microbes are safe for human consumption. LAB regarded as safe for human use need to comply with specifications regarding origin (healthy human gastrointestinal tract), non-pathogenicity and antibiotic resistance (Mattilla-Sandholm *et al.*, 2002). Lactobacilli and lactococci are classified as "generally recognised as safe" (GRAS) by the Food and Drug Administration of the USA.

Safety criteria for successful probiotics have been defined in reviews by Lee and Salminen (1995); Donohue and Salminen (1996) and Salminen *et al.* (1998) and include the following:

- Strains used for humans have to be preferably of human origin and isolated from healthy human GI-tract
- Strains should have no history of being pathogenic or associated with diseases such as infective endocarditis of GI-disorders
- They must not carry transmissible antibiotic resistance genes

## 2. Prebiotics

### 2.1 Definition/description of prebiotics

Gibson and Roberfroid (1995) defined a prebiotic as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/ or activity of one or a limited number of bacteria in the colon that can improve host health". Prebiotics can either be found naturally in food products such as onion, garlic, banana, chicory, asparagus, leek and Jerusalem artichoke or are synthesised from simple sugars, such as sucrose or lactose, or can be produced by controlled hydrolysis of starch or other polysaccharides (Gibson, 2001; Van Loo *et al.*, 1995).

### 2.2 Compounds with convincing prebiotic effects

The nutritional properties of prebiotics classify them as functional food ingredients and they can also play an important role in food product development due to their physiological properties (Roberfroid, 2000). The only food-ingredients that are currently known to have convincing prebiotic effects are non-digestible oligosaccharides (NDO's). These oligosaccharides include those that contain fructose, xylose, soya, galactose, glucose and mannose residues. Most research has been done on oligosaccharides containing fructose e.g. inulin-fructans (Roberfroid, 2001). Oligosaccharides are defined as glycosides that have between three and ten simple sugars linked together and contain low levels of monosaccharides, di- or polysaccharides (Playne & Crittenden, 1996).

The properties of oligosaccharides depend on their chemical structure, molecular weight and levels of mono- and disaccharides. These properties influence food-freezing temperature and mouthfeel (Playne & Crittenden, 1996; Roberfroid, 1993).

However, the physiological properties of oligosaccharides such as reduced energy value, potential hypocariogenic properties, dietary fibre like effects and improving growth of beneficial microflora are the most important for the use of oligosaccharides to fortify food (Playne & Crittenden, 1996; Roberfroid, 1993).

### 2.2.1 Inulin

Inulin (Fig.2.2) contains 10% mono- and disaccharides (sucrose and fructose). Ninety percent of the oligosaccharides found in chicory roots, the best natural source of inulin, is in the form of inulin

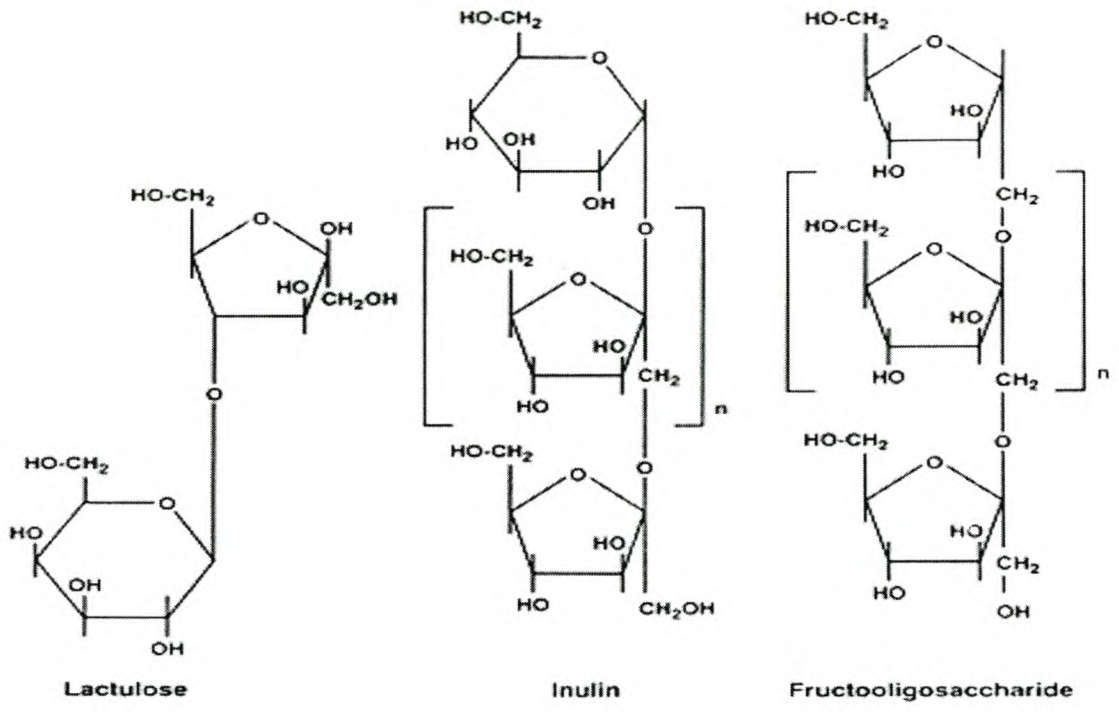


**FIGURE 2.2** Chemical structures of the prebiotics lactulose, inulin, and fructo-oligosaccharide (Roberfroid, 1993).

The nutritional properties of inulin include the following:

- It is a non-digestible food ingredient and the energy value recommended for food labelling is 6.28kJ/g (Roberfroid, 1999a).
- It may be classed as a dietary fibre, because it is resistant to the digestive processes and fermented by colonic bacteria, which increases the faecal biomass, produces short-chain fatty acids and decreases the pH in the colon (Roberfroid, 1993).
- It stimulates the growth of bifidobacteria and inhibits the growth of bacteria, which is less beneficial (Roberfroid, 1999b)

Increased intakes will therefore result in increased dietary fibre content, increased bifidus-promoting capacities and reduced total dietary energy content.

The following physical characteristics of inulin ensure that it can be used effectively to fortify food products:

- It has a neutral taste and is colourless, and therefore has minimal influence on the organoleptic characteristics of the product. If necessary inulin can be combined with high intensity sweeteners to give it a more sugar-like sweetness (Niness, 1999).
- It is highly soluble in water and can be used to increase water viscosity at a concentration below 40% (Niness, 1999). At a concentration of 40-45% inulin forms a gel in water that is firm but with a fatty mouthfeel. In this form it is stable in acidic conditions and at high temperatures. Therefore inulin can be used in fat-free products to give these products a smooth creamy texture and taste (Wouters, 1998).

### 2.2.2 Other oligosaccharides

There are twelve classes of other food grade oligosaccharides, which have the same physiological properties as inulin, including fructo-oligosaccharides, galacto- oligosaccharides, lactulose, laktosucrose, palatinos- oligosaccharides, glycosyl sucrose, malto- oligosaccharides, isomalto-oligosaccharides, cyclodextrins, gentio- oligosaccharides, soybean oligosaccharides and xylo-oligosaccharides (Playne & Crittenden, 1996).

Oligosaccharides other than inulin that are frequently used as prebiotic food ingredients, include the following:

- Fructo-oligosaccharides (FOS) (Fig. 2.2), which contain mostly glucose and fructose units modifiy the nutritional and organoleptic (appearance, taste and smell) properties of foods, particularly low-energy foods. For food labelling purposes the energy value of FOS is set at 6.28 kJ/g (Roberfroid, 1999a), although human studies suggest an energy value of 9.5kJ/g (Molis *et al.*, 1996). FOS provides 30 – 50% of the sweetness of table sugar and is more soluble than sucrose. Sucrose is often replaced by a combination of FOS with high intensity sweeteners as it provides a balanced sweetness profile and masks the aftertaste of sweetness such as aspartame or acesulfame K (Wiedmann & Jagar, 1997).
- Galacto-oligosaccarides (GOS) which contain glucose and galactose units are found in human milk but can be manufactured commercially. The natural occurring form in milk can stimulate the growth of bifidus flora in breast-fed infants (Rubaltelli, 1998).
- Lactulose (Fig.2.2) which contains galactose and fructose units is a pharmaceutical preparation that can be used to treat constipation and hepatic encephalopathy, as it is not absorbed in the upper intestine. It can also be included in infant milk formulas for its bifidogenic effects (Kontula *et al.*, 2000; Clausen & Mortensen, 1997; Baskaran *et al.*, 1999).
- Lactosucrose contains galactose, fructose and glucose units and is also a pharmaceutical preparation that has potential bifidogenic effects (Clausen & Mortensen, 1997; Baskaran *et al.*, 1999).

### 2.2.3 Resistant starch

Resistant starch is also a carbohydrate, which is naturally found in raw potatoes and bananas and in processed foods, such as pasta and bread (Wisker, 2000). Resistant starch is fermented by large bowel microflora and not absorbed in the small intestine (Asp, 1997). It can therefore be seen as a dietary fibre with prebiotic effect and has an estimated energy value of 11.7 kJ/g (Behall & Howe, 1996). Resistant starch can be used as a fat mimetic or to increase dietary fibre content of food. It will not alter the texture and appearance of food, because of its white colour, bland taste and micro-particulate structure (Würsch, 1999).

## 2.3 Physiological and metabolic impact of prebiotics and associated effects

Prebiotics have the potential to positively influence human health by escaping digestion in the small bowel and by playing a role in the establishment of normal microflora in the large intestine (Crittenden, 1999). The growth and activity of beneficial organisms (probiotic bacteria) in the gastrointestinal tract are stimulated by prebiotics and therefore contribute to the health benefits of probiotic bacteria as mentioned in Section 1.3.1. However, prebiotics also have health benefits not directly related to the enhancement of the growth and inhibitory activity of probiotics. These benefits include improvement of mineral uptake and reduction of plasma cholesterol levels and constipation (Crittenden, 1999) which are largely brought about by the stool bulking effect of prebiotics as well as the stimulating effect it has on the production of short-chain fatty acids.

### 2.3.1 Increased in biomass and stool bulking

Inulin-type fructans resist digestion in the upper part of the gastrointestinal tract because of their β-configuration of the enomeric C-2 in the fructose monomers. The non-digestible oligosaccharides (NDO's) remain in solution in the chyme and contribute to osmotic pressure, resulting in increased water flow. Colonic microflora ferment the NDO's and produce gases, short-chain fatty acids and lactate. These processes also contribute to an increase in biomass and consequently to an increase in faecal bulk and dry matter, which improves gastrointestinal motility (Van Loo *et al.*, 1999). The latter is illustrated by the work of Gibson *et al.* (1995) who studied the effect of oligofructose intake on human biomass and stool bulking. Volunteers were given 15g oligofructose/d for a period of two weeks. An increased stool weight corresponding to 1.5 – 2g increase in stool weight per gram oligofructose ingested, was observed. However, the intestinal transit time was not effected (Van Loo *et al.*, 1999: 123). In another controlled study done by Gibson *et al.* (1995), the subjects' stool output was 92g/d before inulin intake and 123g/d after 15g/d inulin intake (Cummings *et al.*, 2001). Van Loo *et al.* (1999) reported that 1.5 – 2g NDO's/day resulted in faecal bulking and in a normalization of stool frequency.

### 2.3.2 Production of short-chain fatty acids

In prebiotic metabolism, the anaerobic microflora in the colon convert undigested carbohydrates to SCFAs, including acetate, propionate and butyrate. The formation of different proportions of these SCFA's is associated with fermentation by different populations of the colonic ecosystem, which are stimulated by different carbohydrates. Research done on *in vitro* fermentations indicate that inulin-type fructans typically increase production of acetate and butyrate, galacto-oligosaccharides increase production of acetate and propionate, and xylo-oligosaccharides increases the production of acetate only (Campbell *et al.*, 1997). This is also illustrated by the work of Wang and Gibson (1993) who compared the *in vitro* production and molar ratios of SCFA's from seventeen carbohydrate sources by using fecal innocula from six healthy volunteers. The molar ratios of acetate: propionate: butyrate of the two established prebiotics (FOS and inulin) were 78:12:8 and 72:18:8. In a similar *in vitro* study the faeces of two subjects who had been eating 20g FOS daily for four weeks, were analysed. The molar ratios of acetate: propionate: butyrate at 12h was found to be 63:12:25 (Luo *et al.*, 1996). The production of SCFA's is associated a number of health benefits, including promotion of probiotic bacterial growth, increased mineral absorption, improved lipid metabolism, suppression of carcinogenesis and immunomodulation.

#### 2.3.2.1 Promotion of probiotic bacterial growth

The production of SCFA's and lactic acid lead to a drop in the pH of the large intestine. This effect is beneficial for the organism as it constitutes an ideal medium for the development of the bifidogenic flora and, at the same time, limits the development of bacteria which are considered to be pathogens, thus preventing or decreasing disease conditions associated with these organisms (Schley, 2002) (see Section 1.3.1 for health benefits of probiotic bacteria).

#### 2.3.2.2 Improved mineral absorption

Results of several studies done on mineral absorption indicate that NDO's improve the bioavailability of minerals (Ohta *et al.*, 1993; Delzenne *et al.*, 1995; Scholz-Ahrens *et al.*, 1998; Lemort and Roberfroid, 1997). In different research studies all the results indicated that the increased absorption originates mainly at the level of the large intestine and that it results in increased bone density (Baba *et al.*, 1996).

NDO's bind to minerals that are not absorbed in the small intestine. In the colon these minerals may be released from the carbohydrate matrix and be absorbed. Colonic fermentation of NDO's results in a high concentration of short-chain carboxylic acids, which facilitates the colonic absorption of minerals, particularly calcium and magnesium. NDO's may also have an osmotic effect that transfers water into the large bowel, thus increasing the volume of fluid in which these minerals can dissolve, which in turn improves mineral absorption and balance (Roberfroid, 2000).

The effect of NDO's on mineral absorption is illustrated by human feeding studies where a significant effect of inulin-type fructans on calcium absorption was found. In the first study nine men (mean age: 21.5 ± 2.5y) ingested 850mg $Ca^{2+}$/day and a dietary supplement of 40g/day of inulin. They experienced a significant increase (± 12%) in the apparent absorption of calcium without any significant change in urinary excretion. In the second study, twelve males between the ages of 15 and 18 years consumed 16.8 g of oligofructose/day. Their calcium balance, as measured by the double stable isotope technique, increased 11% with no significant effect on urinary excretion (Coudray *et al.*, 1997). Similar effects for magnesium and iron absorption have been reported for animals (Van Loo *et al.*, 1999).

A further mechanism through which NDO's might benefit the absorption of minerals, involves the enhanced degradation of phytic acid (Lopez *et al.*, 2000). The latter compound binds mineral cations, rendering them unavailable for absorption. Results from studies done on phytic acid indicated that fermentable carbohydrates in diets could enhance phytic acid breakdown and improve cation absorption. The results of a recent study supports this possibility as improved mineral absorption (calcium+20%, magnesium+50%, copper+45%) and mineral status (in blood, liver and bone) were found in rats that ingested inulin (100g/kg diet) (Younes *et al.*, 2001).

#### 2.3.2.3 Improved lipid metabolism/profile

The mechanisms by which prebiotics influence blood lipids are not clearly understood at present (Rastall & Gibson, 2002). Two hypothesis have been formulated to explain the possible mechanism by which serum cholesterol is lowered by prebiotics. The **first hypothesis** involves delayed gastric emptying that slows down chyme entrance into small intestine. The rate of carbohydrate and lipid absorption is decreased, with modulated (decreased) insulin secretion and lipoprotein formation (Lupton, 2000). The **second hypothesis** refers to a two-fold increase in the portal concentration of both acetate and propionate because of the production of short-chain fatty acids in the large intestine. Acetate and propionate are absorbed by the portal vein and transported to the liver. Propionate inhibits HMG CoA reductase (rate-limiting enzyme for cholesterol synthesis) activity in the liver with a consequent decrease in cholesterol synthesis (Lupton, 2000). This hypothesis is supported by studies done by Takase *et al.* (1994), Delzanne and Kok (1999) and Roberfroid (2000) who reported that propionate inhibited fatty acid biosynthesis and the formation of serum LDL cholesterol in individuals consuming prebiotic supplements. Davidson *et al.* (1998) also reported that cholesterol concentrations in rats are decreased by long-term administration of FOS. Preliminary data indicates that inulin (18g/day for 3weeks) may also lower both total and LDL serum cholesterol in slightly hypercholesterolemic subjects (Davidson *et al.*, 1998).

#### 2.3.2.4 Suppression of carcinogenesis

As was mentioned in Section 1.3.4, it is believed that microflora play a role in the development of bowel cancer. Short-chain fatty acids can potentially decrease the risk of colon cancer by the following mechanisms:

- Butyrate stimulates apoptosis in colonic cancer cell lines and is the preferred fuel for healthy colonocytes (Prasad, 1980; Kim *et al.*, 1982). It is therefore desirable to increase the level of butyrate formation in the large gut and prebiotics are known to have this effect (Olano-Martin *et al.*, 2000).
- The formation of phenol, indoles, amines and ammonia, which could promote the development of colon cancer, is the result of protein and amino acid metabolism by certain microorganisms in the colon. Prebiotics have been shown to effectively reduce the levels of these compounds by inducing a shift in bacterial metabolism in the colon (Ballongue *et al.*, 1997; Rastall & Gibson, 2002).
- The conversion of primary bile acids to secondary bile acids by microflora can increase the risk for the development of colon cancer. Short-chain fatty acids produced after the ingestion of prebiotics lowers the pH to lower than 6.5, which inhibits this conversion (Heijnen, 1998).

The colon cancer reducing properties of FOS, GOS and resistant starch have been investigated in animal and human trials. FOS reduced toxic enzyme levels (Bouhnik *et al.*, 1996) and resistant starch reduced sterols, secondary bile acids and genotoxic enzyme levels (Hylla *et al.*, 1998). However, a recent study on GOS found no significant changes in markers for carcinogenesis (Alles *et al.*, 1999). It is therefore clear that knowledge on the effects of prebiotics on colon cancer is still inadequate and that more research in this regard needs to be conducted (Rastall & Gibson, 2002; Van Loo *et al.*, 1999).

#### 2.3.2.5 Immunomodulation

Probiotics stimulate both non-specific host defense mechanisms and certain cell types involved in the specific immune response (see Section 1.3.3). As prebiotics stimulate the growth and activity of probiotics, the intake thereof may increase immune function, as is illustrated by the following studies.

- The growth and health status of children (4 –24 months) receiving a standard infant cereal supplemented with FOS (0.55g/15g of cereal) or placebo was compared in a double blind, randomized controlled study. One hundred and twenty-three children who attended a day care center were enrolled and completed the study. The daily FOS intake of the group receiving the fortified cereal was 1.2 g. Febrile events and any cold symptoms, runny nose, antibiotic use and day care absenteeism were significantly reduced in the experimental group. Diarrhea-associated symptoms such as fever, medical attention required, vomiting, discomfort and regurgitation were also reduced. It was concluded that consumption of a cereal supplemented

with FOS was associated with fewer infectious periods and less severe diarrheal disease (Saavedra *et al.*, 1999).

- Haschke *et al.* (2001) investigated the effects of a milk-containing infant cereal supplemented with a prebiotic mixture of FOS and inulin Prebio1 on the immune response of 8-months-old infants after measles vaccination in a double randomized controlled study. The infants were randomly assigned to receive cereal with (1g/25g cereal) or without prebio1 during a 10-week period. All infants were vaccinated with live attenuated measles vaccine after four weeks of cereal feeding. Blood was collected before and 16 weeks after vaccination for IgG measles antibody measurement. Post-vaccination IgG antibody titers were significantly higher in the group receiving Prebio1 (Haschke *et al.*, 2001).

## 2.4 Manufacturing of prebiotics

Oligosaccharides can be manufactured using a range of extraction and chemical and enzymatic synthesis methods and cheap substrates (Roberfroid, 2001).

Extraction of oligosaccharides from biological material is the simplest approach to manufacture prebiotics, e.g. inulin is extracted from chicory on a commercial basis (De Leenheer, 1994; Koga *et al.*, 1993b). Chicory chips are extracted with hot water and the extracted chips are sold as animal feed. Proteins, peptide, anions, colloids and phosphates are then removed from the chips by liming and carbonation at alkaline pH. Anion and cation exchange chromatography is used to demineralise the inulin and it is decolourised by chromatography on activated carbon. The final product is sterilised, concentrated and spray-dried (De Leenheer, 1994).

The only prebiotic manufactured using a chemical approach is lactulose. Fructose is formed by a base-catalysed Lobry de Bruyn-Alberda van Ekenstein isomerisation of the glucose moiety of lactose as follows: Galβ1 → 4Glc → Galβ1→ 4Fru. The reaction results in both the pyranose and furanose forms of lactulose. The reaction is catalysed by either sodium hydroxide or borate (Timmermans, 1994) and degradative side reactions are minimised by carefully controlled reaction rates (Rastall & Gibson, 2002).

The most common methods currently used to manufacture include polysaccharide hydrolysis and enzymatic transfer reactions. Chemical methods of carbohydrate synthesis are generally inferior to enzyme-mediated approaches for industrial production, as side reactions of the chemical method result in unwanted colour and flavour compounds that must be removed in further refining steps. The field of food-grade oligosaccharides production is proving to be an area for innovation in enzyme technology and new processes are likely to be developed in the future (Rastall & Gibson, 2002).

Polysaccharide hydrolysis is used to manufacture (FOS) from inulin and xylo-oligosaccharides (XOS) from xylan. In Europe FOS are manufactured by partial enzyme hydrolysis of chicory inulin (De Leenheer, 1994). Inulin contains chains terminating in either a reducing fructose residue or a non-reducing glucose residue. FOS produced from inulin therefore have a degree of reducing activity (De Leenheer, 1994). In Japan xylo-oligosaccharides are manufactured commercially by enzymatic hydrolysis of xylan from corncobs (Koga *et al.*, 1993a). The xylan is extensively hydrolysed to the disaccharide xylobiose, its repeating unit. Smaller quantities of higher oligosaccharides are also formed. Xylose and high molecular weight components are removed by membrane processes to purify xylobiose (Rastall & Gibson, 2002).

Enzymatic transfer reactions are used to manufacture isomalto-oligosaccharides, lactosucrose and some FOS (Playne & Crittenden, 1996). Cheap sugars such as sucrose and lactose are utilised as donors and acceptors in the following manufacturing procedures:

- FOS are build up by using fructosyltransferase from *Aureobasidium pullulans* or *Aspergillus niger* or are used from a 60% (w/v) sucrose solution at 50-60°C in an immobilised cell-based reactor (Yun, 1996). Glucose and sucrose can be removed by ion-change chromatography to ensure product qualities.
- Galacto-oligosaccharides (GOS) are manufactured commercially from lactose using ß-galactosidase (Matsumoto, 1993). ß-galactosidase is a hydrolase enzyme and works by transferring galactose from lactose to water. GOS are formed under conditions of high lactose concentration when lactose is utilised as an alternative acceptor to water (Timmermans, 1994).
- Lactosucrose is made by incubating a mixture of sucrose and lactose with ß-fructofuranosidase at high concentrations (Kitahata & Fujita, 1993).
- Isomalto-oligosaccharides (IMO) are manufactured by a more complex process of enzymatic synthesis (Yatake, 1993). Starch is hydrolyzed to malto-oligosaccharides and used as a substrate in an enzymatic synthesis reaction. Glucose is removed from the mixture using chromatography to give the final IMO products (Rastall & Gibson, 2002).

## 2.5 Dose of prebiotics required for health effects

The levels of prebiotic oligosaccharides in food such as onions, garlic, banana, asparagus, leek and Jerusalem artischoke are too low to have any significant effects on health (Gibson, 2001). Therefore dietary fortification is important to ensure the different benefits of prebiotics (Gibson, 2001). Dosages of 8g to 40g prebiotics for one to three weeks have been assessed for their effect on lipid profile and mineral absorption in humans (Table 2.4).

**TABLE 2.4:** The effect of the consumption of different types and doses of prebiotics on lipid metabolism and mineral absorption

| Study | Effect on lipid metabolism and mineral absorption | Author |
|---|---|---|
| 18 non-insulin-dependent diabetic subjects were given 8 gram FOS for 14 days | No reduction of LDL-cholesterol and fasting blood glucose levels was observed | Yamashita *et al.* (1984) |
| Healthy adults were given 9g inulin/day for 2 weeks | A reduction in serum triacylglycerol and cholesterol levels was found | Canzi *et al.* (1995) |
| Nine adult volunteers consumed 40g inulin/day for 20 days | A 58% increase (from 21.3 to 33.7%) in calcium absorption was observed | Coudray *et al.* (1997) |
| 18 non-insulin-dependent diabetic subjects were given 15g FOS/day for 20 days | No reduction of LDL-cholesterol and fasting blood glucose levels was observed | Alles *et al.* (1998) |
| Healthy but slightly hyperlipidaemic subjects were given 18g inulin/day for 3 weeks | A regulating effect on total and LDL-cholesterol levels was found | Davidson *et al.* (1998) |
| 54 healthy but slightly hyperlipidaemic volunteers consumed 10g inulin/day for 15 days | No effect on cholesterol was found. However, a 19% decrease in fasting serum triacylglycerol and a 10% decrease in insulin levels was observed after eight weeks | Williams (1998) |
| Twelve volunteers consumed 15g FOS/day for one week. | A significant increase of 25% in calcium absorption was found | Van den Heuvel *et al.* (1999) |

These results clearly indicate that the higher doses for longer periods were the most beneficial. Generally, research indicates that prebiotic intake between 4-40gram/day is efficient to have a significant effect on human health (Gibson, 2002).

### 2.6 Negative effects of increased prebiotic intake

Excessive intake of prebiotics may result in excessive gas production in the gastrointestinal tract (Cummings *et al.*, 2001). Carbon dioxide and hydrogen are the main gases produced by gut microorganisms as products of prebiotic fermentation (Cummings *et al.*, 2001). Gas production leads to unwanted symptoms as reported in the following human trials with prebiotic feedings.

- In a study done by Stone-Dorshow and Levitt (1987), 112 subjects took 15g FOS daily for 12 days. Symptoms of abdominal pain, eructation, flatulence and bloating were significantly more severe when compared with a group who only took sucrose.
- Two studies in which FOS was consumed at doses of 5 and 20g/day showed dose-related increases in breath hydrogen and mild flatulence (Gibson *et al.*, 1995; Luo *et al.*, 1996).
- In a study concerning inulin intake, breath-hydrogen excretion increased and symptoms such as flatulence, rumbling, stomach and gut cramps and bloating significantly increased in 64 women consuming a dose of 14g/day. The symptoms were reported to be unacceptable in 12% of the volunteers.

Prebiotics with different chain lengths, degree of branching and degree of polymerization could be developed to decrease the problem of gas formation and related symptoms (Cummings *et al.*, 2001).

### 2.7 Prebiotic containing products available on the market

Prebiotics can be used in the fortification of foods or be combined with probiotics in supplements (Douglas, 2003: 2003/11/26 accessed). Prebiotics are usually encapsulated with probiotic bacteria in capsule form to protect and increase the level of growth substrate for the probiotic in the gastrointestinal tract.

A wide range of foods can be used as carriers of prebiotics because it is simple to use, less costly than probiotics and easy to introduce in the food vehicle (Saavedra, 2002; Gibson, 2000). Examples of foods that can be fortified with prebiotics include the following: beverages and fermented milks, health drinks, bakery products, spreads, sauces, infant formulae and weaning foods, medical foods, cereals, biscuits, confectionary, cakes, deserts, snack bar, soups and pet food (Gibson, 2001).

In South Africa prebiotics, mainly FOS, are also manufactured and used in foodfortification. The only prebiotic products available in South Africa include inulin and FOS manufactured by ORAFTI in Belgium and distributed in South Africa by SAVANNAH Fine Chemicals. The range includes Raftiline®GR (inulin), Raftilose®L95 (Oligofructose) and Raftilose®Synergy1 (combination of inulin and oligofructose). No conclusive list of products containing prebiotics available in South Africa could be found in the literature in South Africa.

### 2.8 Summary of key characteristics of probiotics and prebiotics

The key characteristics of true probiotics and prebiotics are summarized in Table 2.5.

**TABLE 2.5:** The key aspects of probiotics and prebiotics

| Probiotics | Prebiotics |
|---|---|
| • Should meet safety guidelines<br>• Should survive passage through the gastrointestinal tract<br>• Should establish itself permanently in the small intestine and colon<br>• Should provide one or more specific health benefits<br>• Should selectively utilize a prebiotic as a source of energy | • Must not be hydrolysed or absorbed in the upper part of the gastrointestinal tract<br>• Must be selectively fermented by potentially beneficial bacteria in the colon<br>• Should alter the composition of the colonic microbiota towards a healthier composition<br>• Should preferably induce additional effects which are beneficial to the host health |

## 3. Synbiotics

Synbiotics refers to the combination of live microbial preparations (probiotics) with specific substrates (prebiotics) (Fuller, 1989). This combination could improve the survival of the probiotic organism, as its specific substrate is readily available for fermentation (Collins & Gibson, 1999). The health effects of synbiotics would therefore include both the health effects of probiotics and prebiotics described in this chapter. This possibility is supported by a number of studies, including the following:

- In a study done by Fooks and Gibson (2001) a range of probiotics including *L. plantarum* 0407 and *L. pentosus* 905, *L. reuteri, L. acidophilus* and *B. bifidum* was tested for their ability to inhibit the growth of some common enteropathogens including *Escherichia coli*, *Campylobacter jejuni* and *Salmonella enteritidis*. The effect of prebiotics including FOS, inulin, XOS and mixtures of inulin: FOS (80:20 w/w) and FOS: XOS (50:50 w/w) on probiotic antimicrobial activity against pathogens was also determined. The impact of added prebiotics depended on the probiotic strain involved as well as the type of prebiotic. Results indicate that *L. plantarum* 0407 and *B. bifidum* Bb12 tended to inhibit pathogen growth, particularly with *E. coli*. *B. bifidum* numbers were enhanced by 1-log cycle, in the presence of FOS and the FOS: XOS (50:50) mixture. Both *L. plantarum* and *B. bifidum* numbers increased after 24-h fermentation when they were grown with *C. jejuni,* regardless of the carbohydrate source used. In conclusion this study showed that lactobacilli and bifidobacteria species can inhibit some important pathogenic species *in vitro* and that this antagonism is influenced by the prebiotic provided (Fooks & Gibson, 2001).
- In a trial done by Ahmad *et al.* (2000) fifty-eight healthy children with acute gastroenteritis received a low lactose formula with or without a combination of *L. rhamnosus* and FOS. The duration of diarrhea was shortened by one day in the group that received the probiotic and prebiotic supplement.
- Fisberg *et al.* (2000) completed a second study with 626 one to six year old children, with mild to moderate malnutrition. The children received a nutritional supplement with or without a synbiotic preparation containing *L. acidophilus, B. infantis* and FOS. The children were evaluated during a monthly visit for a period of four months. The data recorded included supplement intake, height, weight and stool pattern, incidence and duration of illness and episodes requiring antibiotics. The number of 'sick days' recorded was significantly lower in the group receiving the synbiotic supplement.

Synbiotics which promote host health can therefore be applied in the maintenance of the balance of the gut flora in healthy individuals, as well as in restoring the equilibrium in individuals whose gastrointestinal microflora have been altered as a result and/or disease, age, or diet (Fooks *et al.*, 1999).

# 4. The market for functional and probiotic and prebiotic containing products

Probiotic containing functional foods are well established and are known to consumers (Stanton *et al.*, 2001). However, the food manufacturer should satisfy the consumer demands to succeed in marketing probiotic containing products. Probiotic drinks have been the key growth sector in Europe, U.S.A. and Japan and the popularity of dose-delivery systems for probiotic containing drinks have also resulted in research efforts targeted to develop probiotic foods outside the dairy sector (Stanton *et al.*, 2001). The market of probiotic and prebiotic containing products in Europe, United States, Japan and South Africa is discussed with reference to products available, consumer awareness and legislative control.

## 4.1 European market

According to Hilliam (1998), the key focus of the functional food market in Europe has been the development of probiotic and prebiotic containing products. This has lead to a rapid growth in the development of probiotics and prebiotics. To assess the functional food market, Leatherhead Food RA undertook a study in nine countries, including the United Kingdom, France, Germany, Spain, Belgium, Netherlands, Denmark, Finland and Sweden (Hilliam, 1998). The probiotic market, especially dairy products such as yoghurts and fermented milks, accounted for 65% of the European functional food market in 1997, valued at 889 million US$ (Hilliam, 1998). In 1999, the sales of probiotic dairy products increased to 1.35 billion US$ (Hilliam, 2000). The total European probiotic market comprising the four main application areas including dairy, animal feed, supplements and infant nutrition, was estimated at 4.3 billion US$ in 2003 and will grow to reach 13.79 billion US$ in 2010 (Patton, 2003: 2003/11/26 accessed).

According to Hilliam (2000) the probiotic yoghurt market in all the countries totalled more than 250 million kg in 1997. France represented the largest market with sales of 90 million kg, valued at 219US$ million in 1997 and 240US$ in 1999 (Hilliam, 1998; Hilliam, 2000). Furthermore, it was found that the German market for probiotic yoghurts is growing rapidly as the market volume increased from 5 million US$ in 1995 to 419 million US$ in 2000, whereas the market in the United Kingdom grew by 26% (Hilliam, 2000).

The leading brand in the yoghurt sector is LC1 from Nestlé, with a 60% market share, followed by Actimel from Danone with a 25% market share and Mueller's Procult brand (Mueller, Germany) (Stanton *et al.*, 2001). Nestle's LC1 contains the *L. acidophilus* strain La1 and is available as a set cultured milk or as a drinking yoghurt. Researchers from Nestlé conducted human studies on this particular *Lactobacillus* strain and it was subsequently chosen for inclusion in milk products because of its strong probiotics characteristics. Results of these studies indicated that the immune system of the user is stimulated, leading to the product claim, "helps the body protect itself". This product was launched in France in 1994 and is currently available in most European countries (Young, 1996).

Hilliam (1996) investigated consumer awareness of probiotic containing products in Europe and the United Kingdom. He found that 80% of French respondents are aware of probiotics and 46% claimed that they have bought products containing probiotics. Only 7% of Germans and 15% of respondents in the United Kingdom claimed to have heard of probiotics. According to Cathro and Hilliam (1993), only 16% of French respondents, 9% of German respondents and 3% of United Kingdom respondents claimed that they have heard of prebiotics.

In Europe no specific legislation concerning the marketing and labelling of functional foods exists, although laws on misleading health claims and food safety are being enforced. These laws affected the Danish dairy company, MD Foods as they claimed cholesterol-reducing properties for Gaio yoghurt, a cultured dairy product. The Advertising Standards Authority responded to the complaints of a consumer organization and the product had to be withdrawn (Young, 1996).

### 4.2 Market in United States

In the United States the key area of functional food development is vitamin and mineral fortification of foods. However, the market for probiotic and prebiotic containing foods is growing especially in the form of diary products such as fluid milk, yoghurt and kefir. The yoghurt product sales increased with 12.8% and reached $2.39 billion in 2000. The sales of yoghurt shakes and drinks increased with 270% and reached $75.7 million in mass-market sales (Heller & Weir, 2001). According to Patton (2003: 2003/11/26 accessed) the probiotic market including dairy products, animal feed, supplements and infant food, in the United States is estimated at 14.39 billion US$ and will grow to reach 39.4 billion US$ in 2010. Despite these changes/increases, it was found that for the trimester ending July 2001, that probiotics ranked in the 5$^{th}$ or lowest level of marketing monitored in the United States (STS, 2002a). However, according to STS's TrendSense market-timing model, probiotics should show a growing appeal in the natural and health food channel if research activity continues (STS, 2002a).

Little information regarding consumer awareness of probiotics and prebiotics in the United States is available. According to Stanton *et al.* (2001) and the Hartman Group (2002) consumer awareness is low although it is still growing.

As far as legislation is concerned, companies in the U.S. are strictly prohibited from making claims on foods and supplements that suggest the product can be involved in "diagnosis, cure, mitigation, treatment, or prevention of disease, etc." with the exception, however, of health claims that are approved by the Food and Drug Administration (FDA) for use on food labels (Hasler, 1996). However, to date no claims regarding the potential health benefits of probiotics and prebiotics has been approved by the FDA and thus no legislative recognition of probiotics and prebiotics exists (Berner & O'Donnell, 1998).

### 4.3 Japanese market

The original functional food market in Japan was dominated by soft drinks and dietary fibre, with probiotics the significant functional ingredient in many of the dietary fibre products (Young, 1996). In 1993 a functional drink, containing bifidobacterial cultures, whey minerals, xylo-oligosaccharides and dietary fibre, named Bikkle, was launched and achieved sales of 11 billion yen in the first year (Young, 1996). Although the fermented milk, Yakult (Yakult, Japan) is classified as a functional food in Europe, the presence of probiotics in isolation from other functional ingredients does not carry functional food status in Japan (Young, 1996). It is expected that in the future the major functional food ingredients in Japan will still be probiotics and prebiotics (Stanton, 2001). Consumer awareness in Japan of probiotics and prebiotics is very high (Young, 1996).

No information regarding awareness awareness of the Japanese consumer of probiotic-and prebiotic containing products could be traced.

As far as Japanese legislative aspects are concerned, a program for the approval of functional foods exists and is known as 'foods for specified use' (FOSHU) (Berner & O'Donnell, 1998). A FOSHU is defined as a food that is expected to have certain health effects. The application of such a product is evaluated by the Japan Association of Health and Nutritious Foods, by academic experts, and finally by a committee appointed by the Ministry of Health and Welfare. The manufacturer is then licensed to label the health benefits of the product (Shinohara, 1995). In 1995, seventy products were approved by FOSHU and forty of these products contain oligosaccharides for improvement of intestinal microflora (Oku, 1996).

### 4.4 South African market

No statistics regarding the market share of functional foods containing probiotics and prebiotics in South Africa are available. Probiotic products such as yoghurt made with AB cultures (*L. acidophilus* and *B. bifidum*) and infant follow-up formulas, such as Nestlé NAN2 and NAN3, enriched with *Lactobacillus* and *Bifidobacterium*, are currently found on the market. Only one reference could be traced in the literature which reported on the availability of a few probiotic containing supplements available in South African pharmacies (Table 2.6) (Du Toit, 2003). However, this is not an exhaustive list of such products available in the country.

**TABLE 2.6:** Probiotic supplements on the South African market

| Manufacturer | Product | Probiotic cultures | Cfu/g |
|---|---|---|---|
| Biopro | Biopro Reuteri | *Lb. reuteri* | *N/a |
| Bioflora | Infantiforte | *B. infantis* | $1 \times 10^8$ |
| Bioflora | Kiddieforte | *B. longum, B. bifidum, Lb. acidophilus* | $1 \times 10^8$ |
| Bioflora | Combiforte | *B.longum, B. bifidum, Lb. acidophilus* | $1 \times 10^8$ |
| Cipla Medro | Lactovita | *Lb. sporogenes* | $6 \times 10^8$ |
| Addock Ingram Ltd. | Inteflora | *Saccharomyces boulardii* | 0.15g |
| Pharma-Dynamics | Culturelle capsules/ chew-tabs | *B. longum, Lb. rhamnosus* | $1 \times 10^7$ |
| Pharma-Dynamics | Culturelle paediatric sachets | *B. longum, Lb.acidophilus, Streptococcus thermophilus* | $1 \times 10^7$ |

*N/a = Not available

No information regarding awareness of the South African consumer of probiotic-and prebiotic containing products could be traced.

As far as legislative aspects are concerned, regulations are being finalised within the Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act no. 54 of 1972; http://www.doh.gov.za; 2003/05/02 accessed), which will regulate probiotic and prebiotic health claims for labelling purposes. The permissible information to accompany a probiotic claim is the following: "Probiotics such as *bifidobacteria* and the *lactobacilli* improve the intestinal microbial balance, and consequently the health and functioning of the digestive tract. They manufacture B vitamins, inhibit the growth of harmful pathogens and may, when ingested on a regular basis as part of a prudent, balanced diet, assist in improving the immune status, the digestion of lactose, and may help reduce the risk of colon cancer. The probiotic microbial count should exceed $1 \times 10^6$ colony forming units (cfu) per gram product for foodstuffs. Permitted species are *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*." For foods and formulae for infants younger than one year, the probiotic bacterial count should exceed $10^6$ cfu per gram product for foodstuffs and the only permitted organism is *Bifidobacterium infantis*.

Prebiotics, mainly fructo-oligosaccharides, are also manufactured in South Afrcia and used in food supplementation. No conclusive list of prebiotic containing products available in South Africa could be found in the literature. Market analysis by the primary researcher indicated that the only prebiotic products currently available in South Africa include inulin and fructo-oligosaccharides manufactured by ORAFTI in Belgium and distributed in South Africa by SAVANNAH Fine Chemicals. The range includes Raftiline®GR (inulin), Raftilose®L95 (Oligofructose) and Raftilose®Synergy1 (combination

of inulin and oligofructose). According to the Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act no. 54 of 1972; http://www.doh.gov.za; 2003/05/02 accessed), the proposed information to accompany a prebiotic claim is the following: "Prebiotics are food components that escape digestion by normal human digestive enzymes and reach the large intestine where they may create conditions that will promote the growth of indigenous, colonic bacteria, also referred to as probiotics and are considered to be beneficial. The product should contain at least 3 g "prebiotic" per daily serving. The amount and sources of 'prebiotics' such as fructo-oligosaccharides, galactosylsucrose or galacto-oligosaccharides from whey must be declared on the label. Fructo-oligosaccharides mainly from chicory, onion, garlic, asparagus, Jerusalem artichoke and soya beans and galacto-oligosaccharides from whey and galactosylsucrose must be stated on the label."

## 5. Marketing of functional foods

Kotler (1994) defines marketing as "a social and managerial process by which individuals and groups obtain what they need and want through creating, offering and exchanging products of value with others". Marketing is also concerned with the orientation of products or services to the market. An important component of this decision-making process is associated with consumer awareness and buying behaviour of consumers in the target market (Du Plessis, 1999). It is important to know how consumers perceive a product, what their values, needs and expectations are and how consumer attitudes are formed and how learning takes place. For example, the influence of advertising on buying behaviour, where target consumers buy their products, how they decide on a specific product and how much they are willing to pay for such a product influence the decision-making processes of marketers. Therefore, the process a marketer should follow in the marketing of a product include market segmentation, the selection of target markets and positioning the product in terms of desired attributes to meet these needs, development of appropriate marketing strategies to communicate the value, price positioning, and finally product distribution (Du Plessis, 1999).

### 5.1 Market segmentation

Market segmentation involves the segmentation of markets based on the identification of subgroups of customers that are internally homogeneous in some relevant aspects e.g. their need and wants and different from other market segments (Kotler, 2000; Van Trijp & Meulenberg, 1996). Factors that are considered in segmentation include demographics, geographics and psychographics.

**Demographic characteristics**, such as age, gender, marital status, income, occupation and education, are the most common bases for market segmentation and are important in locating a target market (Du Plessis, 1999). This type of information provides the most accessible and cost-effective way to identify target markets because it mostly involves public-domain data (Du Plessis, 1999).

Demographic measurements are therefore more or less indispensable to the market industry (Du Plessis, 1999).

Age groups as defined by the census or in five-year increments are a useful way to understand and segment a market. Analyzing age cohort groups or generations will often provide more meaningful segments and marketing strategies than the other demographic segments (Hawkins *et al.*, 2001). A generation or age cohort is defined as a group who has experienced a common social, political, historical and economic environment. Age cohorts function as subcultures because their shared histories produce unique shared values and behaviors (Hawkins *et al.*, 2001). For these reasons age has been found to affect the consumption of products ranging from beer to vacations. "Our age shapes the media we use, where we shop, how we use products, and how we think and feel about marketing activities" (Hawkins *et al.*, 2001).

Gender segmentation has long been applied in marketing for example brands might be accompanied by appropriate flavor, packaging and advertising cues to reinforce a gender image or certain features are designed to appeal to a specific gender (Kotler, 2000). In this regard it is important to bear in mind that many changes in gender related factors have occurred because of the continued impact of dual-income households. One consequence for marketers is that women are not as readily accessible through traditional media as they once were. Working women do not have so much time available to watch television or listen to the radio. For these reasons many advertisers now emphasize magazines in their media schedules, especially those specifically aimed at working women (Schiffman & Kanuk, 1997).

As far as the influence of education and income are concerned, it is well recognised that education influences what one can purchase by partially determining a person's income and occupation. Education can also influence how a person thinks, makes decisions and relates to others and therefore has a strong influence on a person's tastes and preferences (Hawkins *et al.*, 2001; Ibid & Smith, 1996). It must be borne in mind that a household's income level combined with its accumulated wealth determines its purchasing power. As far as occupational status is concerned, the number of professional workers such as engineers, scientists and lawyers has grown rapidly over the past fifteen years. Likewise, managerial, marketing, sales, health care and social services jobs have also seen rapid growth. In contrast, traditional blue-collar occupations have grown slowly if at all (Person, 1993). Occupation also has a direct influence on preferences for products, media and activities, and the income generated provides the means to acquire them (Mulhern *et al.*, 1998). Therefore services targeted at professional, managerial or service worker have experienced greater growth in demand than those targeted at the traditional blue-collar worker (Hawkins *et al.*, 2001). Hawkins *et al.* (2001) suggested that income is generally more effective as a segmentation variable when used in conjunction with other demographic variables.

The living Standards Measure (LSM) is used in South Africa to measure social class, or living standard, regardless of race, income or education. The LSM quantifies the ownership of certain durable goods and access to services to yield a composite measure of social class. There are eight LSM groups ranging from group 1 with the lowest living standards to group 8 with the highest (Du Plessis, 1999).

In **geographic segmentation** the market is divided by location. The theory behind this approach is that people who live in the same region, suburb or neighbourhood, have broadly similar lifestyles, needs and wants, and these differ from the needs of people living in other areas (Du Plessis, 1999). Geographic segmentation is most often applied in conjunction with other types of measurement such as demographics, and is then broadly referred to as "geodemographic". This approach is useful when the differences in product consumption correspond closely with demographic or lifestyle "types" of people who live in different areas or suburbs (Du Plessis, 1999).

**Psychographic characteristics** refer to the inner or intrinsic qualities of the individual consumer and strategies for consumer segmentation are thus based on specific psychologic variables. Where demographics help to locate a target market, psychographics help to describe how the members of that market think and feel (Schiffman & Kanuk, 1997). Consumers may therefore be segmented in terms of their needs and motivations, personality, perceptions, learning, level of involvement and attitudes (Schiffman & Kanuk, 1997). Psychographic segmentation has become increasingly popular and most published studies on segmentation are based on some form of lifestyle or psychograophic data (Du Plessis, 1999).

### 5.2 Target positioning

Market segmentation is followed by target positioning which refers to an assessment of the market potential of each segment. One or more target segments are subsequently selected for a specific brand and promotional appeal. The brand is then positioned to satisfy the needs of target customers better than competitive offerings (Rath, 1999). For example the target positioning of probiotic and prebiotic containing products involve health conscious consumers, between the ages of 18 and 49 years and in a high socio-economic group.

### 5.3 Market communication strategy

The target market profile and product characteristics determine the marketing communication strategy (Du Plessis, 1999). For example the person selling the product, publicity, advertisements or sales promotion techniques such as competitions, sponsorships, brochures, leaflets or special offers might be used as marketing communication. Menrad *et al.* (2000) maintain that in the marketing of functional foods a strong need for specific information and communication activities to consumers has been identified because consumers' knowledge and awareness of the health effects of newly

developed functional ingredients is limited. A crucial success factor for the marketing of functional foods is not only to target information at consumers, but also at opinion leaders, e.g. medical doctors and nutrition advisors (Menrad *et al.*, 2000). According to Menrad *et al.* (2000) the success of information marketing of health products campaigns also depends on whether the message of the health effect dissiminated to the consumer via advertisements, labels and other sales promotion techniques is simple and easy to understand. Specialist terminology and medical details should therefore be avoided.

### 5.4 Price positioning

"Price is the amount of money and/or other items with utility needed to acquire a product" (Cant *et al.*, 2002). The price of a product should cover manufacturing and marketing costs and generate sufficient contribution to overheads and profits. Price positioning thus depends on the expected quantities that can be sold at that price level (Cant *et al.*, 2002). For the price positioning of for example probiotic and/or prebiotic containing products, marketers should consider probiotic culture or prebiotic cost, packaging, distribution, advertisements and profits (Sanders, 1998). Consumers also evaluate prices in relation to the product's perceived quality and the prices of competitive substitute products (Cant *et al.*, 2002). Marketers should therefore know how much the consumers in the target market are willing to pay for the product (Sanders, 1998).

### 5.5 Distribution

An additional requirement for good marketing is the availability and distribution of the products to the target market at locations where it is convenient for procurement (Menrad, 2003). The marketer of the product should establish effective distribution channels i.e. when and where to supply products and physical distribution including transport and storage (Cant *et al.*, 2002; Van Trijp & Meulenberg, 1996). Good channel decisions require a sound knowledge of where target customers shop for the product in question (Cant *et al.*, 2002). The market success of functional food products depend on the use of high-volume distribution channels e.g. supermarkets, general retail stores or discount retailers (Menrad, 2003). In Europe these are the most important distribution channels for functional foods. Most consumers are not willing to go to specific shops just to buy functional food products and expect these products in retail outlets (Menrad, 2003).

## 6. Future prospects

Diet is a major focus of public health strategies aimed at maintaining optimum health throughout life, preventing the early onset of chronic diseases such as gastrointestinal disorders, cardiovascular disease, cancer, osteoporosis, as well as promoting healthier ageing. Probiotics in combination with prebiotics may be become important strategy in the prevention and treatment of these diseases (Bezkorovainy, 2001). The increasing consumer health consciousness and expenditure strengthens

this possibility, as socio-economic factors are responsible for the expanding worldwide interest in functional foods. Therefore the market for functional food products containing probiotics and prebiotics can be developed and expanded (Mattilla-Sandholm *et al.*, 2002).

Although the probiotic and prebiotic concept is currently widely accepted in the scientific and industrial fields, further research input is required. Matllia-Sandholm *et al.* (2002) proposed that the future scientific and technological research areas should involve the following:

- Investigations of the mechanisms of probiotic and prebiotic action in the GIT and development of diagnostic tools and biomarkers for the assessment of probiotics and prebiotics
- Examination of the effects of probiotics on GIT diseases, GIT infections, and allergies
- Ensuring the stability and viability of probiotic products by developing feasible manufacturing and storage technology
- Development of technology for non-diary, novel or artificial probiotic fortification
- Evaluation of the role of probiotics as functional food in healthy consumer groups and bearing in mind consumer needs.

The possibility that the properties of prebiotics could be manipulated is also being investigated, allowing molecules with desired functionality to be manufactured and implemented in the functional food market (Rastall & Martin, 2002). Another important factor that should receive attention is the continued growth of the market for probiotic and prebiotic containing products. Consumers should be educated regarding the health benefits of probiotics and prebiotics to increase their awareness of these products. Therefore marketers of probiotic and prebiotic containing products should ensure a good marketing strategy.

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# CHAPTER 3

# MARKET AND PRODUCT ASSESSMENT OF PROBIOTIC AND PREBIOTIC CONTAINING PRODUCTS AVAILABLE ON THE SOUTH AFRICAN MARKET

# Market and product assessment of probiotic/prebiotic containing functional foods and supplements manufactured in South Africa

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**Abstract**

Probiotic (live microbes) and prebiotics (non-digestible food-ingredients) that benefit the consumer's health by improving their intestinal microbial balance are fast gaining interest as functional foods and supplements. It is important that such products are correctly labelled to provide the consumer with correct information. In this study probiotic and prebiotic containing products manufactured in South Africa were identified. Probiotic and prebiotic health and content related claims stated on the labels of products were evaluated according to available scientific evidence, the proposed South African regulations within the Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act no. 54 of 1972; http://www.doh.gov.za; 2003/05/02 accessed) and microbial assessment. A range of products was identified which included probiotic and/or prebiotic containing supplements (capsules), food items fortified with probiotics and/or prebiotics and fermented foods containing probiotics e.g. dairy products. Most of the health related claims on the labels of the identified products do not comply with the proposed South African regulations. However, results also indicate that the proposed South African regulations should be reconsidered to include another five claims, for which scientific sound evidence is available. Probiotic strains and viable cell number claims stated on the labels of the products mostly comply with the proposed South African regulations. The claims regarding prebiotic type and concentration stated on labels of the products all comply with the proposed South African regulations with the exception of three products. The actual viable cell content of three out of five probiotic supplements readily available on the South African market, did not comply with the content claim stated on the label. However, this problem did not seem to affect the inhibitory activity of the probiotic strains against indicator strains isolated from faeces of patients diagnosed with AIDS.

*Key words:* market assessment, product assessment, probiotics, prebiotics

## Introduction

Consumers are becoming more aware of functional foods and supplements and the potential role of these products in a balanced diet and ensuring health (Mattilla-Sandholm *et al.*, 2002). Functional foods can be defined as foods that contain physiologically active components, which provide health benefits beyond basic nutrition (Clydesdale, 1999) by affecting one or more functions in the body in a targeted way (Roberfroid, 1999). "The component that makes the food 'functional' can either be an essential macronutrient with specific physiological effects such as resistant starch or omega-3 fatty acids or an essential micronutrient" (Roberfoid, 1999). "It also includes food components that have some nutritive value, but are not classified as 'essential', such as oligosaccharides or food components with no nutritive value, such as live microorganisms (e.g. probiotics) or plant chemicals (phytochemicals)" (Roberfoid, 1999). A food product can be naturally functional i.e. containing an active component or can be made functional by increasing the concentration of a natural component, by adding a component not naturally present in the product, by replacing or reducing a component naturally present e.g. fat, or by improving the bioavailability of a component (Roberfroid, 1999).

"A dietary supplement is defined as a product intended for ingestion as a supplement to the diet" (Smolin & Grosvenor, 2000). Supplements may contain one or more of the following ingredients: vitamins, minerals, herbs, botanicals, or other plant-derived substances; amino acids, enzymes, concentrates and extracts. Dietary supplements can be manufactured as pills, tablets, capsules, gelcaps, liquids and powders (Smolin & Grosvenor, 2000).

At present the functional food market in many countries seems to be dominated by gut health products, in particular probiotic and prebiotic containing products (Menrad, 2003). Some of the most popular functional foods on the market include Nestlé LC1 yoghurt, Danone Actimel and Mueller's Procult brand in Europe; Nestlé LC1 yoghurt in the United States and a probiotic containing milk, Yacult, in Japan (Kopp-Hoolihan, 2001; Temmerman *et al.*, 2002). No similar scientific information regarding popular functional foods in South Africa could be traced.

Probiotics are defined by Fuller (1989) as "a live microbial food supplement that beneficially affects the host animal by improving its intestinal microbial balance". Reported potential health benefits of probiotics include alleviation of lactose intolerance, immune enhancement, inhibition of pathogen colonization, reduction of colon cancer, reduction of serum cholesterol levels, improved digestion and reduction of gastrointestinal disorders such as diarrhea and constipation (Crittenden, 1999; Fuller & Gibson, 1995; Kopp-Hoolihan, 2001; Saavedra *et al.*, 1994; Salminen *et al.*, 1998). Probiotics are available in various forms of pharmaceutical preparations e.g. (powders, liquid suspensions and tablets), or are incorporated in for example fermented food products to produce functional foods (Fuller, 1989; Svensson, 1999).

The latest trend in the functional food market is to combine probiotics with prebiotics to enhance the effect of probiotics (Menrad, 2003). Prebiotics are defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/ or activity of probiotic bacteria in the colon' (Gibson & Roberfroid, 1995). However, prebiotics also have health benefits that are not related to the simultaneous intake of probiotics. These include increased mineral absorption and improved bone health, a decrease in serum triglycerides and constipation relief (Roberfroid, 2000). Inulin and fructo-oligosaccharides are amongst the most common prebiotics included in breakfast cereals, nutritional drinks or used in combination with probiotics in nutritional supplements (Roberfroid, 2001). The prebiotic potential of inulin and fructo-oligosaccharides may be ascribed to their molecular structure. The linear chains of these compounds mainly consist of ß(2→1)-type linked fructose molecules while the other types of prebiotics are branched and less readily accessible for bacterial hydrolyses (Van Loo *et al.*, 1999).

As the market of probiotic containing products, especially probiotic supplements, is still growing, long-term experience with the use of particular strains is not available (Mattilla-Sandholm *et al.*, 2002). Possible dangers such as contamination and the presence of potentially pathogenic species such as *E. feacium* and *E. faecalis* have been found in probiotic products (Alcid *et al.*, 1994). Furthermore, the appropriateness of *E. feacium* as a probiotic is still controversial (Hamilton-Miller & Shah, 2003). The production and marketing of probiotic functional foods should therefore be strictly controlled and carefully monitored to ensure safe and correctly labelled products (Sanders & Huis in't Veld, 1999; Saarela *et al.*, 2000). Information on the label of the product, especially regarding the composition and identity of the probiotic strains included, need to be correct to guarantee safety and functionality (Temmerman *et al.*, 2002). Recent studies conducted on probiotic supplements and dairy products in Europe revealed gross irregulatories in this regard. In most of the cases the identity and number of viable strains recovered did not correspond to the information on the label (Hamilton-Miller *et al.*, 1999; Holzapfel *et al.*, 1998; Reuter, 1997; and Temmerman *et al.*, 2002).

One solution to address the problem of an unacceptable product content is to improve the control of the probiotic strain content of products via legislation. However, despite the large market segment occupied by probiotic foods and supplements in Europe, no specific regulation regarding the labelling of these products exists (Richardson, 1996). Although this particular market has not yet been that well developed in the United States, where the United States Food and Drug Administration (FDA) strictly regulates the labelling and marketing of conventional foods containing probiotic bacteria. As a result, no statements regarding health benefits for probiotics may be stated on the labels of products. In South Africa permissible statements regarding health benefits of probiotic and prebiotic claims are included in the proposed South African regulations governing the labelling and advertising of

Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act No. 54, 1972[3], http://www.doh.gov.za, 2003/05/02 accessed). However, despite the fact that this type of regulation is being finalised, limited information is available regarding the probiotic and prebiotic containing product market in South Africa.

In view of the above, the aims of this study were to complete a market and product assessment of probiotic and prebiotic containing products manufactured in South Africa. This firstly involved identifying the range of products available on the South African market. Secondly, the health and probiotic/prebiotic content related claims that are made on the labels of the identified products were evaluated based on available scientific evidence, proposed South African regulations and microbial assessments.

## Materials and Methods

### Identification of probiotic and prebiotic products on the South African market

For the purposes of this study "probiotic and prebiotic containing products manufactured in South Africa" is defined as all products manufactured in South Africa which contain either imported probiotic strains and/ or prebiotics (most products) or probiotic strains and/ or prebiotics produced in South Africa (minority of products). For the identification of such products available on the South African market during the time period of 1 February to 1 September 2003, the following information sources were scrutinized or visited: general outlets (e.g. Pick 'n Pay, Woolworths, Checkers, Spar), health food stores, web-sites, published information including scientific literature, advertisements and pamphlets obtained at conferences and shops. Every identified product that contained either probiotics and/or prebiotics was listed and the following information was recorded: type of product (tablet, syrup, food item, straw containing probiotics in powdered form), specific target group (if applicable), probiotic strains [strain, number of viable cells (cfu/g)], prebiotic (type and concentration), and current cost/ per 100g. The information was collated in descriptive tables based on target group and/or product type.

### Evaluation of claims on probiotic and prebiotic containing products manufactured in South Africa

For the purposes of this study "claims" are defined as health related claims e.g. treatment and prevention of diarrhea, improvement of lactose intolerance, alleviation of food allergy and so forth as well as probiotic and prebiotic content related claims i.e. strain and viable cell numbers of probiotic and concentration of prebiotics included.

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[3] Referred to as proposed South African regulations from here onwards

*Evaluation of health related claims in proposed South African regulations*

The health related claims regarding probiotics and prebiotics on the label of each identified product were listed and similar claims were grouped together. The wording/content of each of the claims was subsequently compared to the prescribed wording/content claim as is proposed by the South African regulations (Tables 3.1 and 3.2) to determine whether it complied with the regulations.

**TABLE 3.1**: The wording/ content of the probiotic claim proposed in the South African regulations of Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act No. 54, 1972, http://www.doh.gov.za, 2003/05/02 accessed).

| Permissible information to accompany a probiotic claim | Conditions |
|---|---|
| Probiotics such as *Bifidobacteria* and the *Lactobacilli* improve the intestinal microbial balance, and consequently the health and functioning of the digestive tract. They manufacture B vitamins, inhibit the growth of harmful pathogens and may, when ingested on a regular basis as part of a prudent, balanced diet, assist in improving the immune status, the digestion of lactose and may help reduce the risk of colon cancer. | For foods for persons older than 1 year<br>The probiotic microbial count should exceed 1 x $10^6$ colony forming units per gram product for foodstuffs.<br><br>Permitted species are, *Lactobacillus* spp., *Bifidobacterium* spp., *Lactococcus* spp., *Streptococcus salivarius* subspecies *thermophilus*, *Lactobacillus delbrueckii* subspecies *bulgaricus*.<br><br>For foods, formulae for infants younger than 1 year<br>The probiotic bacterial count should exceed $10^6$ colony per gram product for foodstuffs.<br><br>Permitted organism is *Bifidobacterium infantis* only. |

*Evaluation of health claims based on scientific soundness*

The scientific soundness of each of the identified claims was assessed by searching the scientific literature for any published studies that seemed to provide data in support of the claim. However, in this process it was assumed that publication of a paper relating to a claim in a scientific journal is not necessarily final proof of scientific soundness of such a claim. To address this issue, the research quality of each identified paper was assessed based on the study design that was applied. According to Farnworth (2000), it is generally accepted that health claims concerning specific nutrients/ foods/ functional components need to be assessed using a random controlled trial (RCT). The following criteria of RCT's in humans were therefore used to assess the quality of the identified papers (Smolin & Grosvenor, 2000).

1. The measurements used must be objective. Subjective claims, referred to as anecdotal evidence, which include individual testimonials or opinions, are not acceptable objective measurements.
2. The experimental population must be appropriate, i.e. human and the subjects used must be in line with conclusions drawn and recommendations formulated. It is for example not acceptable to use adult subjects and formulate health claims for children.
3. The study must include a control group.
4. The study must include an experimental group.
5. Subjects must be randomly assigned to an experimental and control group.

6. Control subjects must receive a placebo.
7. The study must be at least single-blind, but preferably double-blind.
8. The journal in which the study was published must be peer reviewed.

Although sample size is a very important factor to consider in a study design, it was not possible to specify a minimum sample size for a scientifically sound study for the assessment of probiotic and prebiotic related health claims.

A study was classified as scientifically sound if at least seven out of eight of the mentioned criteria were met. If any two or more of the criteria were not met, the study was classified as lacking in scientific soundness, although not necessarily completely worthless. Although *in vitro* studies supply important evidence regarding microbial activity and potential health benefits, the final proof lies in the execution of well-planned RCT's. *In vitro* studies were therefore not accepted as a scientific basis for the formulation of a health claim.

*Evaluation of content claims regarding strains included, viable cell numbers and prebiotic type and concentration*

The probiotic strains and viable cell numbers, type of prebiotic and concentration thereof were listed for each identified product and compared to the proposed South African regulations in this regard (Table 3.2). This information was included in the mentioned descriptive tables.

**TABLE 3.2:** The wording/content of the prebiotic claim proposed in the South African regulations of Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act No. 54, 1972, htpp://www.doh.gov.za, 2003/05/02 accessed).

| Permissible information to accompany claim | Conditions | Food stuff |
|---|---|---|
| Prebiotics are food components that escape digestion by normal human digestive enzymes and reach the large intestine where they may create conditions that will promote the growth of indigenous, colonic bacteria, also referred to as probiotics and are considered to be beneficial. | At least 3 g "prebiotic" per daily serving. The amount and source of 'prebiotics' such as fructo-oligosaccharides, galactosylsucrose or galacto-oligosaccharides from whey shall be declared on the label. | Fructo-oligosaccharides mainly from chicory, onion, garlic, asparagus, Jerusalem artichoke and soya beans, Galacto-oligosaccharides from whey and Galactosylsucrose. |

*Microbial assessment of viable cell numbers included in five selected probiotic supplements*

Five probiotic supplements readily available in pharmacies were selected to determine the viability (growth and inhibitory activity) of the probiotic strains and to compare the actual viable cell numbers with the "label" claim in this regard.

For determination of viable cell numbers, the samples were taken at time of production and the content of the capsule was resuspended in 10ml sterile distilled water and a 10-fold dilution was prepared. A

total of 100µl of each dilution were plated in duplicate onto De Man Rogosa Sharpe (MRS) agar (Biolab, Diagnostics, Midrand, S.A). Plates from each dilution were incubated aerobically and anaerobically at 37°C. After 24h of incubation, the viable cell numbers were determined by counting the number of white colony forming units (cfu) grown on the plate and compared to the cell numbers indicated on the label.

To further assess the viability of the probiotics included in the products, probiotic strains isolated from each of the five products were screened for inhibitory activity against the following ten indicator strains isolated from the faeces of patients diagnosed with AIDS; *Salmonella typhi, Salmonella typhimurium, Salmonella* Gr.B., *Shigella flexnerii* 1, *Shigella flexnerii* 3, *Shigella sonnei, Shigella boydii, Shigella spp., Yersina* spp., *Vibrio parahemolyticus.* The strains were cultured in MRS broth (Biolab) for 18h at 37°C and 10 µl spotted on MRS agar (Biolab). The plates were incubated for 24h at 37°C and then lawned with active growing strains from the indicator strains, imbedded in soft agar (0.8%, m/v), as described by Van Reenen *et al.* (1998). The plates were incubated at 37°C for 24h and the colonies examined for the formation of zones, which indicate level of inhibitory activity and thus viability. The examination of inhibitory activity was done in triplicate and the means derived.

## Results

*Probiotic and prebiotic containing products on the South African market*

The identified range of probiotic- and prebiotic containing products manufactured in South Africa is listed in Tables 3.3 to 3.8. The products include three fortified infant foods (Table 3.3), seven yoghurt products selected from a variety of dairy products containing live cultures targeted at children and adults (Table 3.4) and sixteen probiotic supplements of which three are targeted at infants/children and thirteen at adults (Table 3.5). A combination of probiotics and prebiotics was found in six supplements of which only one is targeted at children (Table 3.6), two energy drinks and one dairy product targeted at children and adults (Table 3.7). Sixteen food items naturally containing or fortified with prebiotics, including two supplements, two breakfast cereals, eleven nutritional drinks and one muesli bar, were identified. Four of these are targeted at infants/children, four at children and adults and eight at adults only (Table 3.8).

The cost of probiotic products in September 2003 varies between R7.78 and R8.80 per 100g of infant food; between R1.16 and R2.63 per 100ml of dairy product and between R1.30 and R8.81 per capsule. The cost of supplements containing probiotics and prebiotics varies between R1.92 and R3.56. The dairy product containing probiotics and prebiotics cost R1.40 per 100ml. The products containing prebiotics cost R5.78 for 100g of infant food, between R2.76 and R2.95 for 100g of breakfast cereal and between R9.80 and R15.60 for 100g of nutritional drink. The costs of some products were not available and these were the products from web-sites and brochures.

**TABLE 3.3:** South African manufactured infant food fortified with probiotics

| Manufacturer | Product | Probiotic strains | Number of viable cells (cfu/g) | Cost per 100g in Rand (Sept. 2003) |
|---|---|---|---|---|
| Nestlé | NAN 2 probiotic follow-up formula with iron (from 6 months) | *Bifidobacterium** *Lactobacillus** | Not available | 8.80 |
| Nestlé | NAN3 probiotic enriched with $B_L$- a probiotic (from 1year) | *Bifidobacterium** *Lactobacillus** | Not available | 7.78 |
| Nestlé | Growing up milk enriched with $B_L$- a probiotic | *Bifidobacterium** *Lactobacillus** | Not available | Not available |

* Species comply with the proposed South African regulations

**TABLE 3.4:** South African manufactured dairy products containing probiotics*

| Manufacturer | Product | Probiotic strains | Number of viable cells (cfu/g) | Cost per 100ml in Rand |
|---|---|---|---|---|
| Parmalat | Low fat Bulgarian yoghurt | *Lb. acidophilus*† *Streptococcus thermophilus*†, *Lb. bulgaricus*† | Not available | 1.16 |
| Dairybelle | Low fat yoghurt | Original live AB-cultures‡ | Not available | 1.16 |
| Dairybelle | Drinking yoghurt | Live AB-cultures‡ | Not available | 1.20 |
| Fair Cape | Low fat Bulgarian yoghurt | Live probiotic AB-cultures‡ | Not available | 1.80 |
| Fair Cape | Drinking yoghurt | Live probiotic cultures‡ | Not available | 1.66 |
| Woolworths | Low fat yoghurt | HOWARU Bifido probiotic cultures† | $1 \times 10^6$† | 2.26 |
| Woolworths | Ayrshire fat free yoghurt | *S. thermophilus*†, *L. acidophilus*†, *Bifidobacterium*† | $1 \times 10^6$† $1 \times 10^6$† | 2.63 |

* Only a selection of dairy products claiming live AB-culture content were included
† Species and viable cell numbers comply with the proposed South African regulations
‡ Do not comply with the proposed South African regulations

**TABLE 3.5:** South African manufactured probiotic containing supplements

| Manufacturer | Product and target group | Probiotic strains | Number of viable cells (cfu/g) | Delivery form | Cost per unit in Rand (Sept. 2003) |
|---|---|---|---|---|---|
| Bioflora | Acidoflora (adults) | *L. acidophilus*† | Not available | capsules | Not available |
| Bioflora | Bifidoflora (adults) | *B. bifidum*†, *B. longum*† | Not available | capsules | Not available |
| Bioflora | Intestiflora (adults) | *L. acidophilus*†, *B. bifidum*†, *B. longum*† | Not available | capsules | Not available |
| Bioflora | Infantiflora (infants) | *B. infantis*† | 1 X $10^9$† | capsules | 3.16/caps |
| Bioflora | Kiddie-forte (children) | *L. acidophilus*†, *B. bifidum*†, *B. longum*† | 1 X $10^9$† | capsules | 3.16/caps |
| African dynamics | B- immune: probiotics (adults) | *Lactobacillus* sp. † *Bifidobacterium* sp. † | 1 x $10^9$† | capsules | Not available |
| African dynamics | B- immune: little (children, 3-10 years) | *Lactobacillus* sp. † *Bifidobacterium* sp. † | 1 x $10^9$† | chewable tablets | Not available |
| SA Scientific pharma-ceuticals | Vagiforte (women) | *Lactobacillus* sp. † *Bifidobacterium* sp. † | Not available | capsules and tablets | Not available |
| Zoë Care Probiotia Health | Zoë AB-Lactics (adults) | Acidophilus†, Bifidobacterium† | 1.8 x $10^{10}$† | capsules | 2.90/caps |
| Biopro | Reuteri (adults) | *L. reuteri*† | 1 x $10^8$† | Chewable tablets or in straws | 3.42/caps |
| | Lactologic (adults) | *Lactobacillus acidophilus*†, *Bifidobacterium bifidum*† | Not available | syrup | 120/200ml |
| Cipla Medro | Lactovita (adults) | *Lactobacillus sporogenes*† | 6 x $10^9$† | capsules | 1.30/caps |
| NATURA | Acidoflora | Not specified | Not available | capsules | Not available |
| SOLGAR | Advanced Acidophilus Plus | *L. acidophilus*† *Bifidobacterium lactis*† | 5 x $10^8$† | capsules | 1.75/caps |
| SOLGAR | Advanced 40+ Acidophilus | *L. acidophilus*†, *L. bulgaricus*†, *L. paracasei*†, *B. lactis*†, *S. thermophilus*† | 1.5 x $10^9$† | capsules | 2.10/caps |
| Adcock Ingram Ltd. | Inteflora 250 (adults) | *Saccharomyces boulardii** | 0,15g | capsules | 8.81/caps |

* Yeast species do not comply with the proposed South African regulations

† Species and viable cell numbers comply with the proposed South African regulations

**TABLE 3.6.** South African manufactured supplements containing probiotics in combination with prebiotics

| Manufac-turer | Product and target group | Probiotic strains | Number of viable cells (cfu/g) | Prebiotic and concentration | Delivery form | Cost per unit in Rand (Sept. 2003) |
|---|---|---|---|---|---|---|
| Pharma Dynamics | Culturelle: Probiotic supple-ment (adults) | *L.rhamnosus,*† *B. longum*† | 1 X $10^8$† | Fructo-oligosac-charide*† | capsules/ chewable tablets | 1.92/caps |
| Pharma Dynamics | Culturelle: Paediatric sachet (infants; toddlers) | *L.acidophilus*† *B. longum*†, *S.thermo-philus*† | 1 X $10^8$ | Fructo-oligosac-charide*† | capsules | Not available |
| Pharma Dynamics | Culturelle: Vaginal capsules (women) | *L.acidophilus*† *L. paracasei*† | 1 X $10^8$ | Fructo-oligosaccharide*† | capsules | Not available |
| Bioflora | Combi-forte (adults) | *B. bifidum*†, *B.longum*†, *L.acidophilu*† | 1 X $10^9$ | Not specified | capsules | 3.16/caps |
| Viridian | Acidophilus complex with F.O.S | *L.acidophilus*† *B. bifidum*†, *L. bulgaricus*† | 5 X $10^8$ 5 X $10^8$ 5 X $10^8$ | Fructo-oligosac-charide† (40mg/capsule) | capsules | 2.30/caps |
| AIM | Probiotic Flora Food (adults) | *L. salivarius*†, *L. plantarum*† | 1.3 X $10^8$ 1.3 X $10^8$ | Fructo-oligosac-charide† (210 mg/capsule) | capsules | 3.56/caps |

* No content claim regarding concentration

† Species, viable cell numbers and prebiotic and concentration comply with the proposed South African regulations

**TABLE 3.7:** South African manufactured energy drinks and diary products fortified with probiotics and prebiotics

| Manufac-turer | Product and target group | Probiotic strains | Number of viable cells (cfu/g) | Prebiotic and concentration | Cost per unit in Rand (Sept. 2003) |
|---|---|---|---|---|---|
| African Dynamics | B-immune: energy drink (adults and children ) | *Lactobacillus*†, *Bifidobacterium*† | 2.5 x $10^9$† | Fructo-oligosac-charide† (7.5g/100g) | Not available |
| African Dynamics | B- immune: Probiotic maize drink (adults and children ) | *Lactobacillus*†, *Bifidobacterium*† | 1 x $10^9$† | Fructo-oligosaccharide† (3g/100g) | Not available |
| Woolworths | 4 plus fat free Bulgarian yoghurt | HOWARU Bifido probiotic cultures† | 1 x $10^6$† | Inulin*† | 1.40 |

* No content claim regarding concentration

†Species, viable cell numbers and prebiotic type and concentration comply with the proposed South African regulations

**TABLE 3.8:** South African manufactured prebiotic supplements and food items with claimed prebiotic content*

| Manufacturer | **Product and target group** | **Prebiotics** | **Concentration** | **Current cost per unit (Rand)** |
|---|---|---|---|---|
| African Dynamics | B-immune preBiotics (Adults and children ) | Fructo-oligosaccharides (FOS) †, Raftilose† | 1200 mg per capsule† | Not available |
| Nestlé | Lactogen 2 Nestlé (infants) | Fructo-oligosaccharides† | 1.7g/100g† | 5.78 |
| Nestlé | Nespray fortified Prebio[1] (children) | Fructo-oligosaccharides† | Not available | Not available |
| Nestlé | Build-up (Vanilla) Prebio[1] (school children; adults) | Fructo-oligosaccharides† | Not available | Not available |
| Nestlé | Growing-up milk Prebio[1] (+ 1 year/+ 3 years | Fructo-oligosaccharides† | Not available | Not available |
| Nestlé | Cerevita Prebio[1] (general) | Fructo-oligosaccharides† | 6g/100g† | 2.76 |
| Nestlé | Muesli Bar Prebio[1] | Fructo-oligosaccharides† | Not available | Not available |
| Bokomo | Pronutro (general) | Chicory (source of FOS) † | Not available | 2.95 |
| Bioflora | Colon Food (adults ) | Fructo-oligosaccharides† | Not available | Not available |
| African Dynamics | B – immune: immuno-meal (adults) | Fructo-olgisaccharides† | 6.4g/100g† | Not available |
| Zoë Care | Nutrivive Shake 3000 (children, adults, elderly) | Lactilol†, Fructo-olgisaccharides† | Not available | Not available |
| | Slimmer's ideal by choice | Prebiotic fibre‡ | Not available | 15.60 |
| | Target Candida (women) | Prebiotic fibre‡ | Not available | 17 |
| | C strategy | Fructo-oligosaccharides† | 5g/100g† | Not available |
| | Extreme Care with prebiotic fibre (adults ) | Prebiotic fibre‡ | 12g/100g† | 9.80 |
| OPTI-MX Nutrition | Supa Form Nutritional drink (adults) | Fructo-oligosaccharides† | 5g/100g† | Not available |

* None include content claims regarding concentration
† Prebiotic type and concentration comply with the proposed South African regulations
‡ Prebiotic type and concentration do not comply with the proposed South African regulations

## Evaluation of claims on South African manufactured probiotic and prebiotic containing products

*Health related claims on products based on proposed South African regulation and scientific evidence*

The comparison of the health claims stated on the label of probiotic and prebiotic containing products manufactured in South Africa with the South African regulations is presented in Table 3.9. Scientific evidence that seems to support each claim is also included in this table. The studies that complied with a minimum of seven out of eight of the stated criteria for a RCT (Table 3.10) are printed in bold in Table 3.9.

**TABLE 3.9:** Claims stated on the labels of probiotic and or prebiotic containing products, references supporting claims and compliance with the proposed South African regulations

| Claims | Products on which the claim is made | Scientific evidence | Comply with SA regulations |
|---|---|---|---|
| Prevention of diarrhoea in infants | NAN 2, Infantiflora, B-immune little | *** Isolauri *et al.*, 1991; Kaila *et al.*, 1992; Saavedra *et al.*, 1994; Shornikova *et al.*, 1997; *Vanderhoof *et al.*, 1999; Guandalini *et al.*, 2000; Haschke *et al.*, 2001; Hatakka *et al.*, 2001; Szajewska and Mrukowicz, 2001** | No |
| Assists in prevention and treatment of nappy rash | NAN 2, Infantiflora, B-immune little | No references | No |
| Assists in prevention and treatment of constipation | NAN 2, Infantiflora, B-immune little | **Benno *et al.*, 1996** | No |
| Assists in protection of infants in hygienically compromised situations | NAN 2, Infantiflora, B-immune little | No references | No |
| Decrease lactose intolerance symptoms | NAN 2, Infantiflora, B-immune little | Montes *et al.*, 1995; Shermak *et al.*, 1995; **Vesa *et al.*, 1996** | Yes |
| Treatment of disorders of the colon | Bifidoflora | Grill *et al.*, 1995; Goldin, 1996 | No |
| Replenish intestinal flora of adults (after antibiotic treatment) | Combiforte, Intestiflora, Reuteri 100 | **Sittonen *et al.*, 1990; Orrhage *et al.*, 1992;** Bennet *et al.*, 1996; Pochapin, 2000; **Cremonini *et al.*, 2002;** | Yes |
| Helps the body to naturally alleviate diarrhoea in adults. | B-immune, Lactologic, Inteflora, Culturelle Probiotic supplement and Paediatric sachet | **Sittonen *et al.*, 1990, Orrhage *et al.*, 1992**; Bennet *et al.*, 1996; Pochapin, 2000; **Cremonini *et al.*, 2002** | No |
| Improves digestive health, improves stool quality, prevents constipation | B-immune, BifidofloraLactologic, Inteflora, Culturelle Probiotic supplement, and Paediatric sachet, Lactogen Prebio[1] | Gibson and Wang, 1994b; McBain and Macfarlane, 1997; **Saavedra *et al.*, 1999; Haschke *et al.*, 2001** | No |
| Helps the body to naturally alleviate flatulence | B-immune, Lactologic, Inteflora, Culturelle Probiotic supplement and Paediatric sachet | No references | No |
| Reverses the negative effects of antibiotics on the digestive tract | B-immune, Kiddie-forte | No references | No |
| Reverses the negative effects of alcohol on the digestive tract | B-immune, Kiddie-forte | No references | No |
| Reverses the negative effects of stress on the digestive tract | B-immune, Kiddie-forte | No references | No |
| Reverses the negative effects of poor diet on the digestive tract. | B-immune, Kiddie-forte | No references | No |

Table 3.9 (continued)

| Claims | Products on which the claim is made | Scientific evidence | Comply with SA regulations |
|---|---|---|---|
| Inhibits intestinal and food poisoning pathogens including *E.coli*, *Streptococci* and *Salmonella* and feeds friendly bacteria, balances intestinal pH. | B-immune, Probiotic Flora Food, Pronutro/ Extreme Care with prebiotic fibre, Slimmers ideal by choice, Supa Form Nutritional drink, B-immune prebiotics | **Holzhapfel *et al.*, 1995; Cocconier *et al.*, 1997; Fooks and Gibson, 2002** | No |
| Assist in promotion of healthy bowel flora for treatment of acidity, heartburn, indigestion and digestive upsets | Acidoflora (NATURA) | No references | No |
| Very effective in treating Irritable Bowel Syndrome/ Colitis/ radiation-caused enterocolitis | B-immune, Lactologic, Inteflora | **Pelto, *et al.*, 1998; Gionchetti *et al.*, 2000**, Nobaek *et al.*, 2000; **Niedzielin *et al.*, 2001** | No |
| Very effective in treating Crohn's Disease | B-immune, Lactologic, Inteflora | Malin *et al.*, 1997; **Pelto *et al.*, 1998**; Campieri, *et al.*, 2000; Guslandi, *et al.*, 2000 | No |
| Very effective in preventing of dyspepsia | B-immune, Lactologic, Inteflora | No references | No |
| Restore and maintain the normal vaginal flora (mainly lactic acid bacteria) frequently destroyed by the administration of broad-spectrum antibiotics and the use of disinfectants, soaps and deodorants | Vagiforte, Acidoforte, Culturelle Probiotic supplement and Paediatric sachet | Hilton *et al.*, 1992; Hilton and Isenberg, 1995 | No |
| Treatment of food allergies | Kiddie-forte | **Heyman *et al.*, 1995; Majamaa and; Isolauri *et al.*, 2000** | No |
| Treatment of acne | Kiddie-forte | No references | No |
| Boost immune system | Kiddie-forte, B-immune energy/ maize drink, B-immune prebiotics | **Link-Amster *et al.*, 1994; Schiffrin *et al.*, 1997; Mattilla-Sandholm and Kauppila, 1998; Alander and Mattilla-Sandholm, 2000; Fisberg *et al.*, 2000; Haschke *et al.*, 2001** | Yes |
| Treatment during steriod therapy | Kiddie-forte | No references | No |
| Treatment during chemotherapy | Kiddie-forte | No references | No |
| Treatment during radiotherapy | Kiddie-forte | Salminen *et al.*, 1988; **Hendriksson *et al.*, 1995**; Salminen *et al.*, 1995 | No |

* References stated on website of product.

**TABLE 3.10:** Evaluation of the study design of scientific papers on the health benefits of probiotics and prebiotics

| Study | Objective measurement | Appropriate population | Experimental group | Control | Random | Placebo | Blinded | Peer review |
|---|---|---|---|---|---|---|---|---|
| Alander and Mattilla-Sandholm, 2000 | √ | √ | √ | √ | √ | √ | Single | √ |
| Bennet *et al.*, 1996 | x | √ | √ | x | √ | x | x | √ |
| Campieri *et al.*, 2000 | √ | √ | √ | √ | √ | √ | Single | √ |
| Coconnier *et al.*, 1997 | x | x | x | x | x | x | x | √ |
| Cremonini *et al.*, 2002 | √ | √ | √ | √ | √ | √ | Double | √ |
| Fooks and Gibson, 2002 | √ | *In vitro* | √ | √ | √ | x | x | √ |
| Gibson and Wang, 1994 | √ | *In vitro* | √ | √ | √ | x | x | √ |
| Gionchetti *et al.*, 2000 | √ | √ | √ | √ | √ | √ | Double | √ |
| Goldin, 1996 | √ | √ | √ | x | x | x | x | √ |
| Grill *et al.*, 1995 | √ | √ | √ | x | x | x | x | √ |
| Guandalini *et al.*, 2000 | √ | √ | √ | √ | √ | √ | Double | √ |
| Guslandi, *et al.*, 2000 | √ | √ | √ | √ | √ | √ | Single | √ |
| Haschke *et al.*, 2001 | √ | √ | √ | √ | √ | √ | Double | √ |
| Haschke *et al.*, 2001 | √ | √ | √ | √ | √ | √ | Double | √ |
| Hatakka *et al.*, 2001 | √ | √ | √ | √ | √ | √ | Double | √ |
| Hendriksson *et al.*, 1995 | √ | √ | √ | √ | √ | x | x | √ |
| Hilton and Isenberg, 1995 | √ | √ | √ | √ | √ | x | x | √ |
| Hilton *et al.*, 1992 | √ | √ | √ | √ | x | x | x | √ |
| Holzhapfel, *et al.*, 1995 | √ | *In vitro* | √ | √ | x | x | x | √ |
| Isolauri *et al.*, 1991 | √ | √ | √ | √ | √ | √ | Single | √ |
| Isolauri *et al.*, 2000 | √ | √ | √ | √ | √ | √ | Double | √ |
| Kaila *et al.*, 1992 | √ | √ | √ | √ | √ | x | Single | √ |
| Link-Amster *et al.*, 1994 | √ | √ | √ | √ | √ | x | Single | √ |
| Majamaa and Isolauri, 1997 | √ | √ | √ | √ | √ | √ | Double | √ |
| Majamaa *et al.*, 1995 | √ | √ | √ | √ | √ | √ | Single | √ |
| Malin *et al.*, 1997 | √ | x | √ | x | x | x | x | √ |
| Mattilla-Sandholm and Kauppila, 1998 | √ | √ | √ | √ | √ | √ | Single | √ |
| McBain and McFarlane, 1997 | √ | *In vitro* | √ | √ | √ | x | x | √ |
| Montes *et al.*, 1995 | x | x | √ | √ | √ | x | x | √ |
| Niedzielin *et al.*, 2001 | √ | √ | √ | √ | √ | √ | Double | √ |
| Nobaek *et al.*, 2000 | x | √ | √ | x | √ | √ | x | √ |
| Orrhage *et al.*, 1992 | √ | √ | √ | √ | √ | √ | Double | √ |
| Pelto *et al.*, 1998 | √ | √ | √ | √ | √ | √ | Single | √ |
| Pochapin, 2000 | x | √ | √ | x | √ | x | Single | √ |
| Roberfroid, 2000 | √ | x | √ | √ | √ | x | x | √ |
| Saavedra *et al.*, 1994 | √ | √ | √ | √ | √ | √ | Double | √ |
| Saavedra *et al.*, 1999 | √ | √ | √ | √ | √ | √ | Double | √ |
| Salminen *et al.*, 1988 | √ | √ | √ | √ | √ | x | x | √ |
| Salminen *et al.*, 1995 | √ | √ | √ | √ | √ | x | x | √ |
| Shermak *et al.*, 1995 | x | x | √ | √ | √ | x | x | √ |
| Shornikova *et al.*, 1997 | √ | √ | √ | √ | √ | √ | Double | √ |
| Sittonen *et al.*, 1990 | √ | √ | √ | √ | √ | √ | Single | √ |
| Szajewska and Mrukowicz, 2001 | √ | √ | √ | √ | √ | √ | Double | √ |
| Vanderhoof *et al.*, 1999 | √ | √ | √ | √ | √ | √ | Double | √ |
| Vesa *et al.*, 1996 | √ | √ | √ | √ | √ | √ | Single | √ |

√= complied with stated criteria
x = did not comply with stated criteria

These data indicate that only three out of twenty-six claims comply with the proposed South African regulations. Sound scientific evidence seems to be available for all claims permissible according to the proposed South African regulations. However, sound scientific evidence is also available for at least five additional claims, including diarrhea prevention in infants; diarrhea prevention in adults; improvement of digestive health, stool quality, and constipation; prevention of Crohn's disease and treatment of food allergies which are not permissible according to the proposed South African regulations. Furthermore, eighteen claims were identified on the products for which no sound scientific evidence could be traced.

*Claims regarding probiotic strains, viable cell numbers and prebiotic type and concentration included in the products*

Probiotic strain claims made on the labels of identified products (Tables 3.3 – 3.8) all comply with the proposed South African regulations (Table 3.1) with the exception of four dairy products and one supplement. The dairy products produced by Dairybelle and Fair Cape do not specify the species included and only claim live AB-cultures/probiotic cultures. Inteflora 250 marketed by Addock Ingram Ltd. contains a yeast specie, *Saccharomyces boulardii* which is not permissible. The claims concerning the included viable cell numbers vary between 1 $x10^8$ cfu/g and 6 $x10^9$ cfu/g (Tables 3.3 – 3.7), which comply with the proposed South African regulations. The prebiotic type claims made on the identified products (Tables 3.6 – 3.8) include fructo-oligosaccharides, Raftilose and chicory, which all comply with the proposed South African regulations. Three products including Slimmer's ideal by choice, Target Candida and Extreme Care do not specify the type of prebiotic but only claim prebiotic fibre content, which does not comply with the proposed South African regulations. The prebiotic concentration claims varies between 3g and 15g per 100g, which comply with the proposed South African regulations. Only one product, namely Lactogen 2 manufactured by Nestlé, contains 1.7g/100g fructo-oligosaccharides and does not comply with the proposed South African regulations.

*Microbial assessment of viable cell numbers included in five selected probiotic supplements*

The comparison of viable cell numbers stated on the labels of the supplements with the actual viable cell numbers is listed in Table 3.11.

**TABLE 3.11:** Comparison of the actual viable cell numbers with the claims on the labels of five probiotic supplements manufactured in South Africa

| Supplement | Viable cell numbers stated on the label of the supplement (cfu/g) | Actual viable cell numbers identified (aerobic/anaerobic) (cfu/g) |
|---|---|---|
| 1 | $1 \times 10^8$ | $1 \times 10^8$/ $1.7\times 10^8$ |
| 2 | $1 \times 10^8$ | $2.8 \times 10^6$ / $3 \times 10^6$ |
| 3 | $1 \times 10^8$ | $2 \times 10^7$ (aerobic) |
| 4 | $1 \times 10^8$ | $4 \times 10^5$/ $1.4 \times 10^7$ |
| 5 | $1 \times 10^7$ | $1.5 \times 10^7$ |

The viable cell numbers in Supplements 1 and 5 are in line with the viable cell numbers stated on the labels of the supplements. The viable cell numbers in Supplement 2 were two log-cycles lower and in Supplements 3 and 4 one log-cycle lower than the claimed number of viable cells. The viable cell numbers in Supplement 4 were only $4 \times 10^5$ cfu/g when grown in aerobic conditions, which does not comply with the proposed South African regulations.

The results of the screening of the probiotic strains isolated from the five selected supplements against a panel of ten indicator strains are present in Table 3.12.

**TABLE 3.12:** Inhibitory activity of probiotic strains from five probiotic supplements manufactured in South Africa against a panel of indicator strains

| Human pathogens | Supplement 1 | Supplement 2 | Supplement 3 | Supplement 4 | Supplement 5 |
|---|---|---|---|---|---|
| *Salmonella typhi* | ++ | ++ | ++ | ++ | ++ |
| *Yersina sp.* | ++ | + | ++ | + | + |
| *Shigella flexnerii* 1 | +++ | ++ | ++ | ++ | ++ |
| *Salmonella typhimurium* | +++ | +++ | + | +++ | + |
| *Shigella flexnerii* 3 | +++ | +++ | ++ | ++ | +++ |
| *Shigella sonnei* | ++ | ++++ | ++ | ++ | ++++ |
| *Shigella boydii* | ++++ | ++++ | ++ | +++ | ++++ |
| *Salmonella* Gr. B | ++ | +++ | +++ | ++ | +++ |
| *Shigella* sp. | ++++ | +++ | +++ | ++ | ++++ |
| *Vibrio parahemo-lyticus* | +++ | ++ | ++ | ++ | +++ |

\+ indicates 20-25mm zone size
++ indicates 26-30mm zone size
+++ indicates 31-35mm zone size
++++ indicates 36+mm zone size

All the strains showed good inhibitory activity against the panel of indicator strains isolated from faeces from patients diagnosed with AIDS, as is indicated by the zone sizes.

## Discussion

The probiotic and prebiotic containing product market is a fast growing industry worldwide and the list of available products seems to be increasing on a daily basis (Hilliam, 1996). Probiotic and prebiotic products accounted for 65% of the European functional foods market in 1997 (Hilliam,

1998). In the United States food probiotic products are almost exclusively diary products such as fluid milk, yoghurt and kefir, although marketing of probiotics at this point of time is extremely limited (Hartman Group, 2002; Stanton *et al.*, 2001). The original functional food market in Japan was dominated by soft drinks with probiotics the significant, functional ingredient in many of these products (Young, 1996). In these countries the probiotic and prebiotic market seems to be dominated by dairy products including yoghurt and fermented drinks (Hilliam, 1998; Young, 1996). Although not seen as an exhaustive list, a large variety of probiotic and prebiotic containing products manufactured in South Africa were identified in this study. This range of products on the South African market includes probiotic and prebiotic supplements (capsules) and fortified food items, fermented foods containing probiotics e.g. dairy products and probiotics used in combination with prebiotics in supplements and food fortification. Dairy products also seem to be prominent in the market although fortified cereals, especially baby cereals, and supplements also seem to be growing markets.

As far as South African prices for probiotic and/or prebiotic containing products are concerned, it is interesting to note that the price range is much larger for the probiotic and/or prebiotic containing supplements than for the functional foods containing these substances. The consumer needs to be educated about the potential value of different types of products to empower them to make informed decisions in this regard. Factors that need to be considered in deciding on a product include levels of viable probiotic cell numbers, prebiotic concentration and convenience of use. Lee and Salminen (1995) suggested that a daily intake of $10^6$ to $10^9$ cfu/g probiotic bacteria is necessary to achieve therapeutic effects. It would be difficult to consume such large numbers of organisms in the form of for example dairy products and supplements may therefore be more convenient to attain therapeutic dosages in those suffering from disease (Sanders, 1999). However, it must be borne in mind that in addition to probiotics, fermented dairy products provide a nutrient-dense food source, which includes high quality protein, highly absorbable calcium, conjugated linoleic acids, butyric acid and bioactive peptides. Other functional foods like energy drinks are often also fortified with a range of nutrients that could benefit health, which might not be present in a probiotic and prebiotic capsule and/or tablet (Sanders, 1998).

It is important that the health claims stated on the labels of products supply the consumer with reliable information because such claims influence consumer behaviour and potentially affect public health (Clydesdale, 1997). From this research it was evident that quite a number of claims stated on the labels of products cannot be substantiated by scientific evidence and are therefore misleading. Beside the fact that the consumer is being manipulated in to buying a product under false pretences, it could potentially be dangerous if such products are used to treat a condition instead of seeking medical help (Herbert & Kasdan, 1994). Manufacturers and marketers of these products should therefore be held accountable for health related claims on products via appropriate legislation.

Unlike the European situation (Hilliam, 1998), South African legislators have formulated proposed regulations for the labeling of probiotic and/or prebiotic containing products within the Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act No. 54, 1972, http://www.doh.gov.za, 2003/05/02 accessed). When these regulations were applied to assess the acceptability of claims stated on the labels of products identified in this study, it was found that only three out of 26 claims complied with the regulations. However, sound scientific evidence was found for five additional claims not included in the proposed South African regulations. Therefore, if South African manufacturers follow the regulations, the consumer will also be misinformed because a number of proven health benefits will not be mentioned on the labels of products. To rectify this situation, it is thus suggested that the proposed South African regulations be revised to include the following five claims: diarrhea prevention in infants; diarrhea prevention in adults; improvement of digestive health, stool quality and constipation; prevention of Crohn's disease and treatment of food allergies.

The content related claims on the labels of the identified products were mostly in line with the proposed South African regulations. Probiotic cultures most commonly claimed on the labels of supplements and functional foods in South Africa include *L. acidophilus*, *B. bifidum* and *B. longum*, which complies with the proposed South African regulations. The same species are generally claimed to be included in European probiotic supplements (Temmerman, *et al.*, 2002). However, two infant foods in South Africa are fortified with *Bifidobacterium* sp. and *Lactobacillus acidophilus* instead of *B. infantis*, which is specified in the proposed South African regulations for use in infant foods aimed at infants younger than one year. All the probiotic containing products on which information concerning strains is mentioned, comply with the proposed South African regulations concerning strain content, with the exceptions of NAN2 and Inteflora 250. The viable cell number claims on the labels of all the products on which this aspect was mentioned, varies between $1 \times 10^8$ cfu/g and $1.8 \times 10^{10}$ cfu/g, which complies with the proposed South African regulations.

The viable cell numbers of three of the five probiotic supplements tested for actual viable cell numbers were not in line with the numbers stated on the labels of the supplements. This phenomenon is not uncommon as Hamilton-Miller *et al.* (1999), Hoa *et al.* (2000) and Temmerman *et al.* (2002), also reported that the identity and number of viable strains recovered from probiotic supplements and dairy products in the United Kingdom and Europe did not correspond to the information on the label. Hamilton-Miller and Shah (2002) suggested that manufacturers should ensure careful manufacturing practices and proper storage of probiotic containing products to ensure cell survival. Despite the fact that the actual viable cell numbers in these supplements were lower than the viable cell numbers stated on the labels, all the probiotic strains showed good inhibitory activity against the indicator strains isolated from faeces from patients diagnosed with AIDS.

The prebiotic types used in the prebiotic containing products manufactured in South Africa include fructo-oligosaccharides, Raftilose and chicory, which comply with the proposed South African regulations. However, three products did not specify the type of prebiotic but only claimed prebiotic fibre content, which does not comply with the proposed South African regulations. The prebiotic concentration claimed on the labels varies between 3g and 15g per 100g, which complies with the proposed South African regulations. However, one product contained 1.7g/100g fructo-oligosaccharides which does not comply with the proposed South African regulations. However, if the daily portion size consumed is 200g or more, it will comply with the proposed South African regulations.

It is of concern that quite a number of products on the South African market only claim AB-culture content or prebiotic fibre content without specifying probiotic species, viable cell numbers, prebiotic type and concentration. This situation indicates that the proposed South African regulations are not being enforced and that it is not possible for the consumer to make a well-informed decision about the use of these products. It is of vital importance that this situation should be rectified.

**Conclusions and recommendations**

It can be concluded that a large variety of probiotic and prebiotic containing products are found on the South African market. It seems that manufacturers of these products are misleading consumers with a number of health claims that are not scientifically sound. Marketing claims should be based on sound scientific evidence and should comply with legislation in this regard. However, the proposed South African regulations clearly do not include all the scientifically sound health claims and should be revised to ensure that the consumer is provided with correct information. The content related claims on the labels of products mostly comply with the proposed South African regulations although a number of products do not provide this information. Finally, it is evident that viable cell number claims on the labels of products are not always true. However, this problem does not seem to affect the inhibitory activity of the probiotic strains included in the product.

It is recommended that the proposed South African regulations regarding probiotic and prebiotic containing products should be revised and that the manufacturers of these products should subsequently be held responsible to provide the consumer with scientific sound, true and legally correct information.

## Acknowledgements

This study was funded by the Medical Research Council (MRC) and National Research Foundation (NRF)

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# CHAPTER 4

# AWARENESS OF SOUTH AFRICAN CONSUMERS OF PROBIOTIC AND PREBIOTIC CONTAINING PRODUCTS

# Awareness of South African consumers of probiotic and prebiotic containing products.

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## Abstract

The awareness of South African consumers of probiotics and prebiotics was investigated by conducting a survey among a select group of 100 consumers. An interview schedule containing questions on general awareness of probiotics, AB-cultures and prebiotics, products containing these compounds, consumption of such products, possible health benefits thereof and sources of information, was developed and used to interview respondents. It was found that the general awareness of probiotics (18%), AB-cultures (36%) and prebiotics (4%) is low. Those who are aware of probiotics (including AB-cultures) and prebiotics seem to be students, females and younger consumers although their knowledge of the health benefits thereof is limited. It is recommended that manufacturers of probiotic and prebiotic containing products should improve their market strategy in order to increase the general awareness of consumers of probiotics and prebiotics and the associated health effects thereof.

*Keywords:* market survey, consumer awareness, probiotics, AB-cultures, prebiotics

**Introduction**

Several steps are involved in the development of a new food product. According to Baker *et al.* (1988) these steps or stages include the idea development, sensory profiling, consumer sampling, shelf life studies, packaging, production, market testing and marketing. Marketing, the final step in the developing process, may determine the success or failure of a new product. A new product must not only be of good quality but the awareness of consumers should also be raised to persuade him/her to consume the product regularly. Low consumer awareness and knowledge of a product might therefore restrain the growth of the market of the product (Menrad, 2003). Manufacturers should therefore ensure high consumer awareness of products to improve growth of a market.

In general, the market for probiotic and prebiotic containing products and the awareness of these products in improving health is increasing (Mattilla-Sandholm *et al.*, 2002). Probiotics and prebiotics are used in the fortification of food items and in supplements (Roberfroid, 2000). Fuller (1989) defined probiotics as "a live microbial food supplement that beneficially affects the host animal by improving its intestinal microbial balance", whereas prebiotics are defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/ or activity of probiotic bacteria in the colon" (Gibson & Roberfroid, 1995).

In Europe consumers have a strong interest in gut-health foods and are aware of the health benefits of probiotics, with the French being the most aware, followed by the Germans and the British (Hilliam, 1996). France manufactured the first yoghurt containing bifidobacterium in the mid-1980s, which subsequently took about 9% of the yoghurt market (Hilliam, 1996). The marketing strategy involved emphasized the role of probiotics in gut health, lowering blood cholesterol, and improving the body's natural defences (Young, 1997). The German market for probiotic yoghurt is growing rapidly as it increased by 150% during 1996 – 1997, whereas the market in the United Kingdom grew by 26% during the same time period and is estimated at $2 billion (Young, 1997). In contrast with probiotics, European consumer awareness of prebiotics is very low (Cathro & Hilliam, 1993). Prebiotic containing products on the European market are mostly found in the dairy sector. The first prebiotic containing product available on the European market was Nutricia, a fermented milk drink containing *L. caseii* and inulin, which was launched in Belgium in 1994 (Hilliam, 1996). In Japan the awareness of probiotics and prebiotics is high and the functional food market is dominated by soft drinks with probiotics the significant, functional ingredient. In 1993 a functional drink containing probiotics and prebiotics was launched in Japan and achieved sales of 11 billion yen in the first year (Young, 1996). In the United States consumer awareness of probiotics and prebiotics is still growing as the health benefits of these products are not yet recognized in the country's legislation (Stanton *et al.*, 2001). The probiotic portion of the functional food market in the United States has been estimated to be between $50-$200 million during the late 1990's (New Hope Natural Media, Boulder, CO; Arts, 1996). Although a range of probiotic and prebiotic containing products seem to be available on the

South African market (see Chapter 3), no information regarding the awareness of the South African consumers of these products is available.

It is evident that the market for probiotic and prebiotic containing products is growing world wide possibly also in South Africa. The determination of consumer awareness of these products provides important information for marketers and health providers to estimate the growth of the market and create awareness among consumers about the potential health benefits of these products. It was therefore deemed necessary to conduct an exploratory survey to determine the awareness of a select group of consumers in South Africa of probiotics and prebiotics as such, products containing probiotics and prebiotics, potential health benefits as well as sources of information regarding these products.

## Methods

*Study design*

The study design involved a cross sectional survey. Surveys involve the collection of primary data that may include depth and extent of knowledge; attitudes; interest and opinions; past, present or intended behaviour as well as classification of data according to variables such as demographic and socio-economic measures of age, income, occupation and place of residence (Rousseau, 1999).

*Sample*

The geographic area chosen for the survey was the town of Stellenbosch, situated in the Western Cape Province of South Africa. It was argued that as far as market awareness of functional foods is concerned, there is no reasonable indication that consumers in Stellenbosch would show different attitudes and/or behavior to those in similar areas of South Africa. Stellenbosch is a town with 83 000 inhabitants in a high socio-economic group (Living Standards Measurement Group 8) (Stellenbosch, Tourism, 2001).

The target population included individuals who exhibited awareness of the link between health and nutrition by either buying yoghurt (potential probiotic containing product) or any dietary supplement. A convenience sample of one hundred subjects was recruited over a period of two weeks in September 2003. Three main grocery supermarkets, one health store and two pharmacies in Stellenbosch were identified. A subject was approached and willingness to participate determined once he/she made a purchase of yoghurt or one or more dietary supplements. To ensure the inclusion of a wide range of consumers representing different age, gender, occupation and ethnic groups, each targeted outlet was visited at least twice a day, at least twice a week for two weeks (Addendum 1).

*Survey instrument*

The survey instrument which consisted of an interviewing schedule containing closed and open ended questions, was developed using the dendogram technique (Schutte, 2003). A dendogram is a visual presentation that conceptualizes links and illustrates the different issues pertaining to a specific subject. The consumer market segments suggested by Kotler (2000) (Addendum 2) were used as starting point for the development of the dendogram. These segments include two broad groups of variables. First, consumer characteristics that include geographic, demographic and psychographic segments and second, consumer responses to benefits sought or brands used. This study involved mainly the demographic segment and consumer response to brands used. The final questionnaire (Addendum 3) consisted of nineteen questions regarding knowledge of probiotic/AB-cultured/prebiotic containing products and four questions regarding demographics including gender, age, occupation and ethnicity. Consumer awareness of probiotics, *Lactobacillus acidophilus* and *Bifidobacterium* (AB)-cultures and prebiotics was assessed by asking whether the respondent had ever heard of the product(s). If yes, the respondent was asked to name examples, whether he/she had ever consumed it for health purposes, whether he/she believed the product is necessary for a person as well as the respondent's main source of information regarding the product(s). The survey instrument was pilot tested on ten students at the University of Stellenbosch to assess comprehension. No changes to the survey instrument were necessary.

*Data collection and analysis*

All the interviews were conducted by the primary researcher. An appointment with the manager of each targeted outlet was made to obtain permission to conduct the survey in the shop. The primary researcher was stationed at the dairy product or supplement sections during the survey.

Data coding and analyses were performed by the primary researcher. All the data were entered into *Excel*® worksheets. The *SPSS*® 11.0 version and a syntax file were used for data analysis purposes. Frequencies were tallied for close ended and open-ended questions with preset options (categories). Where necessary categories (options) were collapsed. For open-ended questions for which options were not predetermined, similar answers were grouped together and assigned a code. Frequencies were subsequently tallied. The frequency data are presented in the text or were included in frequency tables. To assess the relationship between demographic variables and awareness of probiotics, AB-cultures and prebiotics, cross tabulations were constructed with age, gender, ethnicity, and employment status as classification variables. The Pearsons Chi-square test was used to determine whether there were any significant differences in the profiles of awareness of probiotics, AB-cultures and prebiotics between the age, gender, ethnicity, and occupation status groups. A p-value of $< 0.05$ was accepted as significant.

## Results

The sample of 100 subjects included 38% males and 62% females. Forty one percent were between the age of 10 and 29, 32% between the age of 30 and 49 and 27% between the age of 50 and 69. As far as ethnicity is concerned, 82% were white and 18% of mixed ancestry. Sixty one percent were working, 20% were not working and 19% were students.

Less than a fifth (18%, n=18) of the subjects indicated that they had heard of probiotics. The groups most aware of probiotics include students (significant), subjects aged between 10 and 49 (strong tendency) and females (strong tendency) (Tables 4.1, 4.2 and 4.4). No ethnic differences were evident (Table 4.3). The probiotic products most commonly mentioned by the subjects who had heard of probiotics included yoghurt (94.4%, n=17) and to a lesser extent supplements (5.6%, n=1) and other items which included cheese and animal feed (11.1%, n=2). Of those subjects who had heard of probiotics, 72.2% (n=13) specifically consumed such products, especially yoghurt, to increase their probiotic intake. Reasons cited by these subjects why probiotics are necessary, include improved health, increased beneficial microflora, improved immune system and the inhibition of the growth of Candida (Table 4.5). Their main source of information in this regard was magazines and the labels of products (Table 4.6).

More than a third (36%, n=36) of the subjects indicated that they had heard of AB-cultures. The groups most aware of AB-cultures include females (significant) and working people (strong tendency) (Tables 4.1 and 4.4). No ethnic or age differences were evident (Tables 4.2 and 4.3). Of those subjects who were aware of AB-cultured products, 16.7% (n=6) specifically consumed such products, especially yoghurt, to increase their AB-culture intake. Only a few subjects cited reasons why AB-cultures are necessary. These reasons include improved health and improved beneficial microflora (Table 4.5). Their main source of information in this regard was labels, magazines, word of mouth and their doctor (Table 4.6).

Only a very small number of subjects (4%, n=4) indicated that they had heard of prebiotics. The groups who tended to be aware of prebiotics were females, students, those of mixed ancestry and younger people (Tables 4.1-4.4). Of those subjects who were aware of prebiotics, only one subject specifically consumed Nestlè's muesli bars to increase his/her prebiotic intake. The only reason cited by subjects why prebiotics are necessary is improved health (Table 4.5). Most of the subjects were not able to cite any reasons. Their main source of information in this regard was labels of products and their doctor (Table 4.6).

**TABLE 4.1:** Column % of knowledge of probiotics /AB cultures/ prebiotics by gender

| Question | Male<br>n =38 | Female<br>n =62 | Pearsons Chi-square p-value | Degrees of freedom |
|---|---|---|---|---|
| Have you ever heard of probiotics: | | | | |
| Yes | 10.5 | 22.6 | 0.128 | 1 |
| No | 89.5 | 77.4 | | |
| Have you ever heard of AB cultures: | | | | |
| Yes | 23.7 | 43.5 | 0.008 | 2 |
| No | 65.8 | 33.9 | | |
| Not applicable | 10.5 | 22.6 | | |
| Have you ever heard of prebiotics: | | | | |
| Yes | 2.6 | 4.8 | 0.585 | 1 |
| No | 97.4 | 95.2 | | |

**TABLE 4.2:** Column % of knowledge of probiotics /AB cultures/ prebiotics by age

| Question | 18-29 yrs<br>n = 41 | 30-49 yrs<br>n = 32 | 50-69 yrs<br>n = 27 | Pearsons Chi-square p-value | Degrees of freedom |
|---|---|---|---|---|---|
| Have you ever heard of probiotics: | | | | | |
| Yes | 22.0 | 25.0 | 3.7 | 5.235 | 2 |
| No | 78.0 | 75.0 | 96.3 | | |
| Have you ever heard of AB cultures: | 36.6 | 34.4 | 37.0 | 0.219 | 4 |
| Yes | 41.5 | 40.6 | 59.3 | | |
| No | 22.0 | 25.0 | 3.7 | | |
| NA | | | | | |
| Have you ever heard of prebiotics: | | | | | |
| Yes | 7.3 | 3.1 | 0 | 0.307 | 2 |
| No | 92.3 | 96.9 | 100 | | |

**TABLE 4.3:** Column % of knowledge of probiotics /AB cultures/ prebiotics by ethnicity

| Question | White<br>n = 82 | Mixed ancestry<br>n = 18 | Pearsons Chi-square p-value | Degrees of freedom |
|---|---|---|---|---|
| Have you ever heard of probiotics: | | | | |
| Yes | 18.2 | 16.7 | 0.898 | 1 |
| No | 81.8 | 83.3 | | |
| Have you ever heard of AB cultures: | | | | |
| Yes | 36.4 | 33.3 | 0.957 | 2 |
| No | 45.5 | 50.0 | | |
| Not applicable | 18.2 | 16.7 | | |
| Have you ever heard of prebiotics: | | | | |
| Yes | 3.4 | 8.3 | 0.414 | 1 |
| No | 96.6 | 91.7 | | |

**TABLE 4.4:** Column % of knowledge of probiotics /AB cultures/ prebiotics by occupational status

| Question | Working n = 61 | Non-working n = 20 | Students n = 19 | Pearsons Chi-square p-value | Degrees of freedom |
|---|---|---|---|---|---|
| Have you ever heard about probiotics: | | | | | |
| Yes | 9.8 | 20.0 | 42.1 | 0.006 | 2 |
| No | 90.2 | 80.0 | 57.9 | | |
| Have you ever heard about AB cultures: | | | | | |
| Yes | 42.6 | 30.0 | 21.1 | 0.26 | 4 |
| No | 48.0 | 50.0 | 36.8 | | |
| Not applicable | 9.8 | 20.0 | 18.0 | | |
| Have you ever heard about prebiotics: | | | | | |
| Yes | 1.6 | 0 | 15.8 | 0.14 | 2 |
| No | 98.4 | 100 | 84.2 | | |

**TABLE 4.5:** Frequency % of knowledge of health benefits of probiotics/ AB cultures/ prebiotics (multiple answers)

| Question | Probiotics n = 18 | AB-cultures n = 36 | Prebiotics n = 4 |
|---|---|---|---|
| Why do you think it is necessary for probiotics/ AB-cultures/ prebiotics intake | | | |
| Improve health | 38.9 | 25.0 | 25.0 |
| Increase beneficial microflora | 33.3 | 13.9 | 0 |
| Improve immune system | 11.1 | 0 | 0 |
| Inhibit growth of Candida | 5.6 | 0 | 0 |
| Don't know | 11.1 | 5.6 | 75.0 |
| Other | 0 | 55.6 | 0 |

**TABLE 4.6:** Frequency % of main sources of information of probiotics/ AB cultures/ prebiotics (multiple answers)

| Main source of information | Probiotics n = 18 | AB-cultures n = 36 | Prebiotics n = 4 |
|---|---|---|---|
| Doctor | 11.1 | 5.6 | 25.0 |
| Magazines | 16.7 | 11.1 | 0 |
| Labels | 22.2 | 44.4 | 50.0 |
| Other | 38.9 | 8.3 | 0 |
| Don't know | 11.1 | 30.6 | 25.0 |

## Discussion

The results point to the possibility that consumer awareness of probiotics in South Africa is very low. However, the awareness of AB-cultures seems to be higher than probiotics, which might be explained by the fact that most probiotic containing yoghurt product labels claim to contain AB-cultures, while no reference is made to probiotic cultures as such. Hilliam (1996) found that 80% of French respondents were aware of probiotics and 46% claimed that they had bought products containing probiotics. Only 7% of German respondents and 15% of respondents in the United Kingdom claimed to have heard of probiotics. The South African consumers who were aware of probiotics, mostly seemed to have knowledge about the health benefits thereof and their main sources of information were the labels on products, magazines and their doctor. Hilliam (1996) also found that in France, Germany and the United Kingdom doctors and health professionals are important sources of information concerning functional foods. Other sources of information reported by Hilliam (1996) include television, the national press, women's magazines and brochures.

Prebiotics seem to be an even less known term in South Africa. This could be linked to the fact that prebiotic products mostly state the fructo-oligosaccharides or inulin content but do not refer to the generic term, prebiotics. The few respondents in this study who were aware of prebiotics, did not seem to have any knowledge about the health benefits thereof. According to Cathro and Hilliam (1993) only 16% of French respondents, 9% of German respondents and 3% of British respondents claimed that they had heard of prebiotics. No information regarding their knowledge of the health benefits of prebiotics was reported.

According to Childs (1997) consumer research has identified several common characteristics of functional food purchasers, namely that they are predominantly female, well-educated, high earners, aged between 35 and 55 years and are actively interested in health. Similar trends were identified in this research. In this study it was found that women were significantly more likely to have heard of AB-cultures and tended to have been more likely to have heard of probiotics and prebiotics. Two possible explanations can be put forward for this situation. First, women buy many of the products for household consumption (Engel *et al.*, 1993). Cant *et al.* (2002) report that women buy 80% of consumer goods and make 80% of health care decisions. It can therefore be speculated that women are more exposed to products on the market, including supplements and functional foods, and would therefore have a better chance to have been exposed to probiotic and prebiotic containing products. Second, despite the fact that women are not as readily accessible through the traditional media (television and radio) because many of them work (Schiffman & Kanuk, 1997), they are now more readily reached through magazines (Wellner, 2002). Consequently many advertisers now emphasize magazines in their media schedules, especially those specifically aimed at working women (Schiffman & Kanuk, 1997). It can therefore be speculated that the higher awareness of females could be

explained by the fact that women might be more inclined to read magazines than men, especially health related magazines.

The higher awareness of the working people in this study regarding AB-cultures could possibly be linked to a higher income and higher level of education than that of the non-working group (Schiffman & Kanuk, 1997). Educated consumers are expected to be more informed about health and nutrition and therefore to purchase health foods and supplements (Fotopoulos & Chryssochoidis, 2000). However, the higher awareness of students regarding probiotic and prebiotic containing products is not in line with what was expected, as they are not included in the working group. This finding can possibly be linked to the fact that students might be exposed to relevant information as a result of their studies or through close interaction with other students on campus.

Consumer research indicates that age affects the consumption of products ranging from beer to vacations. Age also shapes the media people use, where they shop, how they use products, and how they think and feel about marketing activities (Goode & Moutinho, 1996). In this study it was found that the younger age groups were more aware of probiotics and prebiotics with the exception of AB-cultures. These results are also contradictory to expectations as it has been reported that consumers older than 50 years are more focused on their health and thus more inclined to buy healthy and functional foods (Sanders, 1998). It can therefore be speculated that South African consumers in this age group are not yet that well informed about the health benefits of probiotics and prebiotics.

The respondents who were of mixed ancestry seemed to be as aware of probiotics and prebiotics as their white counterparts. However, it must be borne in mind that only a small number of these respondents were included in the study. It can only be speculated that the awareness levels of Black and Indian respondents will not be higher than was found for the white respondents and the respondents from mixed ancestry who participated in this study.

It is evident from the results in this study that the existing markets for probiotic (including AB-cultures) and prebiotic containing products in South Africa are mainly students, females and younger people. To expand the market for these products, manufactures should focus on men, working people and older people. Furthermore, to ensure continued growth of the probiotic and prebiotic market in South Africa, it is recommended that manufacturers of these products should raise consumer awareness by employing effective marketing strategies (Menrad, 2003). The latter involves the identification of the target market; determining the communication strategy, for example advertisements or brochures; price positioning and distributing the products to the target market (Du Plessis, 1999). The market strategy for probiotic and prebiotic containing products should amongst others include communicating the health benefits thereof to the target market. This could be done by making reliable scientific studies available to journalists, by developing newsletters aimed at the

public, by promoting the publication of articles in scientific journals and health magazines and by developing informative websites. Another important avenue for communication with the consumer is the product label that could provide important information about the content and nutritional value of the product (Deliza *et al.*, 2003). Marketers must therefore ensure that the labels of probiotic and prebiotic containing products contain legally correct information regarding probiotic strains and/or prebiotic type used as well as permissible health claims. Furthermore, to ensure correct price positioning marketers should know how much the consumers in the target market are willing to pay for the product, which should determine considerations concerning probiotic culture or prebiotic cost, packaging, distribution, advertisements and profit margins (Sanders, 1998). Finally, the market success of probiotic and prebiotic containing products also depends on the availability of high-volume distribution channels such as supermarkets and general retail stores (Menrad, 2003). Although no information in this regard was obtained in this study, Cathro and Hilliam (1993) maintain that consumers expect to buy functional foods in supermarkets and pharmacies and not only in specialized shops such as health food stores.

**Conclusions and recommendations**

It can be concluded that the results of this study indicate that consumer awareness of probiotics and/or AB-cultures and prebiotics in South Africa is very low. Those who are aware of probiotics and prebiotics seem to be students, females and younger consumers. Knowledge of health benefits of especially prebiotics is limited and products, labels, magazines and doctors seem to be the most important sources of information in this regard. Although this study was of an exploratory nature and only a hundred subjects were included, these subjects were selected specifically because they purchased either yoghurt or a health food product/supplement. It is therefore proposed that the general population will be even less aware of the products.

It is recommended that manufacturers of probiotic and prebiotic containing products should improve their market strategy in order to increase the general awareness of consumers of probiotics and prebiotics and the associated health effects. This could be done by providing legally correct health and content claims on the labels of products as well as the publication of scientific sound literature in for example magazines, brochures and newsletters.

## Acknowledgements

This study was funded by the Medical Research Council (MRC) and National Research Foundation (NRF)

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# CHAPTER 5

# EVALUATION OF PROBIOTIC STRAINS FOR POTENTIAL COMMERCIAL USE

# Evaluation of probiotic strains for potential commercial use

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## Abstract

Probiotic lactic acid bacteria and prebiotics are effective in the treatment and prevention of clinical disorders in humans. Twelve strains of a potential probiotic panel were screened for inhibitory activity against indicator strains, *Escherichia coli* and *Salmonella* sp., lactic acid bacteria, *Stapylococcus aureus*, *Listeria innocua*, *Bacillus cereus*, *Clostridium* spp. and *Propionibacterium* spp. from the LMG-panel. Five lactic acid bacteria (LAB), *Lactobacillus plantarum*, *Lactobacillus casei* LHS, *Lactobacillus salivarius* 241, *Lactobacillus curvatus* DF 38 *and Pediococcus pentosaceus* 34 were selected for further study on the basis of their inhibitory activity. The strains inhibited the growth of all the pathogenic bacteria isolated from the faeces of patients diagnosed with AIDS. All five strains were grown effectively in soymilk-base, which has been proposed as a growth medium for probiotic strains for commercial production of probiotic concentrates. Lyophilization of the strains grown in soymilk-base revealed a 2 log-cycle decrease in cell numbers, but without reduction of the inhibitory activity against pathogens isolated from faeces from patients diagnosed with AIDS. Growth of these strains in the presence of the prebiotics Raftiline®GR (inulin), Raftilose®L95 (oligofructose) and Raftilose®Synergy1 (inulin and oligofructose) (SAVANNAH Fine Chemicals) revealed that 1% (m/v) Raftilose®Synergy1 acted as the best substrate. The inhibitory activity of the LAB against indicator strains isolated from the faeces of patients diagnosed with AIDS was also enhanced when combined with the selected prebiotic.

*Keywords:* lactic acid bacteria, probiotic strains, prebiotics, soymilk

**Introduction**

Various factors such as diet, stress and medication factors may disturb the balance of the normal flora in the gastro-intestinal tract (Fuller & Gibson, 1997). Should this occur, the numbers of health-promoting bacteria such as lactobacilli and bifidobacteria decrease, leading to domination by pathogenic bacteria. Overgrowth of these pathogens may lead to clinical disorders, including cancer, inflammatory disease, ulcerative colitis or other gastrointestinal disorders (Fooks *et al.*, 1999).

Probiotic lactic acid bacteria, i.e. "viable cells that beneficially affect the host by improving its intestinal microbial balance" (Fuller, 1989), have the ability to colonize the gastrointestinal tract and compete with pathogenic bacteria (Crittenden, 1999; Fujiwara *et al.*, 1997; Kailasapathy & Chin, 2000). Probiotic strains compete with pathogens for available nutrients and other growth factors (Rolfe, 2000), antagonize pathogens through production of antimicrobial and antibacterial compounds, such as bacteriocins and butyric acid (De Vuyst & Vandamme, 1994; Dodd & Gasson; 1994; Kailasapathy & Chin, 2000), and reduce the intestinal pH, which in turn stimulates the growth of other lactic acid bacteria (Langhendries, *et al.*, 1995). Probiotic bacteria may also stimulate the immune system (Isolauri *et al.*, 1991; Isolauri *et al.*, 1995; Rolfe, 2000), and lead to the production of lactase (Rolfe, 2000).

For commercial production of probiotic concentrates, the probiotic strains need to be prepared according to specific methods and the cells stabilised in growth-supporting media to ensure metabolic activity. A few studies have been conducted on non-dairy stabilising substrates such as soymilk (Kamaly, 1997; Mattilla-Sandholm *et al.*, 2002). Soymilk contains fermentable sugars and several microbial growth-stimulating compounds and is relatively inexpensive (Kamaly, 1997).

Probiotic strains are freeze-dried or spray-dried when used in special formulations such as capsules or tablets, food fortification or when used as starters in the production of fermented foods (Saarela *et al.*, 2000). Freeze-drying is the most popular method since many strains cannot tolerate the relatively high temperatures that are used in spray-drying (Mattilla-Sandholm *et al.*, 2002). However, many probiotic strains loose their viability after prolonged storage at room temperature (Myllärinen, *et al.*, 1998).

Probiotic strains are available in various forms of pharmaceutical preparations, mostly as encapsulated cells, or are incorporated in functional foods. The latest trend is to include probiotic cells with prebiotics (Fooks *et al.*, 1999). Prebiotics are generally defined as 'non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, especially *Lactobacillus* and *Bifidobacterium* spp.' (Gibson, & Roberfroid, 1995). Prebiotics may thus render probiotic cells a competitive advantage over other bacteria normally present in the gastrointestinal tract (Collins & Gibson, 1999; Crittenden, 1999) and

may help to maintain a balance of gut flora in healthy individuals or restore the equilibrium in individuals with an altered microflora due to disease, age, or variation in diet (Fooks *et al.*, 1999).

This paper reports on the identification of viable probiotic strains, soymilk-base as an appropriate growth medium and determination of the effect of lyophilization (freeze-drying) for potential commercial use. The growth and inhibitory activity of the five LAB in the presence of prebiotics was also determined.

## Materials and Methods

*Bacterial strains and growth conditions*

The lactic acid bacteria (LAB) strains from the culture collection of the Department of Microbiology, University of Stellenbosch, that were screened against indicator strains from the species of *Bacillus, Clostridium, Enterococcus, Lactobacillus, Leuconostoc, Listeria, Pediococcus, Propionibacterium, Salmonella, Shigella, Staphylococcus, Streptococcus, Vibrio* and *Yersinia* are listed in Table 5.1.

**TABLE 5.1:** Lactic acid bacteria screened for inhibitory activity

| **LAB strains** | **Origin** |
|---|---|
| *Lactobacillus plantarum* 423; 411R; 81 | Sorghum beer |
| *Lactobacillus salivarius* 241 | Pig intestine (ileum) |
| *Lactobacillus curvatus* DF38 | Italian salami |
| *Lactobacillus casei* LHS | Sherry |
| *Enterococcus fecalis* 92 | Pig faeces |
| *Enterococcus* sp. B3 | Pig faeces |
| *Pediococcus pentosaceus* ATCC 43200 | Cucumber brine |
| *Pediococcus pentosaceus* 34 | Unknown |
| *Pediococcus* sp. | Department culture collection |
| Probiotic x | Commercial product |

The indicator strains, *Escherichia coli* and *Salmonella* sp. (Department of Microbiology, University of Stellenbosch, Stellenbosch, South Africa) and strains from the LMG- panel (Lab voor Microbiologie, University of Ghent, Ghent, Belgium) and a panel of indicator strains isolated from patients diagnosed with AIDS (Tygerberg Hospital, Cape Town, South Africa) used in this process are listed in Table 5.2.

**TABLE 5.2**: Indicator strains used for the screening of the lactic acid bacteria

| **Indicator strains** | **Origin** |
|---|---|
| *Bacillus cereus* LMG 6910 | Pasteurised milk |
| *Clostridium sporogenes* LMG 8421 | Soil |
| *Clostridium tyrobutyricum* LMG 13571 | Cheese |
| *Enterococcus faecalis* LMG 8222 | Urine |
| *Escherichia coli* | Pig faeces |
| *Lactobacillus acidophilus* LMG 8151 | Acidophilus milk |
| *Lactobacillus bulgaricus* LMG 6901 | Bulgarian yoghurt |
| *Lactobacillus casei* LMG 6904 | Cheese |
| *Lactobacillus curvatus* LMG 9198 | Milk |
| *Lactobacillus fermentum* LMG 18026 | Milk |
| *Lactobacillus helveticus* LMG 6413 | Swiss Emmental cheese |
| *Lactobacillus plantarum* LMG 6907 | Pickled cabbage |
| *Lactobacillus reuteri* LMG 9213 | Adult intestine |
| *Lactobacillus sakei* LMG 9844 | Sauerkraut |
| *Leuconostoc cremoris* LMG 6909 | Hansen's dried cheese starter powder |
| *Listeria innocua* LMG 11387 | Bovine, brain |
| *Pediococcus pentosaceus* LMG 9445 | Cheese curd |
| *Propionibacterium acidipropionici* LMG 16446 | Sheep milk |
| *Propionibacterium* sp. LMG 13573 | Unknown |
| *Salmonella* sp. | Pig faeces |
| *Staphylococcus carnosus* LMG 13567 | Unknown |
| *Streptococcus thermophilus* LMG 6986 | Pasteurised milk |
| | |
| *Salmonella* Gr.B. 003 | Faeces of patient with AIDS |
| *Salmonella typhi* 001 | Faeces of patient with AIDS |
| *Salmonella typhimurium* 002 | Faeces of patient with AIDS |
| *Shigella boydii* 007 | Faeces of patient with AIDS |
| *Shigella flexnerii* 1 | Faeces of patient with AIDS |
| *Shigella flexnerii* 3 | Faeces of patient with AIDS |
| *Shigella sonnei* 006 | Faeces of patient with AIDS |
| *Shigella spp.* 008 | Faeces of patient with AIDS |
| *Vibrio parahemolyticus* 010 | Faeces of patient with AIDS |
| *Yersina* spp. 009 | Faeces of patient with AIDS |

All lactic acid bacteria were cultured in MRS broth (Biolab, Biolab Diagnostics, Midrand, S.A.). The *Clostridium* spp. was cultured in Reinforced Clostridium Medium (Biolab), the *Propionibacterium* spp. in GYP broth (Van der Merwe *et al.*, 2003) and all other species in Brain heart Infusion (BHI) broth (Biolab). Incubation conditions were as indicated by the respective culture collection catalogues.

*Screening for inhibitory activity of lactic acid bacteria*

The twelve LAB were cultured in MRS broth (Biolab) for 18h at 37°C and 10 µl spotted on MRS agar (Biolab). The plates were incubated for 24h at 37°C and then lawned with active growing cells of the indicator strains *E. coli* and *Salmonella* sp. as well as the LMG-panel listed in Table 5.2. The cells were imbedded in soft agar (0.8%, m/v), as described by Van Reenen *et al.* (1998). The plates were

incubated at 37°C for 24h and the colonies examined for the formation of inhibition zones. All tests were conducted in triplicate. Strains with the broadest spectrum of inhibitory activity were selected for further studies and were tested for inhibition against each other. Inhibitory activity was confirmed by testing the cell-free supernatant of 18h-old cultures from MRS broth (Biolab). Ten µl of the supernatant was spotted onto MRS agar (Biolab), lawned with each selected LAB strain as described before and examined for the formation of inhibition zones after 24h. The inhibitory activity of the selected strains was also tested against indicator strains isolated from faeces of patients diagnosed with AIDS (Table 5.2).

*Growth in soymilk-base and growth and inhibitory activity after lyophilization*

The five selected LAB were cultured in MRS broth (Biolab) at 37°C for 24h and then inoculated into 10% (m/v) of a commercially available soymilk-base that was sterilized by autoclaving. Viable cell numbers were counted on MRS agar plates. After 24h growth at 37°C, the cultures were harvested by centrifugation (9000 x g; 30min, 4°C), the pellet resuspended in soymilk-base and freeze dried. Viable cell numbers and inhibitory activity against the pathogens isolated from the faeces of patients diagnosed with AIDS (Table 5.2) were determined as before.

*Effect of prebiotics on the inhibitory activity of lactic acid bacteria*

The five selected LAB from the screening for inhibitory activity were grown in 10ml MRS broth (Biolab), without dextrose, and supplemented with 2% (w/v) of a 5% (w/v) suspension of filter-sterilised Raftiline®GR (92%, w/v, inulin; 8%, w/v, glucose/fructose/sucrose), Raftilose®L95 (95%, w/v, oligofructose, 5%, w/v, glucose, fructose, sucrose) and Raftilose®Synergy1 (combination of 92%, w/v, inulin and oligofructose, 8%, w/v, glucose, fructose, sucrose) respectively prebiotics were from SAVANNAH Fine Chemicals (ORAFTI, Tienen, Belgium). The tubes were inoculated with 15 µl of a 12h-old lactic acid bacterial culture and incubated for 24h. The control was MRS broth (Biolab), without dextrose and prebiotics. Growth was recorded by optical density readings ($OD_{600}$). Changes in culture pH were recorded after 24h of incubation at 37°C. The prebiotic which stimulated the growth of the five selected strains the best was selected for further studies. The effect of 0.5% - 3.0% (w/v, with 0.5% intervals) of the selected prebiotic on inhibitory activity of the selected strains was tested against *E. coli* and *Salmonella* sp. were tested as described before (Van Reenen *et al.*, 1998). The prebiotic concentration that yielded the best inhibitory activity was included in MRS broth (Biolab) and the strains tested against the indicator strains isolated from the faeces of patients diagnosed with AIDS (Table 5.2).

## Results

*Screening for inhibitory activity of lactic acid bacteria*

The screening of twelve LAB strains for inhibitory activity against the indicator strains indicated that the spectrum of inhibitory activity differed for each strain (Table 5.3, p.114). The indicator strains not effected by the LAB strains included *L. casei*, *L. curvatus*, *L. planrarum*, *L. reuteri*, *P. pentosaceus*, *S. thermophilus*, *P. acidipropionici.* The following five strains with the best inhibitory activity were selected for further studies: *Lactobacillus plantarum* 423, *Lactobacillus casei* LHS, *Lactobacillus salivarius* 241, *Lactobacillus curvatus* DF38 and *Pediococcus pentosaceus* 34.

These strains had no inhibitory effect on each other (results not shown) but did show inhibitory activity against all the indicator strains isolated from AIDS patients (Table 5.4, p.115).

*Growth in a soymilk-base and growth and inhibitory activity after lyophilization*

Soymilk-base as growth medium supported the growth of the five selected LAB strains, as is indicated by a two log-cycle increase over 24h (Table 5.5).

**TABLE 5.5:** Cell counts of selected LAB grown in soymilk-base before and after lyophilization

| **Five selected LAB** | **Soymilk (control)** | | **After lyophilization** |
|---|---|---|---|
| | **0 hrs (cfu/g)** | **24 hrs (cfu/g)** | **48 hrs (cfu/g)** |
| L. *plantarum 423* | $4.1 \times 10^5$ | $2.5\times 10^7$ | $3.6 \times 10^5$ |
| *L. casei* | $1.1\times 10^5$ | $4\times 10^7$ | $2 \times 10^5$ |
| *L. curvatus* DF 38 | $8\times 10^4$ | $4.7\times 10^7$ | $3.2 \times 10^5$ |
| L. *salivarius* | $1.5\times 10^5$ | $3.1\times 10^8$ | $2.1\times 10^6$ |
| *P. pentosaceus* 34 | $2.1 \times 10^5$ | $3 \times 10^7$ | $2.4\times 10^5$ |

The cell numbers of the LAB strains were reduced by two log-cycles when lyophilized (Table 5.5). Lyophilized cells grown in MRS broth (Biolab) yielded a spectrum and level of inhibitory activity against pathogens isolated from patients diagnosed with AIDS in the same order as cells that have not been lyophilized (Table 5.4).

**TABLE 5.3:** Inhibitory activity of LAB against *E. coli*, *Salmonella* sp. and strains from the LMG-panel.

| **Inhibitory strains** | **Indicator strains** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E. coli* | *Salmonella* sp. | *L. acidophilus* | *L.bulgaricus* | *L. fermentum* | *L. sakei* | *P.pentosaceus* | *L.cremoris* | *E. faecalis* | *Staphylococcus carnosus* | *Listeria innocua* | *Bacillus cereus* | *Clostridium sporogenes* | *Clostridium tyrobutyricum* | *P.acicipropionici* |
| *L.plantarum* 423 | + | + | + | + | + | + | - | - | + | + | + | + | + | + | + |
| *L.plantarum* 411R | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L.plantarum* 81R | + | - | + | + | + | + | - | - | + | + | + | + | + | + | - |
| *L.curvatus* DF 38 | + | + | - | + | - | + | - | + | - | - | + | - | - | - | - |
| *P.pentosaceus* ATCC 43200 | - | + | - | + | + | + | + | + | + | + | + | - | - | - | - |
| Probiotic x | - | - | - | + | - | + | - | - | - | + | + | + | - | + | - |
| *P. pentosaceus* 34 | + | + | - | + | - | + | + | + | + | + | + | + | - | - | - |
| *P. pentosaceus* sp. PBC | + | + | - | - | + | + | + | + | - | - | + | + | - | - | - |
| *Enterococcus* sp. B3 | - | - | - | - | - | + | + | + | - | - | + | + | - | + | - |
| *E. feacium* | - | - | - | - | - | + | - | - | - | + | + | + | - | + | - |
| *L.salivarius* 241 | + | + | - | - | - | + | + | + | + | + | + | + | + | + | - |
| *L. casei* LHS | + | + | - | + | + | + | + | + | + | + | + | + | + | + | - |

+ = growth inhibition; - = no growth inhibition

[1] Only the indicator strains against which inhibitory activity was found, are included in the table.

**TABLE 5.4**: Inhibitory activity of LAB against indicator strains isolated from faeces of patients diagnosed with AIDS.

| **Inhibitory strains** | *Salmonella typhi* | *Yersina* sp. | *Shigella flexnerii* 1 | *Salmonella typhimurium* | *Shigella flexneri 3* | *Shigella sonnei* | *Shigella boydii* | *Salmonella* Gr.B | *Shigella* sp. | *Vibrio parahemolyticus* |
|---|---|---|---|---|---|---|---|---|---|---|
| When cultured in MRS broth | | | | | | | | | | |
| *L. plantarum* 423 | + | ++ | +++ | ++++ | ++++ | ++ | ++ | ++ | ++ | ++ |
| *L. salivarius* 241 | ++ | ++ | ++ | + | ++++ | + | ++ | ++++ | ++ | + |
| *L. curvatus* DF 38 | +++ | ++++ | ++++ | +++ | ++++ | ++ | +++ | +++ | ++ | + |
| *P. pentosaceus* 34 | +++ | +++ | ++++ | ++ | +++ | ++ | ++++ | +++ | + | ++ |
| *L. casei* LHS | +++ | ++ | +++ | ++++ | +++ | ++ | +++ | ++ | + | ++ |
| When grown in the presence of 1% (w/v) Raftilose®Synergy1 | | | | | | | | | | |
| *L. plantarum* 423 | ++++ | ++++ | ++++ | +++ | ++++ | +++ | +++ | ++++ | +++ | ++ |
| *L. salivarius* 241 | ++++ | ++++ | ++++ | +++ | ++++ | +++ | +++ | ++++ | ++ | ++ |
| *L. curvatus* DF 38 | ++++ | ++++ | ++++ | +++ | ++++ | +++ | ++++ | +++ | ++ | ++ |
| *P.pentosaceus* 34 | +++ | +++ | ++++ | +++ | ++++ | +++ | +++ | +++ | +++ | ++ |
| *L. casei* LHS | ++++ | +++ | ++++ | +++ | ++++ | +++ | ++++ | ++++ | ++ | ++ |
| After lyophilization | | | | | | | | | | |
| *L. plantarum* 423 | + | ++ | ++ | +++ | +++ | ++ | ++ | ++ | ++ | ++ |
| *L. salivarius* 241 | ++ | ++ | ++ | + | +++ | + | + | +++ | ++ | + |
| *L. curvatus* DF 38 | +++ | +++ | ++++ | ++ | +++ | ++ | ++ | ++ | ++ | + |
| *P. pentosaceus* 34 | ++ | +++ | ++ | ++ | +++ | ++ | +++ | ++ | + | + |
| *L. casei* LHS | +++ | ++ | ++ | ++++ | ++ | + | +++ | ++ | + | ++ |

Inhibition zone sizes: + = 20-25mm zone size; ++ = 26-30mm zone size; +++ = 31-35mm zone size; ++++ = larger than 36mm

*Effect of prebiotics on the inhibitory activity of lactic acid bacteria*

The growth of LAB strains in MRS medium supplemented with prebiotics was much more vigorous than in the control medium (without dextrose) as reflected by a rapid decrease in pH and an increase in cell density (Table 5.6). This effect was recorded for all three prebiotics with the best results recorded for Raftilose®Synergy1.

**TABLE 5.6:** Changes in pH and cell density ($OD_{600}$) after 24h growth of LAB in MRS broth, in the presence and absence of prebiotics

| **The selected LAB** | | **MRS without dextrose** | **MRS with Raftiline®GR** | **MRS with Raftilose®L95** | **MRS with Raftilose® Synergy1** |
|---|---|---|---|---|---|
| *Lb. plantarum* 423 | pH | 6.42 | 6.30 | 6.31 | 5.36 |
| | OD | 0.046 | 0.058 | 0.048 | 1.355 |
| *Lb. salivarius* 241 | pH | 6.40 | 4.96 | 5.16 | 4.96 |
| | OD | 0.025 | 1.287 | 1.033 | 1.289 |
| *Lb. casei* LHS | pH | 6.43 | 5.22 | 5.40 | 5.07 |
| | OD | 0.068 | 0.918 | 0.922 | 1.205 |
| *Lb. curvatus* DF 38 | pH | 6.43 | 5.22 | 5.53 | 5.22 |
| | OD | 0.077 | 1.077 | 0.942 | 1.216 |
| *P. pentosaceus* 34 | pH | 6.44 | 5.78 | 5.50 | 5.59 |
| | OD | 0.032 | 1.029 | 1.170 | 1.180 |

A concentration of 1% (w/v) Raftilose®Synergy1 had a greater effect on the inhibitory activity of the LAB than 0.5% (w/v). However, further enhancement of activity was not found for any of the five strains with further increases in Raftilose®Synergy1 concentration (Figure 5.1).

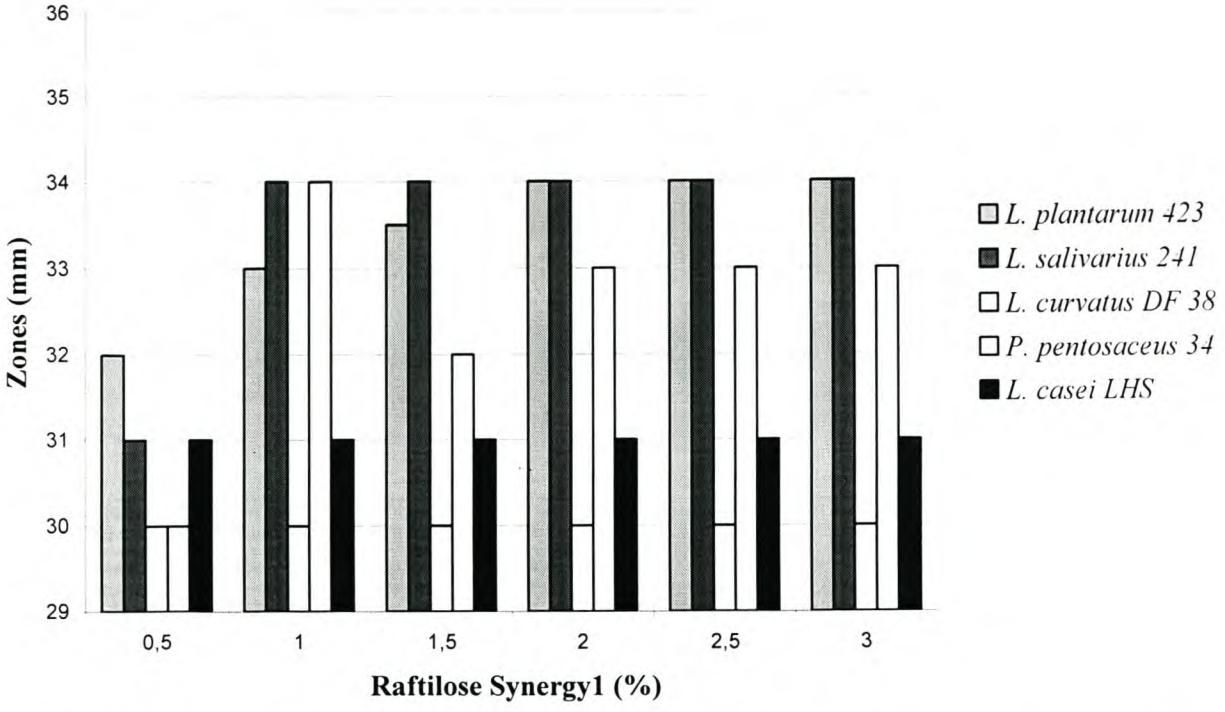


**Fig. 5.1:** The inhibitory effect of five selected LAB in combination with different concentrations of Raftilose®Synergy1 on *E. coli* and *Salmonella* sp. growth

The inclusion of 1% (w/v) Raftilose®Synergy1 in the MRS medium (Biolab) increased the inhibitory activity of the five selected LAB strains against the indicator strains isolated from the faeces of patients diagnosed with AIDS. The inhibition zone sizes were increased to 31-35mm and greater than 36mm (Table 5.4).

## Discussion

In the development of an effective probiotic concentrate for commercial purposes, it is essential to identify viable LAB and ensure optimal cell counts and inhibitory activity after production. In this research five LAB were identified from a panel of twelve LAB that showed effective inhibitory activity against indicator strains under normal conditions. Of these five, *L. curvatus* DF 38 and *P. pentosaceus* 34 tended to inhibit the indicator strains isolated from the faeces of patients diagnosed with AIDS to a greater extend than observed for *L. plantarum* 423, *L. casei* LHS and *L. salivarius* 241, as reflected by larger inhibition zone sizes. The inhibitory spectrum of these five was similar to that reported for other similar LAB such as *L. plantarum* 0407, *L. pentosus* 905, *L. reuteri* SD2112, and *L. acidophilus* La5 against *E. coli*, *C. jejuni* and *S. enteritidis* (Fooks & Gibson, 2002). The effective competitive exclusion of pathogens by LAB strains may involve a number of mechanisms, such as the production of organic acids and a range of antimicrobial compounds or variations of the same type antimicrobial compound. Organic acids such as lactic acid and acetic acid in undissociated form

penetrate the cell membrane and dissociate intracellularly to its anionic form and protons ($H^+$) (Silva *et al.*, 1987 Russell & Diez-Gonzlaez, 1998). Accumulation of anions in the cytoplasm interferes with essential cell functions and reduces the intracellular pH. This antagonism by probiotic bacteria against pathogenic bacteria is important to maintain a normal intestinal microbial balance (Roberfroid, 2000). Apart from lactic acid and acetic acid, probiotics produce antimicrobial peptides, such as bacteriocins and/or low-molecular-mass antimicrobial compounds (Saarela *et al.*, 2000). Four distinct classes of bacteriocins have been identified in four distinct classes on the basis of biochemical and genetic characterization including (I) antibiotics, (II) small heat-stable, non-lanthionine peptides, (IIa) Listeria-active peptides, (IIb) poration complexes consisting of two pepeides for activity, (IIc) thiol-activated peptides, (III) large heat-labile proteins, and (IV) complex bacteriocins (Klaenhammer, 1993). *L. plantarum* 423 produces plantaricin 423 which belongs to the group IIa (anti-Listeria) bacteriocins with a broad spectrum of inhibitory activity (Van Reenen *et al.*, 1998). The other selected LAB strains included in this study may produce bacteriocins and/or organic acids with similar inhibitory activity, as indicated by the respective inhibitory zone sizes.

Soymilk has gained interest as a medium for growth and biochemical activities of lactic acid bacteria for commercial production of probiotic concentrates (Kamaly, 1997). Studies done by Wang *et al.* (2002) and Beasley *et al.* (2003) showed that soymilk supports the growth of lactic acid bacteria. In this study the five selected LAB also showed good growth in soymilk-base. It can therefore be deducted that the soybean-oligosaccharides, raffinose and stachyose, present in the soymilk-base, supplied sufficient nutrients for LAB growth and fermentation. This notion is supported by the work of Wang *et al.* (2003), who studied LAB fermentation in soymilk and found that raffinose and stachyose are metabolized by these strains. Soymilk-base can therefore be used as an efficient growth medium for LAB for the commercial production of probiotic concentrates.

Lyophilization (freeze-drying) is the most popular method to dry LAB for storage purposes, although more expensive than spray-drying. With both these drying methods, cell damage may occur (Porubcan & Sellars, 1979). Freeze drying of LAB grown in soymilk-base resulted in a two log-cycle decrease in cell numbers, which could indicate cell damage. However, the fact that no reduction in inhibitory activity agains the indicator strains was found indicates that the reduction in cell numbers did not affect the functional properties of the LAB negatively. It would however be prudent to include protectants such as lactose or sucrose, monosodium glutamate (MSG) and ascorbate, which are usually added to cultures to prevent cell destruction and prolong storage (Champagne *et al.*, 1991; Mäyrä-Mäkinen & Bigret, 1998; Souzu, 1992).

It is clear from the literature that probiotic growth and inhibitory activity can be improved by combination with prebiotics *in vivo*. According to Roberfroid and Delzenne (1998) and Roberfroid

(1999), the inulin/ oligofructose-type products are the prebiotics that have been investigated most extensively for their prebiotic properties. The consistently observed prebiotic potential of inulin and oligofructose may be ascribed to the molecular structure thereof, namely linear chains composed of mainly ß(2→1)-type linked fructose molecules. The other types of prebiotics are branched and less readily accessible to bacterial hydrolyses (Van Loo *et al.*, 1999). In this research the greatest increase in LAB cell numbers and inhibitory activity was observed in MRS broth supplemented with 1% Raftilose®Synergy1, a combination of inulin and fructo-oligosaccharides. Raftilose®Synergy1 contains a carefully selected DP distribution (DP = Degree of Polymerization=chain length of the molecules). It can therefore be speculated that the polisaccharides and oligosaccharides of Raftilose®Synergy1 are more easily hydrolysed by LAB than the prebiotics Raftiline®GR and Raftilose®L95. The inclusion of 1.0% (w/v) Raftilose®Synergy1 enhanced the growth of the five selected LAB (*in vitro*) and increased inhibitory activity against indicator strains isolated from faeces of patients diagnosed with AIDS. The increased inhibitory activity when LAB are grown in the presence of Raftilose®Synergy1 can be explained by the fact that inulin and oligofructose are fermented by the LAB and that the production of antimicrobial substances such as short-chain fatty acids including acetate, propionate and butyrate is increased (Schley & Field, 2002). This increase in short-chain fatty acids and lactic acid leads to a decrease in the pH of the large intestine and benefits the organism as it constitutes an ideal medium for the growth of the LAB. The resulting increase in populations of LAB can also compete with pathogens for nutrients and receptors on the gut wall and, therefore, limit the growth of pathogens (Schley & Field 2002). The inhibitory effect of probiotic strains combined with prebiotics is also illustrated by the work of Gmeiner *et al.* (2000) who studied the influence of a synbiotic mixture consisting of *Lactobacillus acidophilus* 74-2 and 1% (w/v) powered fructo-oligosaccharide preparation on the microbial ecology sustained in a simulation of the human intestinal microbial ecosystem (SHIME reactor). The results indicate that the growth of *L. acidophilus* 74-2 increased by 1 log-cycle and that the growth of *E. coli* and enterobacteria was inhibited. A substantial increase in short-chain fatty acid production was found which resulted in decreased pH values. Although this study and those reported in the literature were conducted *in vitro*, it is proposed that a similar effect will occur *in vivo* (Roberfroid & Delzenne, 1998).

**Conclusions and recommendations**

It can be concluded that probiotic strains with good inhibitory activity *in vitro* are available in South Africa. For the commercial production of probiotic concentrates the identified LAB can be grown successfully in soymilk-base and be lyophilized for storage. It can also be concluded that the presence of 1.0 % (w/v) Raftilose®Synergy1 increases LAB cell numbers and inhibitory activity *in vitro*, and that a similar effect will occur *in vivo*.

Based on the results of this study, it is recommended that for the purposes of the development of effective probiotic and prebiotic containing products, at least three of the five identified probiotic strains should be included. Furthermore, probiotics should be combined with 1.0% (w/v) Raftilose®Synergy1 to optimalize the inhibitory activity of the probiotics *in vivo*.

## Acknowledgements

The study was funded by the Medical Research Council (MRC) and National Research Foundation (NRF).

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# CHAPTER 6

# GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 1. General discussion

Research has shown that a balanced gastrointestinal ecosystem is resistant to colonization by invading species when in balance within its host and therefore improves immune functions and health (Buddington *et al.*, 2002). Certain factors could cause disturbances of this balanced ecosystem leading to greater sensitivity to pathogens and clinical disorders (Fuller & Gibson, 1997). These disorders can be treated or prevented by dietary supplementation of specific compounds known as probiotics (Fooks *et al.*, 1999; Collins & Gibson, 1999). The survival of probiotic strains in the gastrointestinal tract can be improved by available fermentable subtrates such as oligosaccharides and polisaccharides, known as prebiotics (Gibson & Roberfroid, 1995).

Consumers are becoming more aware of nutrition and health and therefore an expansion of the market in probiotic and prebiotic products in Europe has been witnessed (Temmerman *et al.*, 2002). However, very little information in this regard for the South African market could be traced. Therefore, the first objective of this study was to identify probiotic and prebiotic containing products available on the South African market. A large variety of products were subsequently identified, which included probiotic and/or prebiotic containing supplements (capsules), food items fortified with probiotics and/or prebiotics and fermented foods containing probiotics e.g. dairy products.

As part of the marketing of probiotic and prebiotic containing products, content and health related claims are often made on the labels of the products. For the protection of consumers, manufacturers and marketers of these products should be held accountable for health related claims on products via appropriate legislation. South African legislators have formulated proposed South African regulations within the Foodstuffs, Cosmetics and Disinfectants, Act, 1972 (Act no. 54 of 1972; htpp://www.doh.gov.za, 2003/05/02 accessed) for the labeling of probiotic and/or prebiotic containing products. A further objective of this study therefore was to determine to what extent the content and health claims on the identified products comply with the proposed South African regulations. It was found that only three out of 26 health claims made on the labels complied with the proposed South African regulations indicating that manufacturers might be misinforming the public. However, sound scientific evidence was found for five additional claims not currently included in the proposed South African regulations. Therefore, if South African manufacturers follow the current proposed South African regulations, the consumer will be misinformed because a number of proven health benefits will not be mentioned on the labels of products. To rectify this situation, our research suggests that the proposed South African regulations should be revised to include the following five claims: diarrhea prevention in infants; diarrhea prevention in adults; improvement of digestive health, stool quality and constipation; prevention of Crohn's disease and treatment of food allergies. Assessment of the content related claims showed that probiotic cultures most commonly claimed to be included in supplements and

functional foods in South Africa include *L. acidophilus*, *B. bifidum* and *B. longum*, which complies with the proposed South African regulations. However, two infant foods were found to be fortified with *Bifidobacterium* sp. and *Lactobacillus acidophilus* instead of *B. infantis*, which is specified in the proposed South African regulations for use in infant food aimed at infants younger than one year. The content claims regarding viable cell numbers on the labels of the products varies between 1 x $10^8$ cfu/g and 1.8 x $10^{10}$ cfu/g, which comply with the proposed South African regulations. The prebiotic containing products identified in this study claimed to include fructo-oligosaccharides, Raftilose and chicory, which comply with the proposed South African regulations. However, three products were identified on which the type of prebiotic was not specified, although prebiotic fibre content was claimed, which does not comply with the proposed South African regulations. The prebiotic concentration claimed on the labels varies between 3 g and 15 g per 100 g, which complies with the proposed South African regulations. Only one product that contains 1.7 g/100 g fructo-oligosaccharides does not comply with the proposed South African regulations. However, if the daily portion size consumed from the latter product is increased, it might comply with the proposed South African regulations.

Stability and viability of probiotics strains are often problematic when commercialised. Studies done by Hamilton-Miller *et al.*, 1999; Holzapfel *et al.*, 1998; Reuter, 1997; and Temmerman *et al.*, 2002 on the microbial analyses of probiotic dairy products and supplements, demonstrated that the identity and the number of recovered species do not always correspond to the information stated on the product label. A further objective of this study therefore was to test the claims concerning viable cell numbers on the labels of the products emperically. The viable cell numbers of three out of the five probiotic supplements that were tested were not in line with the viable cell numbers stated on the labels of the products. Despite the fact that the actual viable cell numbers in these supplements were lower than the viable cell numbers stated on the labels, all the probiotic strains showed good inhibitory activity against the indicator strains isolated from faeces of patients diagnosed with AIDS.

It is of concern that quite a number of products on the South African market only claim AB-culture content or prebiotic fibre content without specifying probiotic species, viable cell numbers, prebiotic type and concentration. This situation indicates that the proposed South African regulations are not yet being enforced and that it is not possible for the consumer to make a well-informed decision about the use of these products. It is of vital importance that this situation be rectified.

Low consumer awareness and knowledge of probiotics and prebiotics might restrain the growth of the probiotic and prebiotic market (Menrad, 2003). No information regarding the awareness of the South African consumer of these products in South Africa could be traced. Therefore, a further objective of this study was to conduct an exploratory market survey to determine the awareness of the South African consumer regarding probiotics and prebiotics. The results indicate that the awareness of these consumers

of such products is probably low. However, the awareness of AB-cultures seems to be higher than the awareness of probiotics, which might be explained by the fact that most probiotic containing yoghurt product labels claim to contain AB-cultures, but no reference is made to probiotic cultures. Prebiotics also does not seem to be a well-known term in South Africa. This could be linked to the fact that prebiotic products mostly state fructo-oligosaccharide or inulin content but do not refer to the generic term, prebiotics. The consumers who were aware of probiotics, mostly seemed to have knowledge about the health benefits thereof and their main sources of information were the labels on the products, magazines and their doctor. The few respondents who were aware of prebiotics, did not seem to have any knowledge about the health benefits thereof. In this study it was found that females, students and younger people are more aware of probiotics (including AB-cultures) and prebiotics. It is speculated that the higher awareness of females is because women are usually responsible for food shopping and are therefore more exposed to health food products (Wellner, 2002). They are also more inclined to read health magazines than men (Cant *et al.*, 2002). According to Schiffman and Kanuk (1997) working people have a higher income and higher level of education than the non-working group and might be more informed about health and nutrition. Therefore, the higher awareness of students regarding probiotic and prebiotic containing products is not in line with what was expected, as they are not included in the working group. This finding can possibly be linked to the fact that students might be exposed to relevant information as a result of their studies or through close interaction with other students on campus. It was also found that the younger age groups were more aware of probiotics and prebiotics, with the exception of AB-cultures. These results are also contradictory to expectations as it has been reported that consumers older than 50 years are more focused on their health and thus more inclined to buy healthy and functional foods (Sanders, 1998). It can therefore be speculated that South African consumers in this age group are not yet that well informed about the health benefits of probiotics and prebiotics.

Not many viable probiotic starter cultures are produced in South Africa (Personal communication, 2002, Prof. L.M.T. Dicks, Department of Microbiology, University of Stellenbosch). Therefore, the next three objectives of this study were formulated to identify viable probiotic strains, an appropriate growth medium and determine the effect of lyophilization for future commercialisation. For these purposes a panel of twelve potential probiotic strains was screened for potential inhibitory activity against porcine pathogens and strains from the LMG-panel. Based on the results five lactic acid bacteria (LAB) with the best inhibitory activity were selected and screened against a panel of ten indicator strains isolated from faeces of patients diagnosed with AIDS. The five strains showed inhibitory activity against all the indicator strains. Inhibitory activity involves the lactic acid bacterial production of a range of antimicrobial compounds or variations of the same type antimicrobial compound including lactic acid and acetic acid, antimicrobial peptides, such as bacteriocins and/or low-molecular-mass antimicrobial compounds (Saarela *et al.*, 2000).

For commercial production of probiotic concentrates the probiotic strains need to be stabilised in growth-supporting media to ensure metabolic activity. Soymilk has been suggested as a growth-medium because it contains fermentable sugars and several microbial growth-stimulating compounds and is relatively inexpensive (Kamaly, 1997; Mattilla-Sandholm *et al.*, 2002). Soymilk-base was therefore tested as a potential growth medium for the five LAB and it was found that the LAB showed good growth in this medium. This indicates that the soybean-oligosaccharides, raffinose and stachyose present in the soymilk-base, could act as sufficient nutrition for LAB growth and fermentation. These results show that soymilk-base could be used successfully as growth medium for LAB without supplementation with prebiotics.

Probiotic strains are either freeze-dried or spray dried when commercially produced (Saarela *et al.*, 2000). Lyophilization (freeze-drying) is the most popular method and therefore the effect of lyophilization on the inhibitory activity of LAB was also determined. The five LAB were lyophilized after growth in soymilk-base and it was found that this procedure decreased the LAB cell numbers by two log-cycles. However, lyophilized cells grown in MRS broth (Biolab) yielded a spectrum and level of inhibitory activity against indicator strains isolated from patients diagnosed with AIDS in the same order as cells that have not been lyophilized.

Finally, it has been shown that the viability of probiotic strains *in vivo* is enhanced by the presence of prebiotics. Therefore a further objective of this study was to test the growth of the five LAB in the presence of prebiotics (*in vitro*). The effect of three prebiotics namely Raftiline®GR (inulin), Raftilose®L95 (oligofructose) and Raftilose®Synergy1 (a combination of inulin and oligofructose) was investigated. The greatest increase in LAB cell numbers and inhibitory activity was observed with 1.0 % (w/v) Raftilose®Synergy1, which contains a carefully selected DP distribution (DP = Degree of Polymerization=chain length of the molecules). It is speculated that the polisaccharides and oligosaccharides of Raftilose®Synergy1 are more easily hydrolysed by probiotic strains than the compounds in the prebiotics, Raftiline®GR and Raftilose®L95. Further tests showed that the combination of the five LAB and 1.0% (w/v) Raftilose®Synergy1 enhances the inhibitory activity of these strains against the panel of indicator strains isolated from the faeces of patients diagnosed with AIDS. These results can be explained by the increased production of antimicrobial compounds such as short-chain fatty acids including acetate, propionate and butyrate due to the LAB fermentation of inulin and oligofructose (Schley & Field, 2002).

Based on the results of the last four objectives, it can be recommended that for the purposes of the development of effective probiotic and prebiotic containing products, at least three of the five identified

probiotic strains should be included and that the probiotics should be combined with 1.0% (w/v) Raftilose®Synergy1 to optimalize the inhibitory activity of the probiotics *in vivo*.

## 2. General conclusions and recommendations

From the results of this research it can be concluded that a variety of probiotic and prebiotic containing products are available on the South African market. However, it seems that manufacturers of these products are misleading consumers with a number of health claims that appear on the labels of some products that are not scientifically sound. On the other hand, the proposed South African regulations clearly do not include all the scientifically sound health claims. Content claims regarding probiotic strain, viable cell numbers and prebiotic type and concentration were mostly in line with the proposed South African regulations. It can also be concluded that the awareness of South African consumers regarding probiotics and prebiotics is low. The existing market of these products include females, students and younger people whereas the potential target market includes men, working people and older people. A further conclusion is that viable probiotic strains for commercialization in South Africa are available. Five viable probiotic strains that show good inhibitory activity, can be grown in soymilk-base to produce probiotic concentrates and are not negatively influenced by lyophilization, were identified. Finally, it is suggested that the addition of the prebiotic, Raftilose®Synergy1, to products containing these probiotics will enhance the viability thereof *in vivo*.

It is therefore recommended that:

- The proposed South African regulations should be revised in order to ensure that the consumer is provided with correct information on the labels of the products containing probiotics and prebiotics.
- Manufacturers of probiotic and prebiotic containing products should be held responsible to provide the consumer with scientifically sound and legally correct information.
- Consumer awareness of probiotics and prebiotics should be increased using effective, focussed marketing strategies to ensure continued growth of the probiotic and prebiotic market.
- South African manufacturers of probiotic and prebiotic containing products should consider using probiotic strains isolated in South Africa, such as the identified five LAB.
- South African manufacturers of probiotic containing products should also consider the addition of prebiotics to these products to enhance the effectiveness thereof *in vivo*.

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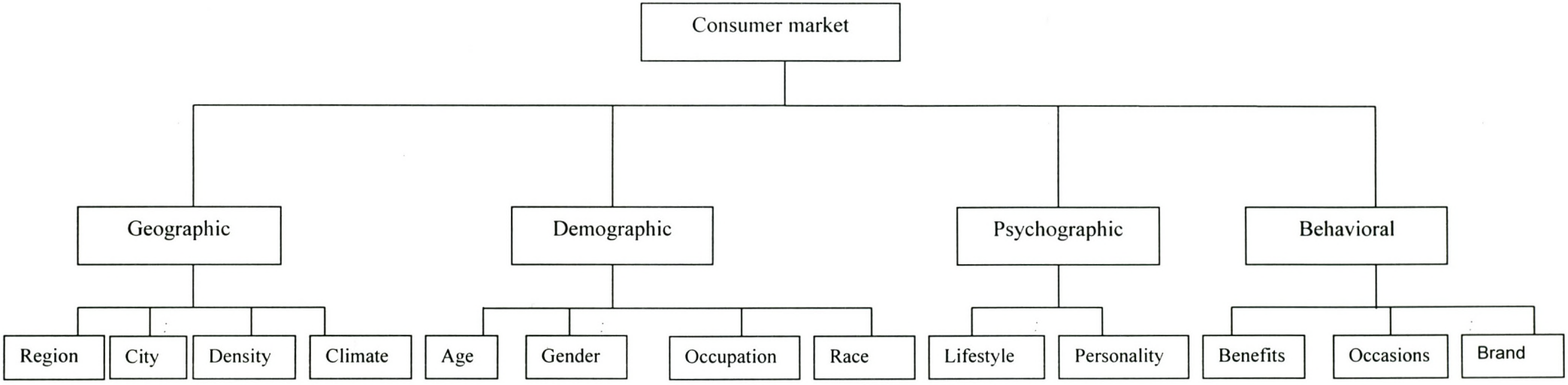
# ADDENDUM 1

**Dendogram: Major segmentation variables for consumer markets**

## Addendum 1

**Fig.1:** Dendogram: Major segmentation variables for consumer markets



# ADDENDUM 2

**Market survey of probiotic and prebiotic supplemented food-items/ supplements**

## Addendum 2

### Market survey of probiotic-and prebiotic supplemented food-items/ supplements

1. Have you ever heard about probiotics?

| | |
|---|---|
| No | 1 |
| Yes | 2 |

1.1 Can you think of any foods/products in which we find probiotics?

| Foods/Products | **No** | **Yes** |
|---|---|---|
| AB cultures in yogurt | 1 | 2 |
| Interflora | 1 | 2 |
| Other: ..... | 1 | 2 |

1.2 Have you ever consumed any of these products specifically to increase your intake of probiotics?

| | |
|---|---|
| No | 1 |
| Yes | 2 |

1.3 If yes, which foods/products?

.....

1.4 In your opinion, why do you think are probiotics necessary for you?

.....

1.5 What is/was your main source of information about probiotics?

.....

2. Have you ever heard about live AB-cultures?

| | |
|---|---|
| No | 1 |
| Yes | 2 |

2.1 Can you think of any foods/products where we find live AB-cultures?

.....

2.2 Have you ever consumed foods/products containing AB-cultures specifically to increase your intake of these cultures?

| | |
|---|---|
| No | 1 |
| Yes | 2 |

2.3 If yes, which foods/products?

.....

2.4 In your opinion, why do you think are AB-cultures necessary for you?

.....

2.5 What is/was your main source of information about AB-cultures?

.....

3. Have you ever heard about prebiotics?

| | |
|---|---|
| No | 1 |
| Yes | 2 |

3.1 Can you think of any foods/products where we find prebiotics?

.....

3.2 Have you ever consumed any of these products specifically to increase your intake of prebiotics?

| | |
|---|---|
| No | 1 |
| Yes | 2 |

3.3 Which product(s)? .....

3.4 In your opinion, why are prebiotics necessary for you?

.....

3.5 What is/was your main source of information about prebiotics?

.....

4. In which of the following age categories do you fall?

| 10-19 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70+ |
|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |

5. With which population group do you associate yourself the most?

| Coloured | African | White | Indian | Other |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

6. What is your current occupation?

| | |
|---|---|
| Professional | 1 |
| Non-professional/Administrative | 2 |
| Labourer | 3 |
| Unemployed | 4 |
| Retired/Pensioner | 5 |
| Housewife | 6 |
| Student | 7 |
| Other: ..... | 8 |

*Thank you for your cooperation*

# ADDENDUM 3

## Time table for market survey

## Addendum 3

**TABLE 1:** Time table for market survey in week one

| Outlet | Mon | Tue | Wed | Thur | Fri | Sat |
|---|---|---|---|---|---|---|
| Neelsie Pharmacy | | 9:00 – 9:30 | 12:00 - 12:30 | | | |
| Boord Pharmacy | 9:00 – 9:30 | | | | | 10:30 – 11:00 |
| Clicks | 10:00 – 10:30 | | | | 12:00 – 12:30 | |
| Spar | | | | 12:00 – 12:30 | 9:00 – 9:30 | |
| Pick 'n Pay | | | 9:00 – 9:30 | | 13:00 – 13:30 | |
| Checkers | 12:00 – 12:30 | 10:00 – 10:30 | | | | |

**TABLE 2:** Time table for market survey in week two

| Outlet | Mon | Tue | Wed | Thur | Fri | Sat |
|---|---|---|---|---|---|---|
| Neelsie Pharmacy | 12:00 – 12:30 | | | 9:00 – 9:30 | | |
| Boord Pharmacy | | | 12:00 – 12:30 | | 9:00 – 9:30 | |
| Clicks | 13:00 – 13:30 | | 10:00 – 10:30 | | | |
| Spar | | 9:00 – 9:30 | | | 12:30 – 13:00 | |
| Pick 'n Pay | 9:00 – 9:30 | | | 12:30 – 13:00 | | |
| Checkers | | 12:30 - 13:00 | 9:00 – 9:30 | | | |