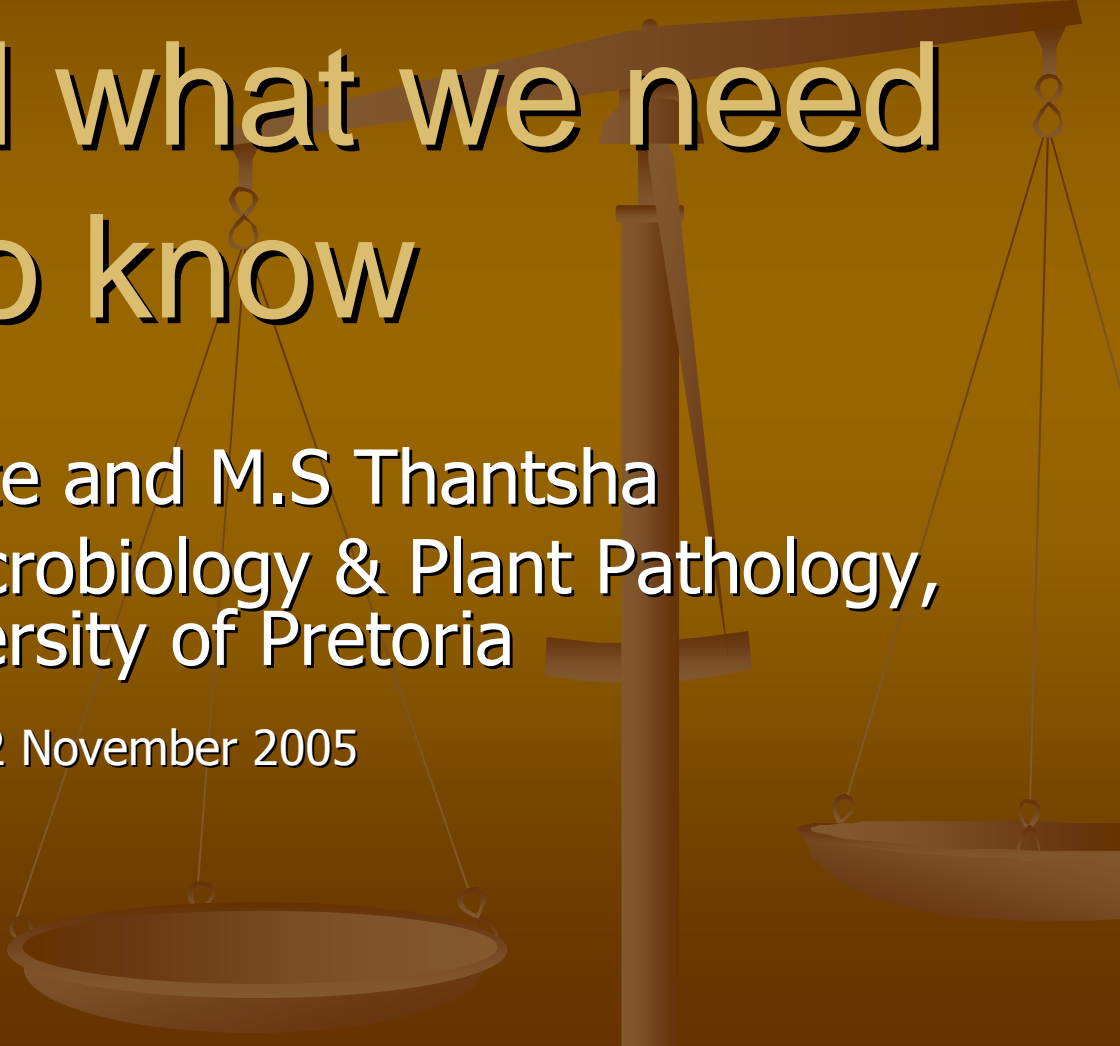
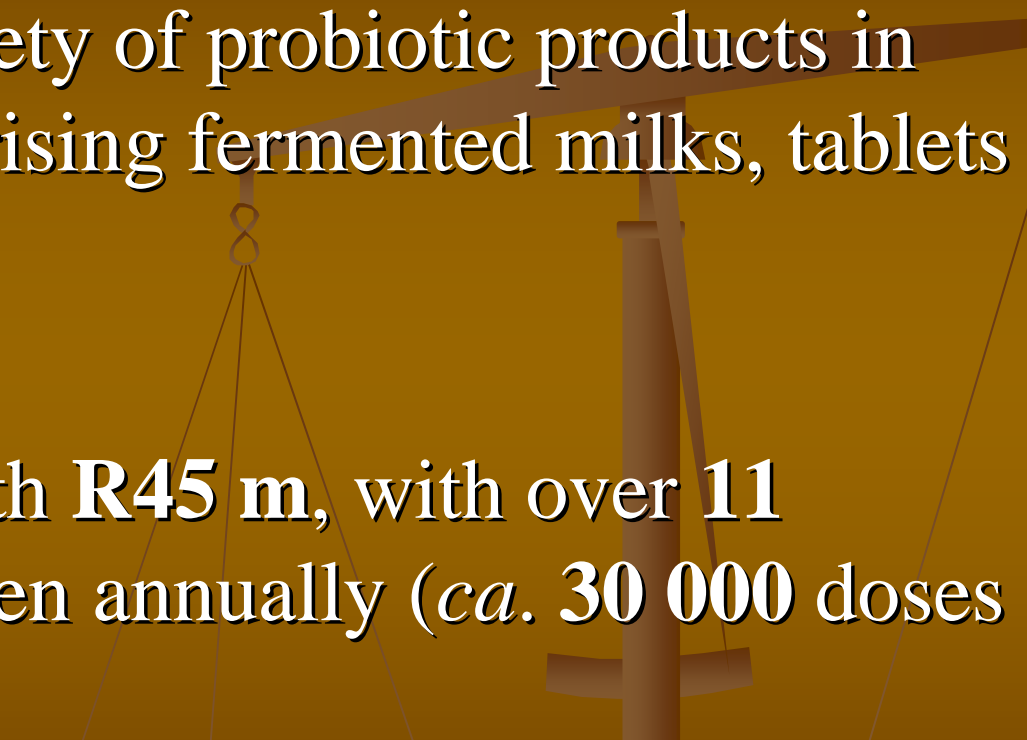


Probiotics: What we know and what we need to know



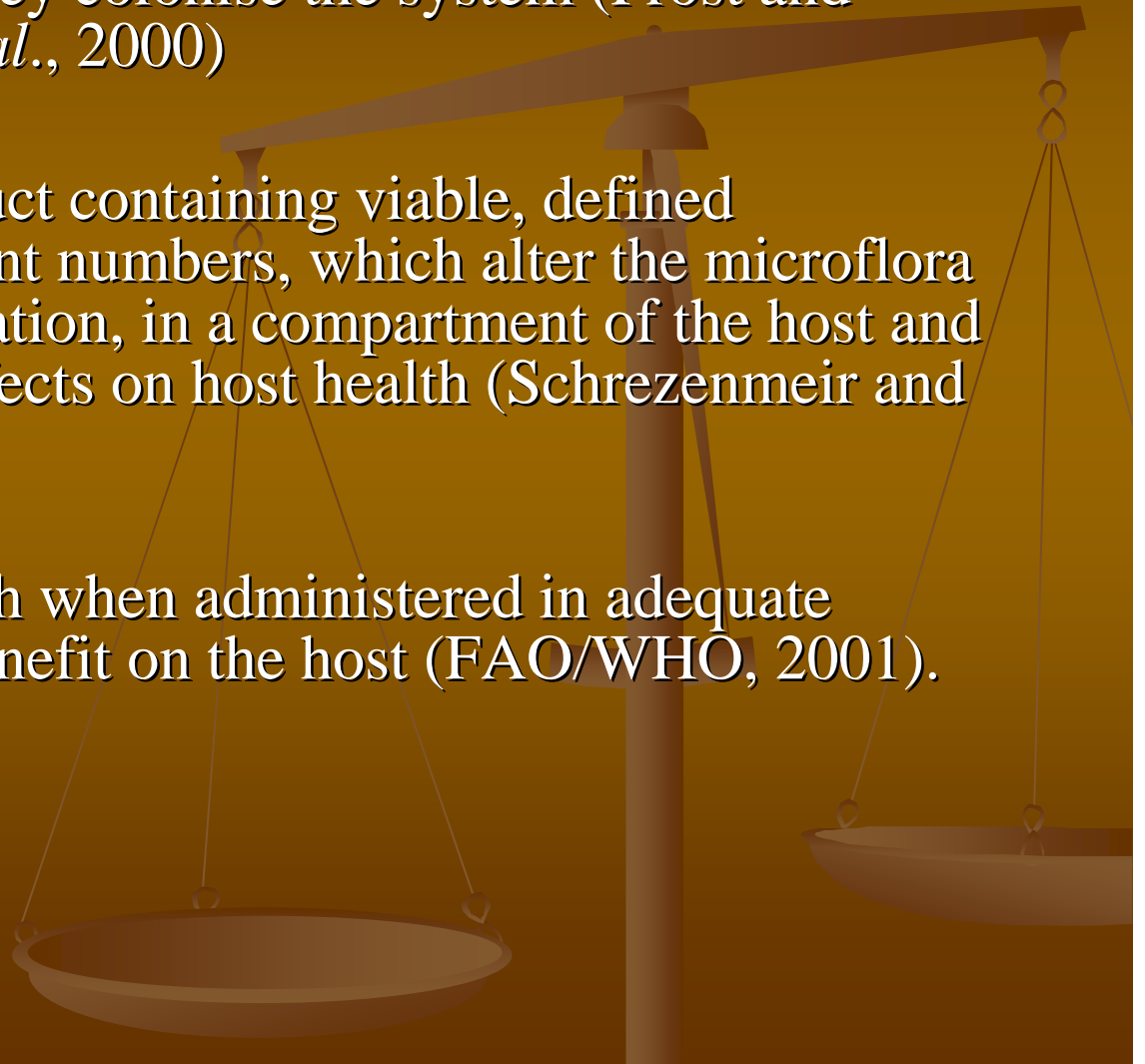
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University of Pretoria

2 November 2005

- 
- SA has large variety of probiotic products in the market comprising fermented milks, tablets and capsules.
 - SA industry worth **R45 m**, with over **11 million** doses taken annually (*ca.* **30 000** doses are daily).

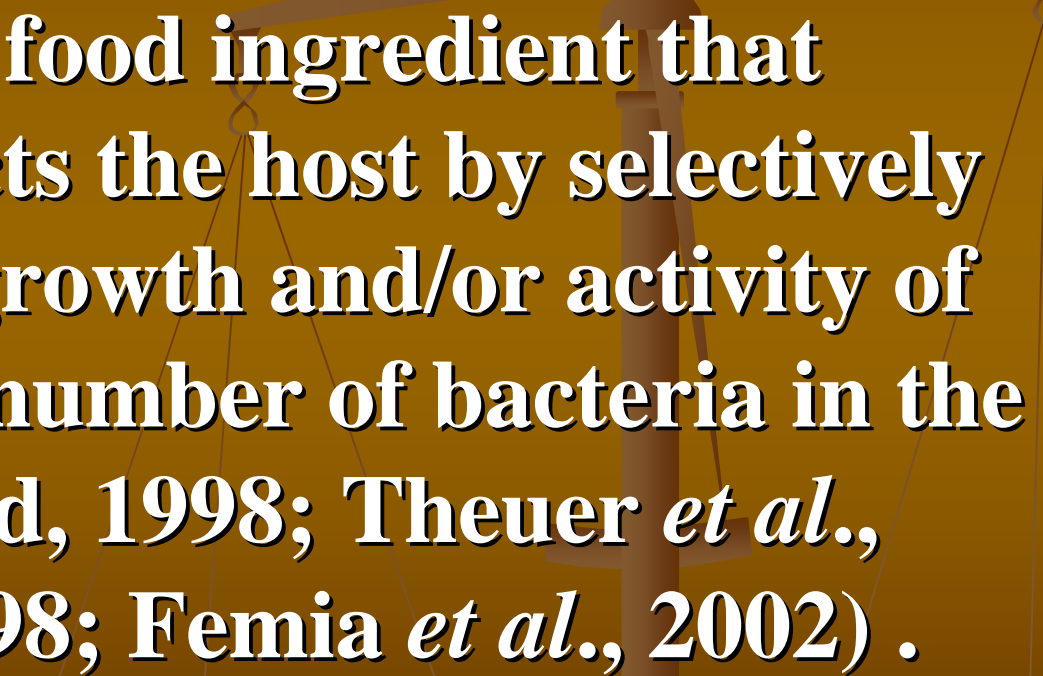
Probiotics

- Live microbial cultures fed by mouth and surviving transit through the large intestine where they colonise the system (Frost and Sullivan, 2000; Saarela *et al.*, 2000)
- A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora by implantation or colonization, in a compartment of the host and by that, exert beneficial effects on host health (Schrezenmeir and de Vrese, 2001)
- Live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001).



Prebiotics

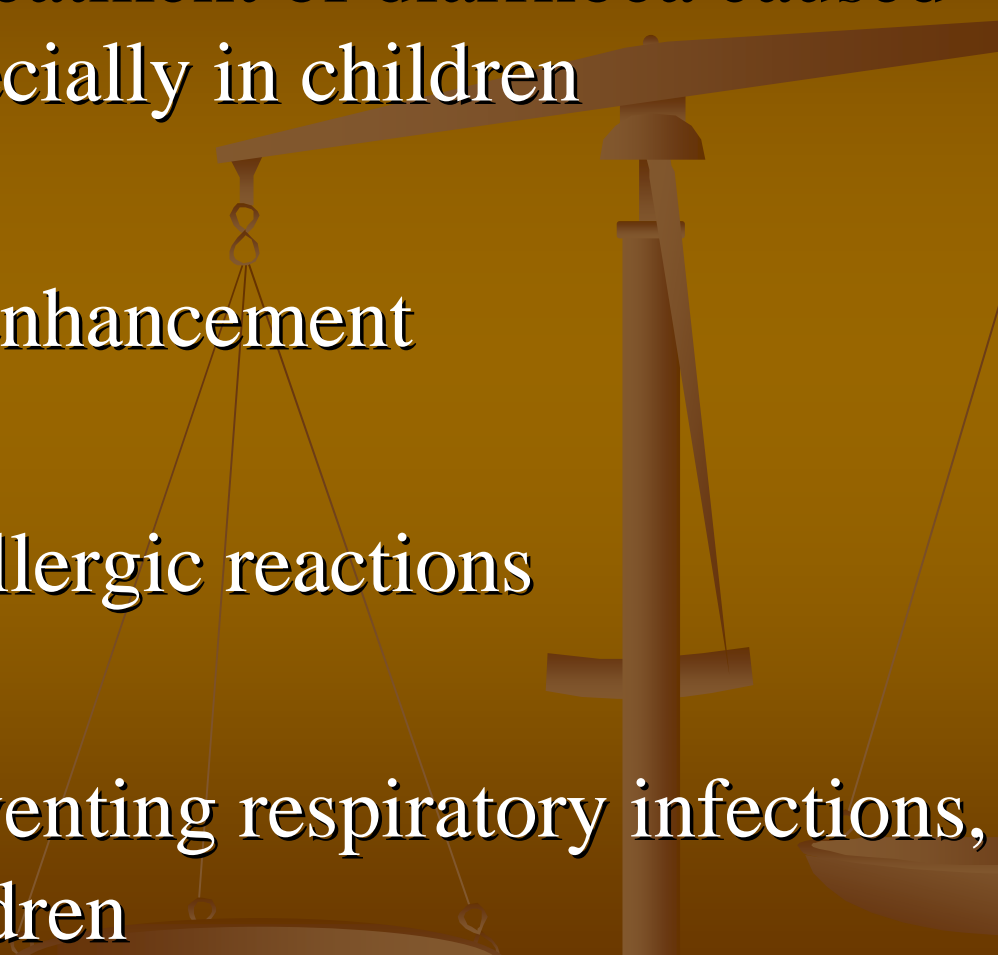
A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Roberfroid, 1998; Theuer *et al.*, 1998; Young, 1998; Femia *et al.*, 2002) .



Most commonly used organisms

- The first and largest group of microorganisms to be regarded as probiotics belonged to the *Lactobacillus* genus.
- Other lactic acid bacteria include the following :
 - **Bacteria:** *Bifidobacteria*, *Enterococci*, *Leuconostoc*, *Pediococci* and *Streptococci*
 - **Yeasts:** *Saccharomyces cerevisiae*, *Saccharomyces boulardii*,
 - **Filamentous fungi:** *Aspergillus oryzae*

Potential benefits

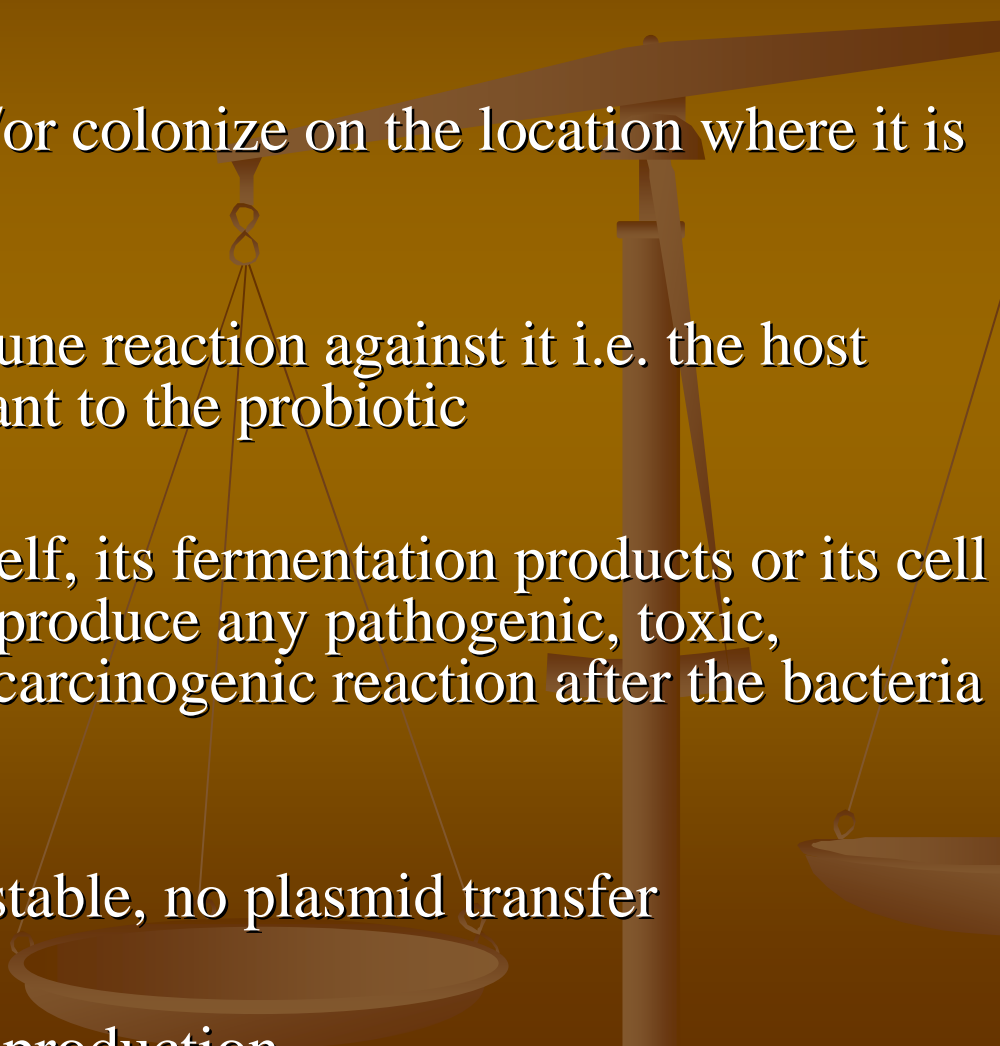
- Prevention and treatment of diarrhoea caused by rotavirus, especially in children
 - Immune system enhancement
 - Reducing some allergic reactions
 - Treating and preventing respiratory infections, especially in children
- 

Potential benefits



- Decreased faecal mutagenicity
- Decrease in the level of pathogenic bacteria
- Decreased faecal bacterial enzyme activity
- Prevention of the recurrence of superficial bladder cancer
- The restoration of the correct balance of natural microflora after stress, antibiotic treatment, alcohol use and chemotherapy

Typical characteristics of a probiotic strain

- It must survive in environmental conditions on the location where it must be active.
 - It must proliferate and/or colonize on the location where it is active.
 - There must be no immune reaction against it i.e. the host must be immuno-tolerant to the probiotic
 - The probiotic strain itself, its fermentation products or its cell components must not produce any pathogenic, toxic, allergic, mutagenic or carcinogenic reaction after the bacteria die off.
 - It must be genetically stable, no plasmid transfer
 - Easy and reproducible production
- 

Survey on South African products

- Lourens-Hattingh and Viljoen (2002) evaluated probiotic bioyoghurts. Levels starter cultures and of *L. acidophilus*, were high and complied with the criterion of 10^6 cfuml⁻¹.

However, the initial counts of *Bifidobacterium bifidum* in all the products tested were lower than 10^6 cfuml⁻¹. This suggested that either the initial inoculum of these organisms was low or that they did not attain the required levels during manufacture.

Survey on South African products

- A study by Elliot and Teversham (2003) indicated that three of nine products tested contained bacteria indicated on the labels, and that only five of the products contained sufficient numbers of organisms to have a probiotic potential.

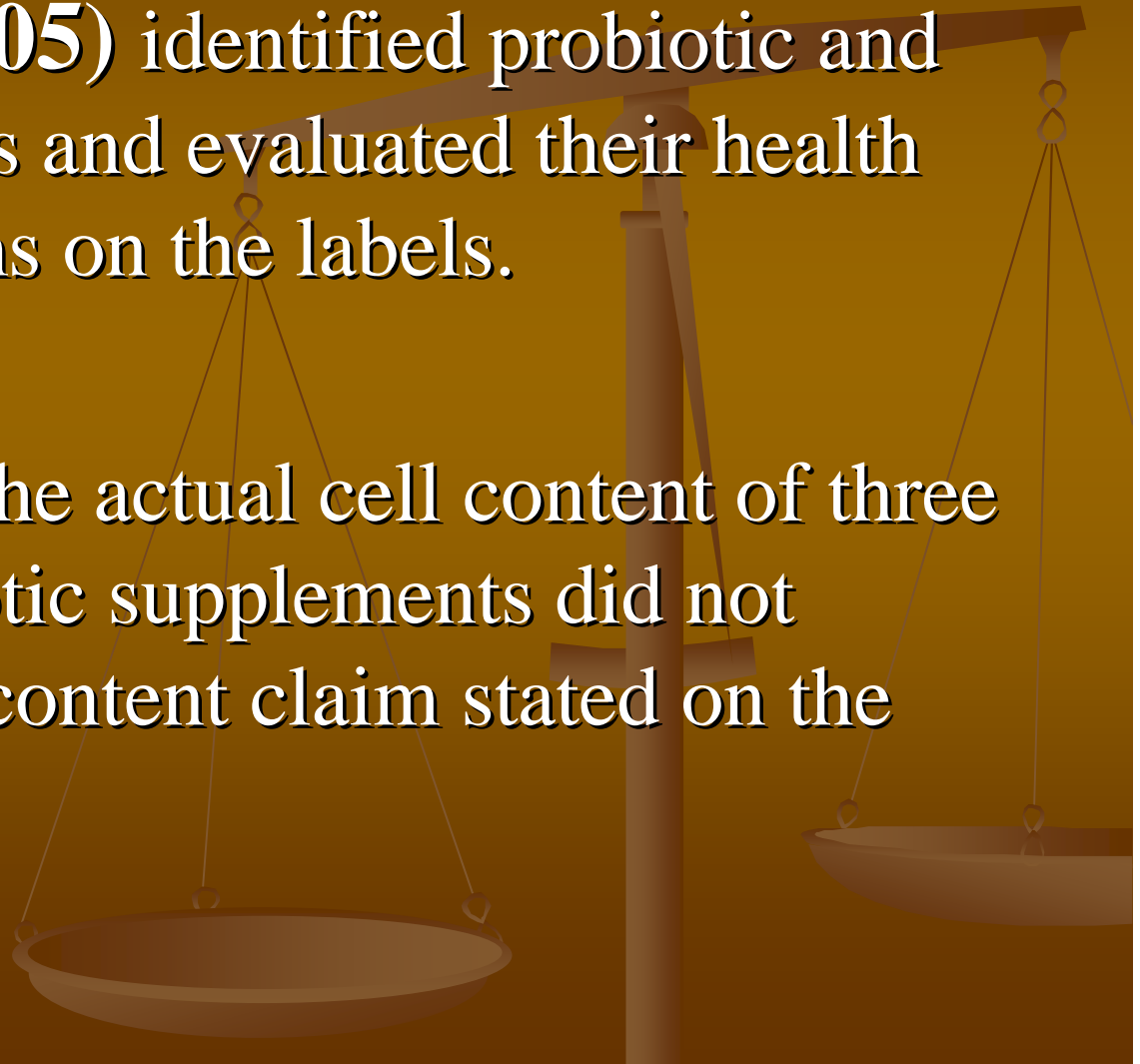


Survey on South African products

- Theunissen and Witthuhn (2004) analysed the probiotic content of 20 different probiotic products using PCR-DGGE. 55% of the tested probiotic yoghurts contained all microbes as indicated on their labels.
- No bifidobacterium was detected in 45% of the yoghurt products that claimed their presence on the product label.
- Apart from showing a poor correlation between the product label and the probiotic content, this study also showed that there is still a problem of viability of probiotic cultures in the food products.

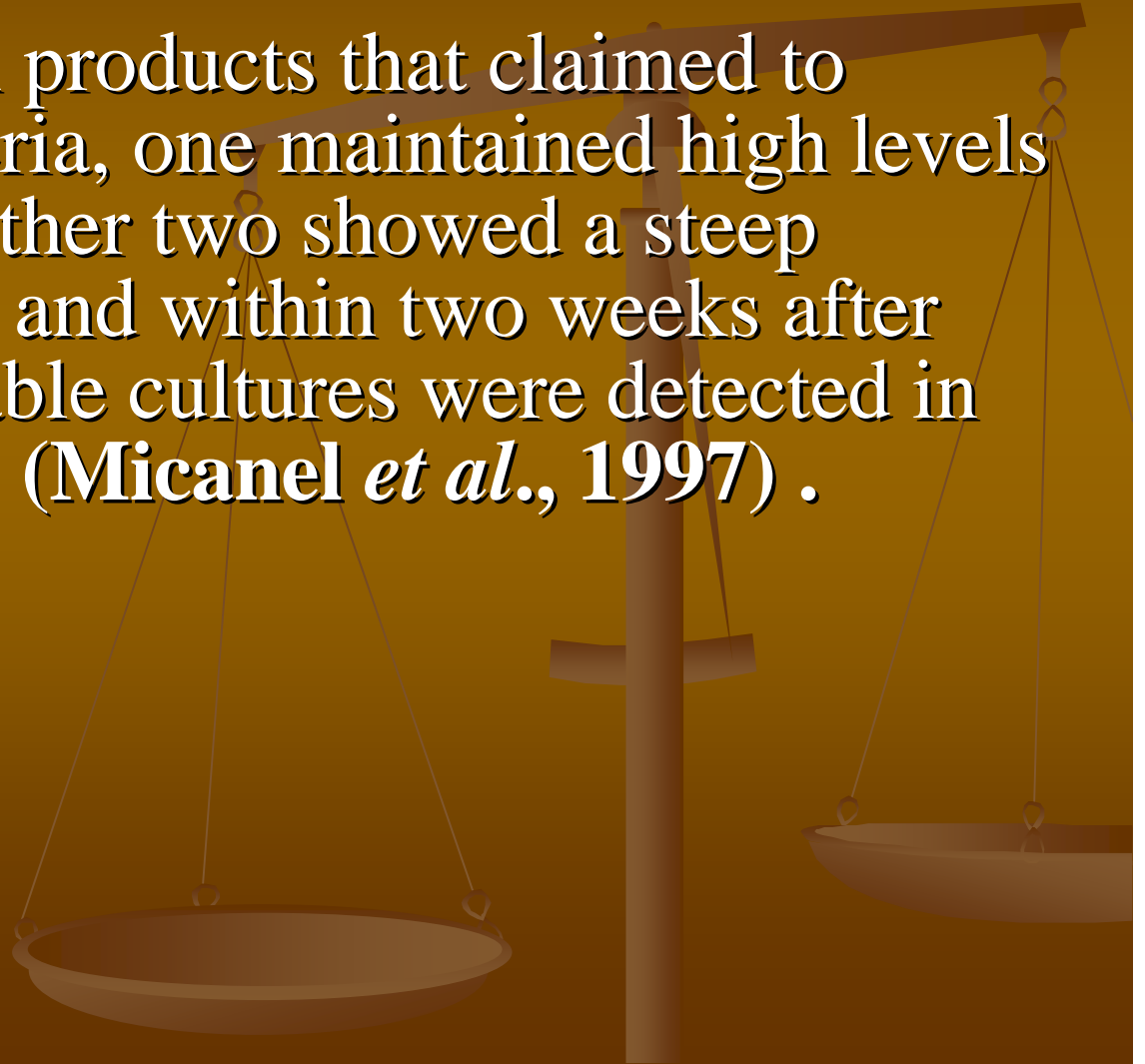
Survey on South African products

- Brink *et al.* (2005) identified probiotic and prebiotic products and evaluated their health and content claims on the labels.
- They found that the actual cell content of three out of five probiotic supplements did not comply with the content claim stated on the label



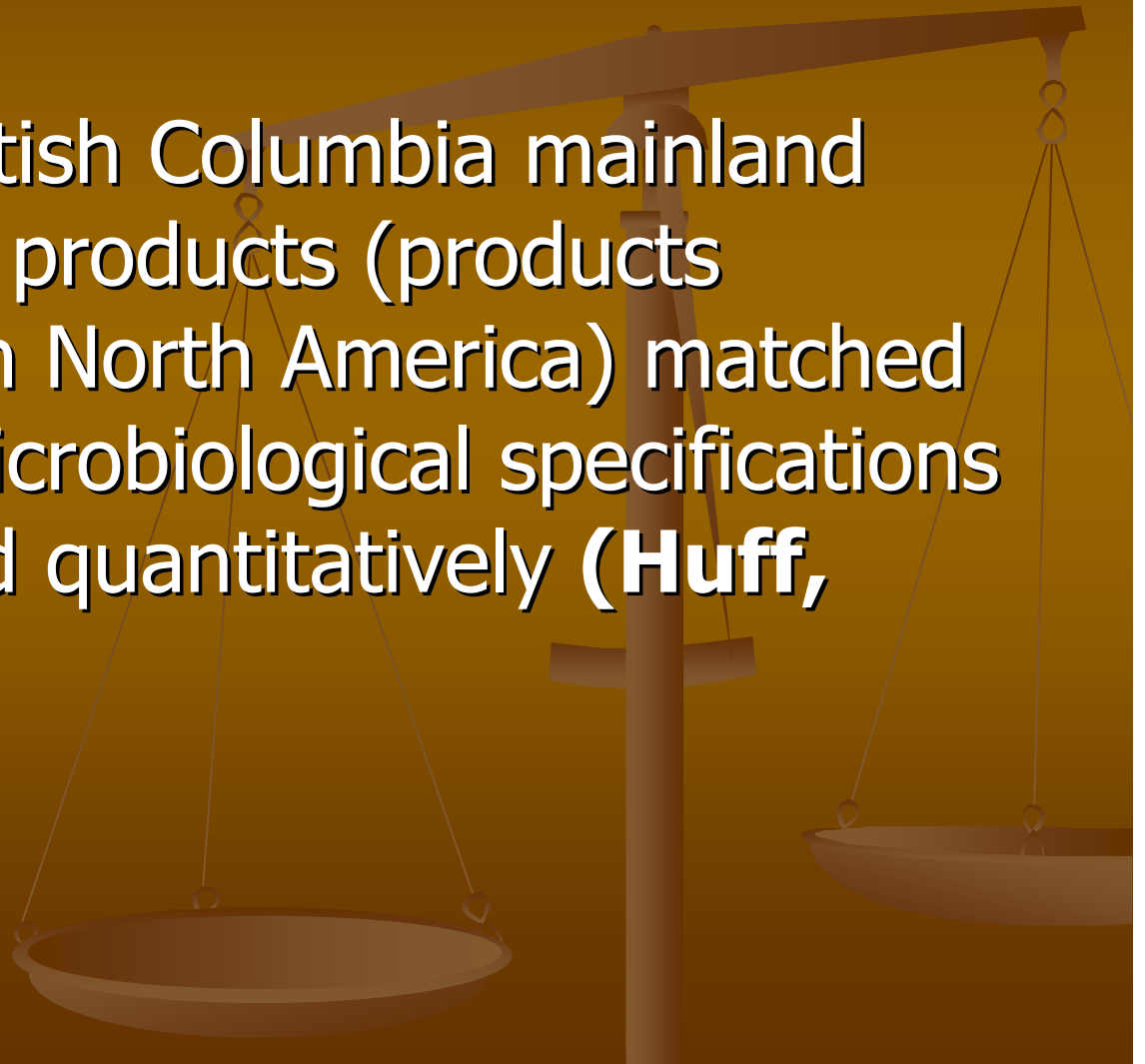
Australia

- Of three Australian products that claimed to contain bifidobacteria, one maintained high levels of $>10^6$ cfu/g, the other two showed a steep decline in numbers and within two weeks after manufacture no viable cultures were detected in one of the products (Micanel *et al.*, 1997) .



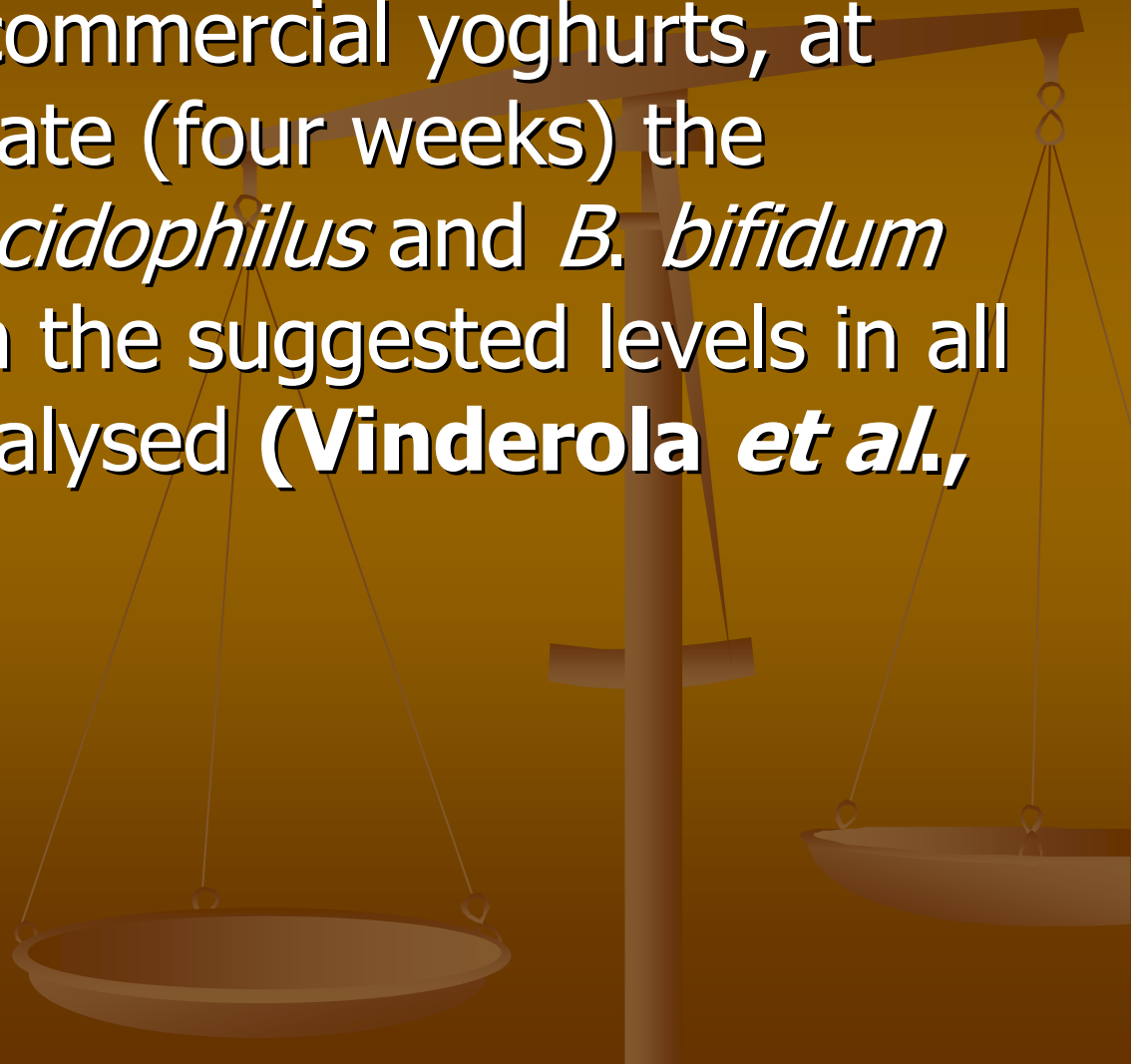
British Columbia

- In the lower British Columbia mainland none of the ten products (products manufactured in North America) matched their labelled microbiological specifications qualitatively and quantitatively (**Huff, 2004**).



Argentina

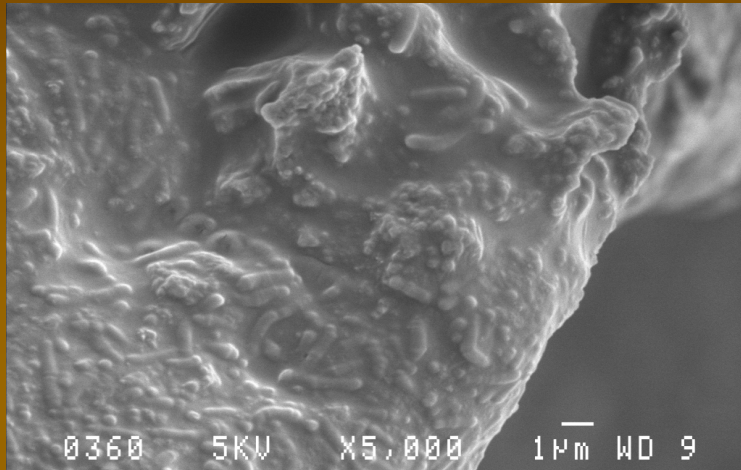
- In Argentinian commercial yoghurts, at the expiration date (four weeks) the contents of *L. acidophilus* and *B. bifidum* were lower than the suggested levels in all the products analysed (**Vinderola *et al.*, 2000**).



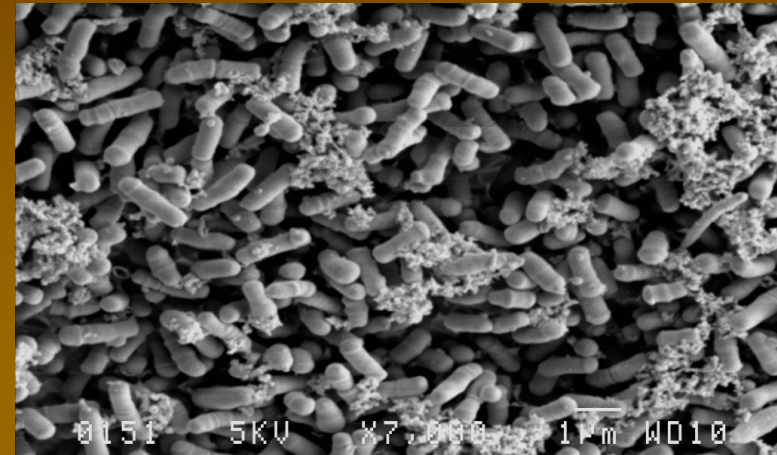
Immobilization of probiotics

- Microencapsulation: technology for packing solids, liquids or gaseous materials in miniature sealed capsules, which can release their contents at controlled rates under specific conditions.
- Microencapsulation to increase shelf life and survival of probiotics in gastrointestinal tract had been tried by various researchers (Doleyres and Lacroix, 2005).

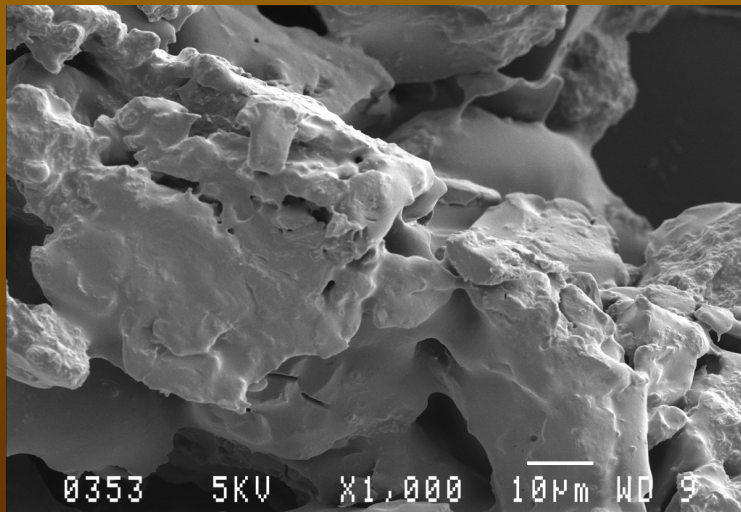
SEM pictures of encapsulated probiotics



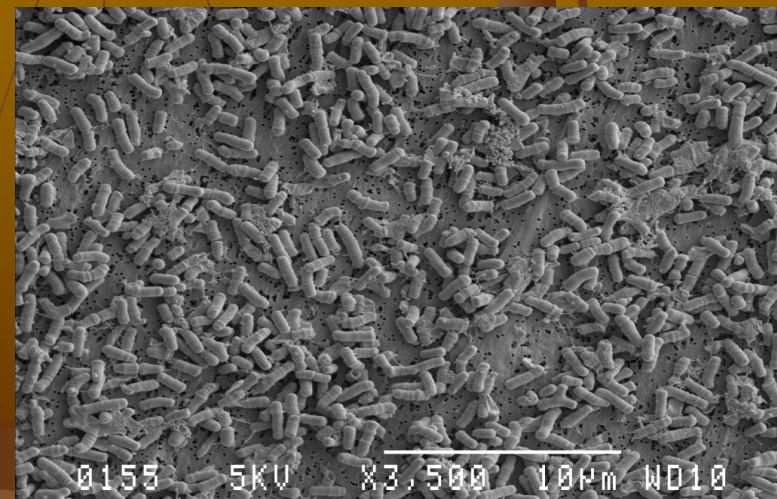
Non encapsulated bacteria powder



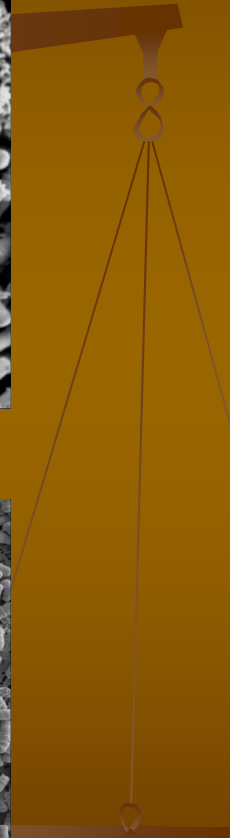
Suspended nonencapsulated bacteria



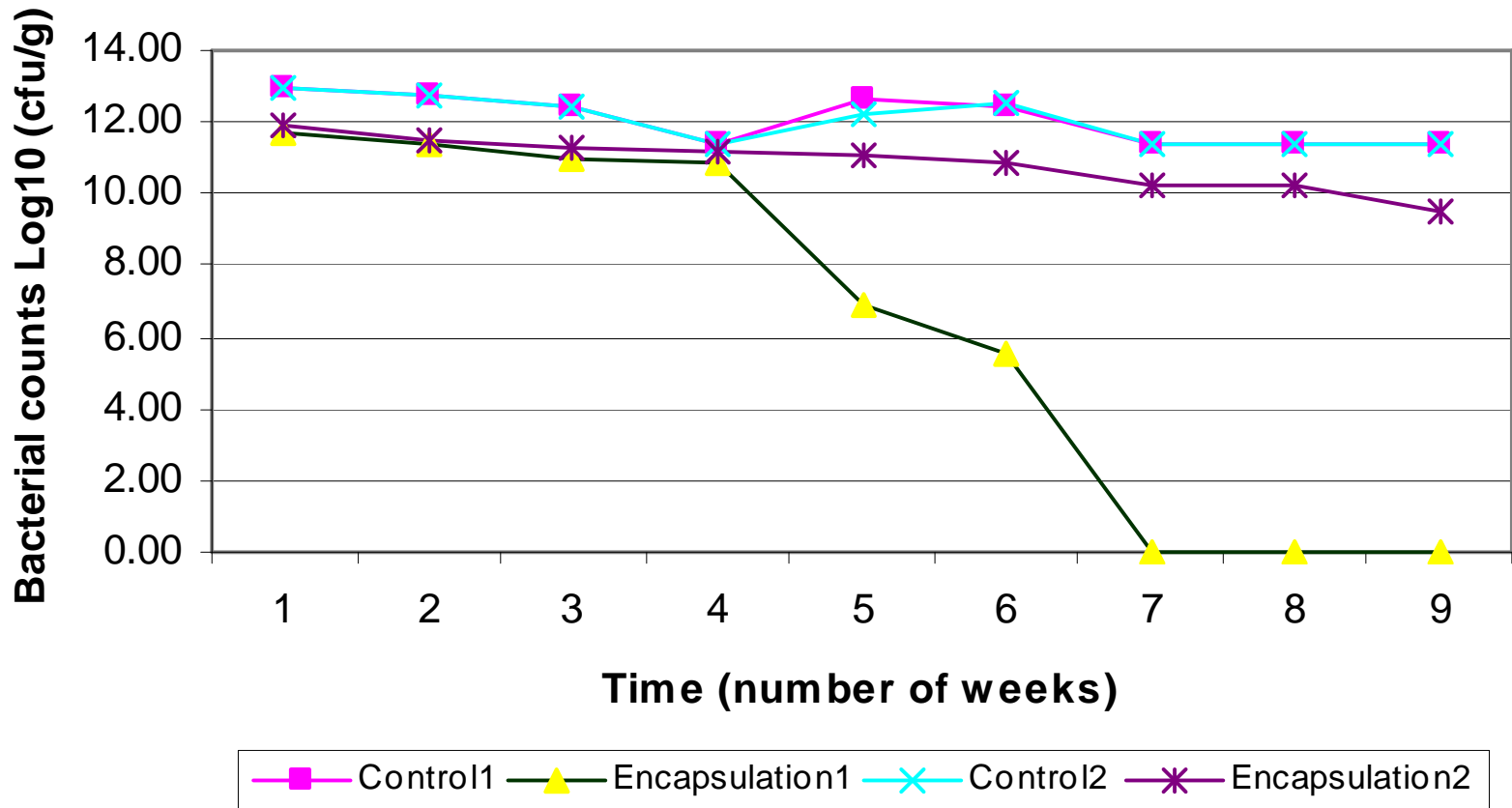
Encapsulated bacteria powder



Suspended encapsulated bacteria

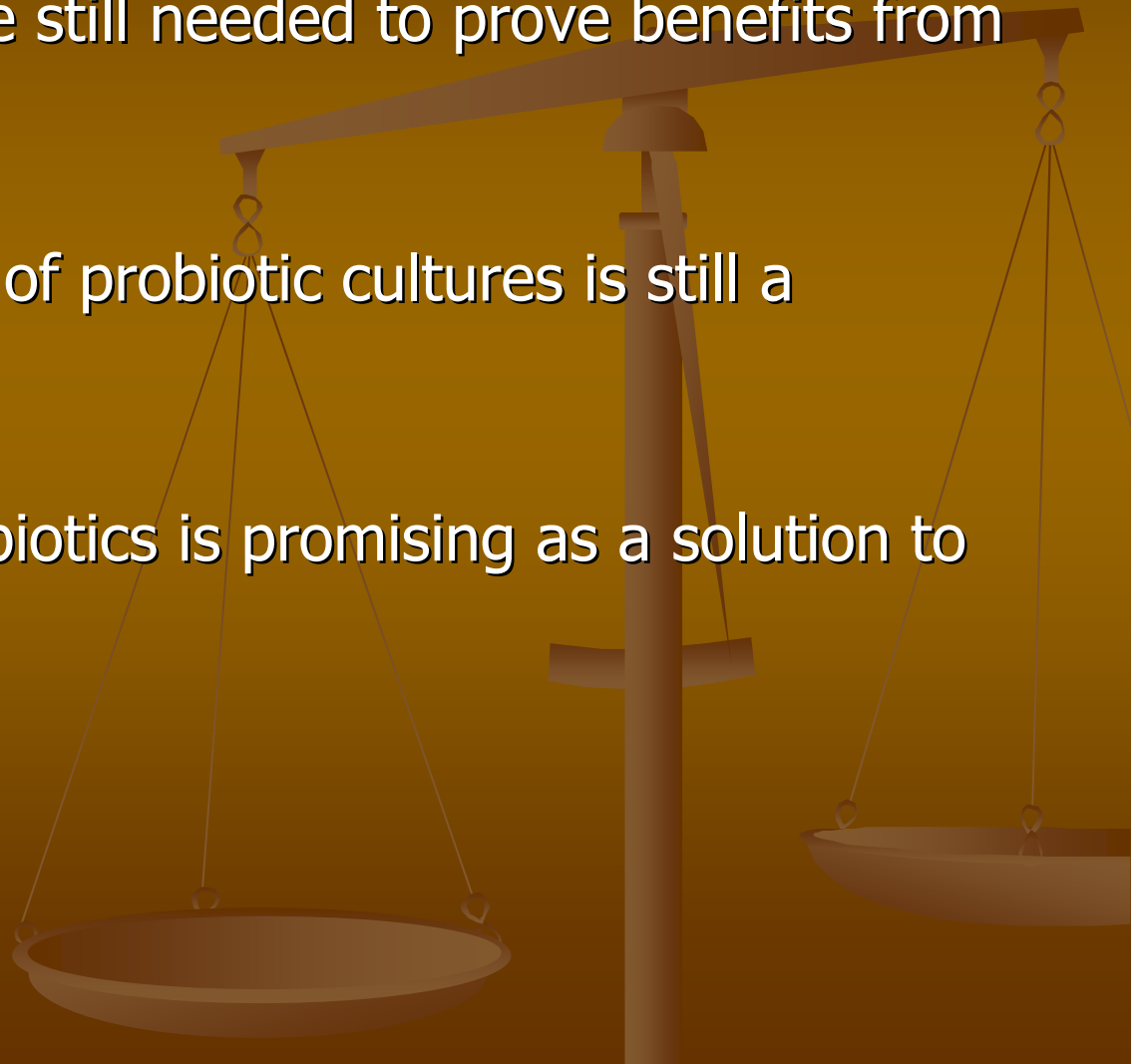


Shelf life of encapsulated probiotics



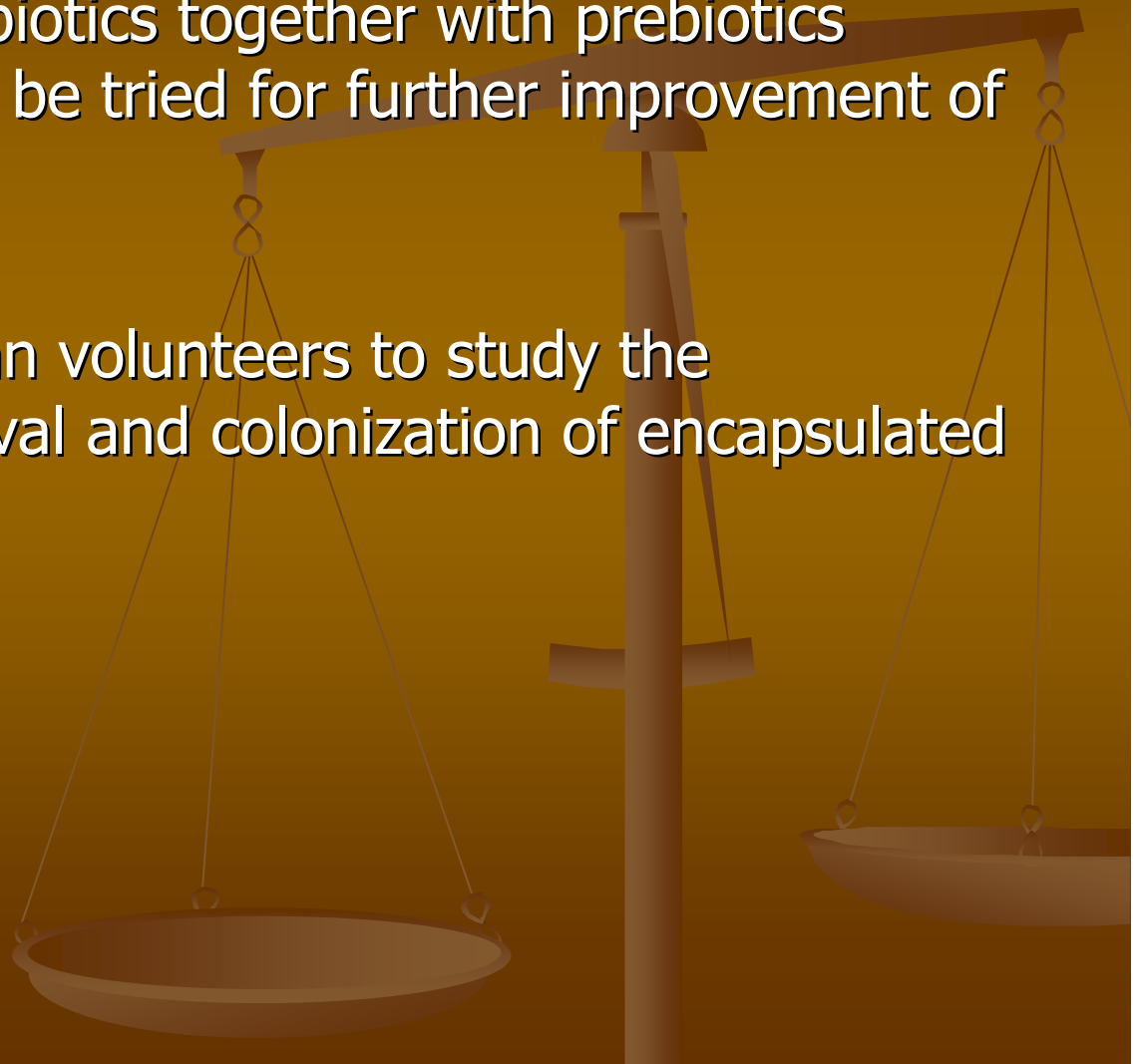
Conclusions

- More clinical trials are still needed to prove benefits from probiotics.
- Stability and survival of probiotic cultures is still a worldwide problem.
- Encapsulation of probiotics is promising as a solution to probiotics' instability.



Pre and probiotics- The future

- Encapsulation of probiotics together with prebiotics within the matrix will be tried for further improvement of shelf life.
- Clinical trials in human volunteers to study the gastrointestinal survival and colonization of encapsulated probiotics (in vivo).



Proven probiotic benefits

Probiotic culture	Clinical use	References
<i>B. bifidum</i> , <i>B. infantis</i>	Immune stimulation	Podoprigora <i>et al.</i> , 1999, Tejada-Simon <i>et al.</i> , 1999, Rowland, 1999
<i>B. longum</i> , <i>B. bifidum</i> BGN4	Cancer	Reddy and Rivenson, 1999, Rowland <i>et al.</i> , 1998, Singh <i>et al.</i> , 1998, Reddy, 1999, You <i>et al.</i> , 2004
<i>B. longum</i> with <i>L. acidophilus</i>	Gastrointestinal discomfort	Colombel <i>et al.</i> , 1987, Orrhage <i>et al.</i> , 1994
<i>B. longum</i>	Lactose intolerance	Jiang <i>et al.</i> , 1996, Rowland, 1999, Golian and Pavelka, 2001, Ouwenhand, 2002, Fooks and Gibson, 2002, Sullivan and Nord, 2005
<i>Lactobacillus GG</i> , <i>B. bifidum</i> with <i>S. thermophilus</i> , <i>B. breve</i> , <i>B. lactis</i>	Diarrhea	Saavedra <i>et al.</i> , 1994, Szajewska and Mrokwowics, 2001, Bae <i>et al.</i> , 2002, Chouraqui <i>et al.</i> , 2004
<i>B. bifidum</i> with <i>S. thermophilus</i> , <i>L. bulgaricus</i> , <i>L. acidophilus</i>	Traveller's diarrhea	Black <i>et al.</i> , 1989
<i>B. longum</i> , <i>B. infantis</i> , <i>B. breve</i> with <i>S. thermophilus</i>	Inflammatory bowel disease, ulcerative colitis	Giochetti <i>et al.</i> , 2000, Mimura, 2002, Kim, 2003, Hart <i>et al.</i> , 2004, Bibilon <i>et al.</i> , 2005
<i>Lactobacillus gasseri</i> PA 16/8, <i>Bifidobacterium longum</i> SP 07/3, <i>B. bifidum</i> MF 20/5	Common cold	De Vrese <i>et al.</i> , 2005
<i>B. infantis</i> , <i>S. thermophilus</i> and <i>B. bifidus</i>	Prevention of necrotizing enterocolitis	Bin-Nun <i>et al.</i> , 2005