Probiotic Cultures – Opportunities and Threats

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Abstract

The article covers the following areas:

- the **definition** of probiotics
- the ever-widening **product** spectrum
- the varying **legislative constraints** across regions
- the key **supply and supplier** issues
- the **market dynamics** as observed by GIRACT and our model for establishing demand estimates across regions and sectors for probiotic strains

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The important issues that define the markets for probiotics, particularly in the Western countries, are as follows:

- a rather strict **legislative** environment which restricts product claims
- advanced **technology** that permits the production of high quality end-products in such sectors as dairy and beverages
- dynamic **producers** of cultures and end-products
- deeply sceptical and confused **consumers**, particularly in Europe.

Let us try and examine the markets for probiotics within the scope of these complex parameters.

Lactic acid bacteria are collectively assigned to the Lactobacteriaceae family of biological organisms. While these may include long and short rods as well as cocci, their physiological characteristics are similar – they are gram positive, do not form spores (with one exception), they are dependent on carbohydrates (including the by-now-famous prebiotics) for their energy supply and they all excrete lactic acid. Unlike many other bacteria, they are easy to isolate given their significant production of lactic acid and their acid-tolerance.

Thus each of the various genera such as Lactobacillus, Bifidobacteria, Streptoccocus, Staphlococcus, etc, has a different shape and properties with respect to its interaction with human beings. Some specific species and sub-species have been found to provide a positive impact in the human digestive system. Thus, the word probiotic, the opposite of antibiotic, was coined.

Antibiotics have been a major tool in management of bacterial infections in the last 50 years. These products are powerful chemicals which act to destroy bacteria throughout the body and consequently may have deleterious side effects on otherwise beneficial gut flora.

Probiotics, defined as viable microorganisms that are beneficial to human health, were first conceptualised by the Nobel prize winning Russian scientist Elie Metchnikoff, at the turn of the 20th century. He believed that the fermenting bacillus (now called *Lactobacillus*) contained in the fermented milk products consumed by Bulgarian peasants positively influenced the microflora of the colon, thus decreasing toxic microbial activities.

Clinical documentation on probiotics is particularly good on the use of specific strains in the management of diarrhoea. While some scientific evidence exists for other benefits, the industry is well aware of the extent of work that still needs to be carried out to establish the credibility of such products at a level where they will achieve legislative approvals in the Western world.

Probiotics are well accepted in Asia (especially Japan and Korea) as an effective prophylaxis against intestinal disorders and many food products are available, particularly in Japan, that claim to impart a positive effect on the digestive tract. Probiotic foods and supplements are also popular in Europe; however, the use of probiotics in foods is largely restricted to the fermented milk sector. In the US, probiotics are found essentially in the supplements sector and, although used in most yogurts, the addition in this case is largely to achieve a mild taste.

DEFINITIONS

It is important to distinguish between probiotic, prebiotic and synbiotic ingredients:

Probiotic: Live microbial strain(s) fed by mouth which beneficially affects the host animal by improving its intestinal microbial balance

Prebiotic: 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

Synbiotic: A mixture of pro- and pre- biotics which beneficially affects the host by improving the survival and implantation of selected live microbial strains in the gastrointestinal tract

MODE OF ACTION

Both probiotics and prebiotics have been involved in various health benefit claims which are both diverse and impressive. These include increased resistance to infection, anti-tumour properties, improved immune status, improved lactose metabolism and lowering cholesterol levels. Changes in microbial composition and activity in the gut may be induced by various mechanisms such as directly antagonistic events, competition for nutrients, immunological influences and occupation of colonisation sites.

Antagonistic agents are usually the end products of bacterial growth such as organic acids or specific antimicrobial compounds. Many strains of lactic acid bacteria are known producers of bacteriocins such as nisin. These compounds are used as food preservatives and may be active in the gut, although the influence of degradative proteases on their activities has not been determined.

Competitive interactions are common amongst intestinal bacteria. As well as synergistic or mutualistic relationships, many of the individual components of the microbiota may also have similar nutritional requirements. In this case, those that have an improved substrate affinity will predominate.

Some probiotics are known to be immunostimulants. Such properties may have beneficial effects on the host.

There is much competition for colonisation sites on the gut wall. It is possible that certain probiotics may preferentially occupy sites where pathogens may otherwise adhere.

The main species which are believed to have probiotic character are *Lactobacilli* and *Bifidobacteria.*

Experiments have shown that Bifidobacteria exert anti-microbial activities against a spectrum of pathogens. This includes the verocytotoxin-producing strain of *E coli* 0157:H7.

For probiotics, measuring changes in numbers of viable cells of a probiotic specie presents problems if the organism being ingested has no special characteristics to distinguish it from indigenous, closely related, strains. Strains with specific antibiotic resistance have been used. However, the colonic ecosystem is able to transfer such resistance to other indigenous organisms, and so this approach is flawed. Modern genetic techniques are now being considered for tracking probiotics through the intestinal tract.

The effect of a probiotic organism on the micro-ecology of the gut depends, to a large extent, on its ability to survive and, preferably, multiply in the gut. Assuming the probiotic health effect has a biochemical basis, the organism should become metabolically stable and active. Studies in humans, however, are almost always done on faecal samples and the significance of results obtained in this way is difficult to assess. The appearance of large numbers of the probiotic spp. in faeces is not necessarily a good index of its activity in proximal regions of the gastro-intestinal tract. Studies indicate that long-term colonisation of probiotics does not occur.

INDICATIONS, HEALTH BENEFITS BY SPECIES/STRAIN

The main criteria used in the selection of probiotic bacteria include:

- non-pathogenic activity
- desired technological and organoleptic properties
- resistance to gastric acid and bile salts
- biological efficiency on humans including studies on adhesion to human intestinal epithelial cells

- interaction with enteropathogenic bacteria
- colonisation of the gut
- stimulation of the immune system

Most products are derived from strains found in the human intestine.

The following health benefits are most commonly cited, with indications of trials referenced in the literature and/or from interviews.

- Cholesterol Reduction
- Recolonisation after Antibiotics
- Resolution of diarrhoea
- Reduction of Irritable Bowel Syndrome
- Reduced Eczema, particularly in the case of children

There is a significant amount of scientific reviews available on this product group and currently a large number of studies are being carried out both at industry and university levels.

One negative and disturbing report published recently, from Dr Merja Rautio of the National Public Health Institute Finland, is the suggestion that a *Lactobacillus Rhamnosus* strain "indistinguishable from LGG" had caused a severe liver abcess - admittedly in a patient with many other life threatening indications. Luckily, the patient did recover!

DOSAGE

Around the world there is increasing scrutiny of food and supplement claims. Both scientific and regulatory authorities are looking for assurance that a probiotic product can deliver live strains at sufficient concentration to the large intestine to provide a benefit to the individual. The current opinion is that levels of 10^6-10^7 cfu/g should be present at the time of consumption and thus 10^7-10^8 at time of manufacture for a claim, stated or implied, to be made. Recently some authorities have found lower levels present in trade samples.

What is the trend? *Yakult* in Japan has recently set the bar at a higher level by increasing the dosage to 2.3x10^8 cfu/g in its standard product, and GIRACT believes that the concentration level will be increased slowly even in the West.

SPECIES/STRAINS AND CLAIMS

Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus salivarus* subsp. *thermophilus* are required fermentative organisms in traditional yogurt in a number of countries and are referenced in the Draft CODEX definition. However, there is evidence that they do not survive passage through the stomach in major numbers. Other species such as *L. acidophilus, L. casei, Enterococcus faecium* and various Bifidobacteria, are more robust and are the basis of probiotic claims.

Although a number of human clinical studies have assessed several probiotic strains, the cholesterol-lowering effects of fermented milk products are equivocal.

More evidence supports their role in cancer risk reduction, particularly colon cancer. This may be due to the fact that lactic acid cultures can alter the activity of faecal enzymes (e.g. ß-glucuronidase, azoreductase, and nitroreductase) that are believed to play a role in the development of cancer. Some research has shown an inverse relationship between the consumption of fermented milk and breast cancer.

The following tables list probiotic cultures for each genus. These have been identified by GIRACT in the key markets across the world as being promoted currently and declaring to have probiotic properties.

Lactobacillus

Specie	Strains
acidophilus	*La2, La5*[1]*, Johnsonii (La1)*[2]*, NCFM, DDS-1, SBT-2062*
bulgaricus	*Lb12*
lactis	*L1A*
plantarum	*299v, Lp01*
rhamnosus	*GG, GR-1, 271, LB21*
reuteri	*SD2112*[3]
casei	*Shirota,Immunitass, 744, 01*
paracasei	*CRL 431*
fermentum	*RC-14*
helveticus	*B02*

1 also known as La1
2 also known as Lj1
3 also known as MM2

Bifidobacteria

This nomenclature is not always consistent. Thus some strains, which were originally referred to as *Lactobacilli* are now being re-classifed as *Bifidobacteria*. Also references to *B lactis* may be the same as *B bifidus, etc.* In short, it is very evident that even basic science is evolving rapidly in this area, which is reflected in the confused manner in which ingredient declaration on labels appear as strains or species or even as the basic genus.

Specie	**Strain**
adolescentis	
longum	*BB536, SBT-2928*
breve	*Yakult*
bifidus	*Bb-11*
essensis	*Danone (Bio Activia)*
lactis	*Bb-02*
infantis	
laterosporus	
subtilis	

Other Genera

ENTEROCOCCI

Enterococcus faecium (or fecium) is the most well-known.

STREPTOCOCCI

Few specific probiotics were identified - most are standard *thermophilus.* However *Streptococcus thermophilus TH-3 and TH-4* are claimed to have probiotic effects.

LACTOCOCCUS

Again, *Lactococcus lactis R-707-1* is claimed to be probiotic.

CAUSIDO CULTURES

These are referenced in ARLA FOOD's *Gaio* products

These lists of species are by no means exhaustive as companies vie with each other to launch new probiotic cultures. However, from the earlier discussions, it must be clear that currently, given the scientific knowledge in this area, it is impossible to tell which cultures are actually probiotic and which cultures are "more" probiotic. The evaluation of the degree of "probioticity", if I am allowed to coin that word, will be the main challenge for the producers, authorities and the consumers in the future.

LEGISLATION

The Codex Alimentarius commission is currently discussing minimal live bacteria concentrations for the strains declared on the labels. These discussions are being followed very closely by the industry who are anticipating the CODEX decisions.

The latest Proposed Draft Standard for Fermented Milks (A-11, 3/2000) defines the specific micro-organisms that must be used in certain fermented milk products. The draft also requires these specific micro-organisms to be present in the final product at concentrations of at least 10^7 cfu/g ; while any additional micro-organism(s) declared on the label must be in concentrations of at least 10^6. The following table list the specific micro-organisms required to be present according to the draft's standards:

Fermented Milk Product	**Specified Micro-organisms**
Yoghurt	Synbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus*
Acidophilus Milk	*Lactobacillus acidophilus*
Kefir	Starter cultures prepared from kefir grains, *Lactobacillus kefiri,* species of the genera *Leuconostoc , Lactococcus* and *Acetobacter*. Kefir grains constitute both lactose fermenting yeasts (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces omnisporus*, *Saccharomyces cerevisae* and *Saccharomyces exiguus*)
Kumys	*Lactobacillus delbruekii* subsp. *Bulgaricus* and *Kluyyveromyces marxianus*

Buttermilks/Filmjölk products do not appear in this list since the cultures used in the production of these products have not been identified as probiotic.

USA

In both food and drug areas, the US regulatory authority is the FDA. In the USA, food products containing probiotic organisms may not systematically claim health benefits. The only method to determine if a product is probiotic in the absence of any claim is to verify if the ingredient list contains organisms known to be probiotic, i.e. a big disincentive.

Probiotic products, as other nutraceuticals, can fall under several Statutes and it is not always clear where the legislation draws the line between the categories.

Example of categories where probiotic cultures may be found are:

- Medical foods
- Food and drinks
- Dietary supplements

The regulatory system is undergoing sweeping changes. Some obsolete categories will cease to exist, while some products will be transferred from one category to another, etc.

Three main Acts may apply to probiotics, plus a proposed new act, which is still in the discussion phase. These are:

Nutrition Labeling and Education Act – NLEA (1990)

Dietary Supplement Health and Education Act – DSHEA (1994)

Food and Drug Administration Modernization Act – FDAMA (1997)

Nutraceutical Research and Education Act - (NREA) (discussion phase)

Yogurt Legislation

It is particularly interesting to review the yogurt legislation in the US.

According to American standards (21CFR131.100), yogurts must contain L. bulgaricus and S. thermophilus. Other strains are allowed and species do not need to be declared, i.e. "starter cultures" is sufficient. There is no minimal live bacteria count required except in the states of California and Oregon, where 2x10ˆ6 cfu/ml are required for probiotic dairy products.

The National Yogurt Association has established a 'Live active culture' seal that helps consumers to recognize yogurt containing live, active cultures. This is a first step in raising American consumer awareness concerning the benefits of live cultures.

To use the seal requires chilled yogurt to have 10^8cfu/ml and frozen yogurt 10^7cfu/ml at the time of manufacturing. However, it is not mentioned that the cultures are bacteria, because of the negative association in the minds of the US public. Further, it does not distinguish between strains so it is not helpful for the consumer seeking a probiotic product.

We have discussed this in more detail in the report, and we have also addressed the legislative complexities in both the European Union and in Japan, which I will not discuss today due to the time constraint.

SUPPLY

Throughout the report, GIRACT has converted the estimates of species usage to "freeze dried weight equivalents", in order to compare like with like across sectors.

There are generally 4 sources of supply:

- captive and/or toll production by major yogurt and dairy drink players or supplement makers around the world (DANONE, NESTLÉ, YAKULT).
- production for captive use under licence from a primary licence holder for a given strain (VALIO, BIOGAIA, MORINAGA)
- supply by traditional suppliers of starter cultures (RHODIA, CHR. HANSEN, SKW and many others)
- supply of frozen cultures or freeze dried powders by "new" entrants who may also use cultures for captive production of supplements (CELL BIOTECH, UAS).

The large majority of the offer is in *Lactobacillus spp* in yogurts, dairy drinks and supplements. *Bifidobacteria* is the other major group of products supplied.

In the yogurt sector, a number of *Lactobacillus* cultures are part of the starter culture mix in yogurt production.

There are a number of producers of strains who are also supplement makers, particularly in the US. These may be making supplements under their own name (captive) or as toll manufacturers for third parties or selling the strain as such (open market).

DEMAND ESTIMATION ACROSS REGIONS AND SECTORS

This is the heart of the work that we have carried out in GIRACT. Given the extremely confused stage of the market with respect to product definitions, legislative status and relevant dosages, we have built two different quantitative models to estimate the demand in food and supplement sectors across sectors and regions. These models have significant number of assumptions, but we have tried to be logical, consistent and transparent in our analyses.

I will continue my examination of the US market, and take you through the supplement sector in the US in order to explain our approach.

Demand in Supplement Sector - USA

The total vitamin/supplement market in the US has been growing strongly and is set to continue.

	US$ mio
1994	4156
1995	4377
1996	4892
1997	5657
1998	6758
1999	7640
growth p.a. 1994-1999	13.1%
2005	11466
growth p.a. 2000-2005	7.0%

Source: GIRACT based on Euromonitor

Supplements are subject to several market forces if we are to forecast development:

- growth from an ageing population ever more seeking "instant health fixes" and credulous concerning pills and drawn towards "Health Foods"
- reduction of marketing support due to stricter requirements for evidence of basis of claims
- increasing volumes for those (few) products which have claims accepted.

PROBIOTIC SECTOR

Probiotic products have a very small market penetration, estimated at 0.5% of the vitamin/dietary supplements sector, with annual sales of around US$ 38 mio in 1999.

SUPPLEMENT FORM

Within this study nearly 60% of the products encountered were in tablet form, and of these, more than a third were in chewable forms.

	%
Tablets:	
- chewable	20
- other	39
Capsules	19
Powder	15
Liquid	7

Source: GIRACT based on database and interviews

PROBIOTIC SPECIES

The range of concentrations found in the market (both in the stores and on the internet) complicate the issue – from 0.1-15x10^9 cfu per dose. GIRACT computed a weighted average load for its market estimates.

TYPICAL BREAKDOWN OF DOSAGES PER CAPSULE

Dosage (bio cfu)	0.05	0.1	2	4	8	12	
Approx. market share (%)	x	x	x	x	x	x	100
Weighted average	x bio cfu per dose						

Companies were awarded ratings, linked to their estimated relative importance in the market place and these applied to the species observed by company, leading to the shares and cfu levels (numbers*10^15 and kg) shown in the table below.

Typical species	**ACI**	**BUS**	**BUM**	**BIF**	**PLA**	**FAE**	**Other**	**Total**
Volume index	xx	xx	xx	xx	xx	x	x	xxx
Share by species (%)	xx	x	xx	xx	x	x	xxx	xxx
Market Volume by species (mio doses)	xxx	xxx	xxx	xx	xx	x	x	xxxx
cfu numbers 10^15	xxx	xxx	xxx	xxx	xxx	xx	xx	xxx
cfu density (bio/gram)	xx	xx	xx	xx	xx	xx	xx	xx
cfu culture/freeze dried (kg)	xxx	xxxx	xx	xxx	xxx	xx	xxx	xxxx

The total consumption is kg in freeze dried equivalent was thus obtained for each of the species/sub-species.

This methodology was followed for estimating the supplement market for probiotics in the USA, W. Europe and Japan. A similar methodology was used to obtain demand estimates in the different food and drink sectors in these regions.

Finally, based on quantitative and qualitative factors, a range of forecasts to the year 2005 was drawn up across regions and species.

WEST RE-INVENTS LONG-TERM COMMITMENT

What are the key conclusions? That the probiotic culture market is worth more than $200 mio across the three key regions of the world. That it is a promising, evolving and a complex market. That it requires significant investment – both in R&D and marketing – to be successful in the long-term.

In fact, it has been a long time since a product category in the food and supplement industry has required such a massive investment and commitment, particularly for a single product concept.

The probiotic product category is surely reminding Europeans of the virtues of longer-term planning in both R&D and marketing – a strength that Europe was so proud of till not so long ago. Just looking at the failed experiments of so many companies in this sector, I am even tempted to say that fly-by-night NASDAQ type of approach is not only dangerous for individual companies but can also be detrimental to the entire industry. A systematic and committed approach, on the other hand, can take the probiotic market very far even in the Western countries.

V. Krishnakumar is the Managing Director of GIRACT, a Geneva-based specialist company in the area of market studies on ingredients and additives. For further information on the market study entitled "Probiotic Cultures – Supply/Demand Patterns in Food and Supplements – W. Europe, USA, Japan", please contact GIRACT at tel: +41 22 7790500, fax: +41 22 7790505 or e-mail: info@giract.com or see our website www.giract.com for more details.

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