PROBIOTICS

for

HEALTH AND WELLBEING

presented by

SABINSA CORPORATION

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INTRODUCTION

Probiotics have a long history of human use, and cultured dairy products, for example, are traditionally consumed in several parts of the world. The FAO/WHO defines probiotics as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2001).

Across the globe, about 20 probiotic strains, singly or in combination, mainly Lactobacilli such as *L. acidophilus*, *L. casei*, *L.reuterii* and others; Bifidobacteria, safe spore forming lactic acid producing bacteria (such as Lactospore®[1]; *Bacillus coagulans* also known as *Lactobacillus sporogenes*), and a probiotic yeast culture *Saccharomyces boulardii*, are used in dietary supplements and functional foods or in mainstream food products.

HISTORICAL PERSPECTIVE

Ancient physicians in the Near and Middle East prescribed soured milk containing lactic acid bacteria for appetite stimulation, as well as in the management of diseases including tuberculosis, gastrointestinal disorders, and liver troubles.

Contemporary interest in probiotics can be partially attributed to Metchnikoff's theory of longevity, which associated prolonged youth and a healthy old age, common in Balkan peasants of those times, with the use of cultured milks in their diet. Metchnikoff, a Russian physician, postulated that the growth of toxin-producing putrefactive organisms in the gastrointestinal tract could be controlled by the implantation of beneficial cultures in the gut (Metchnikoff, E; 1910).

[1] A trademark of Sabinsa Corporation
www.lactospore.com

GUT ECOLOGY AND HEALTH APPLICATIONS OF PROBIOTICS

Humans cannot ever live free from intestinal bacteria. Colonization of the gut with microflora begins in the infant, shortly after birth. The microecology of the human gastrointestinal tract is a complex one, generally involving two kinds of flora:

1. Indigenous beneficial bacteria which have achieved a symbiotic relationship with the host through a long period of evolution.
2. Potentially pathogenic bacteria.

An optimal "balance" in this microbial population is associated with good health in humans (Shahani, KM et al, 1980; Gorbach, SL, 1990 Mitsuoka, T; 1988). This balance of beneficial bacteria versus pathogenic bacteria is referred to as “eubiosis”. For efficient digestion and maximum absorption of nutrients, it is essential that the right balance of microorganisms be maintained. This balance is often compromised during antibiotic therapy when the immune system is weakened by disease, stress or other factors. Available evidence indicates that certain microorganisms, particularly the lactic acid producing organisms that are natural inhabitants of the gastrointestinal tract, or “semi-residents” that help to restore the natural microecological balance, can facilitate a favorable microbial profile in the gut.

HEALTHFUL ROLE OF LACTIC ACID PRODUCING BACTERIA

Research conducted by various individuals during the last century has shown that lactic acid producing bacteria have many beneficial effects to promote human health. In the course of their proliferation and survival in the gastrointestinal tract, these probiotics produce metabolites such as lactic acid and antibiotic-like substances called bacteriocins that suppress the growth of putrefactive microorganisms. Their metabolic activities also help in the pre-digestion of food components and the production of vitamins B, and improve the bioavailability of minerals (Wood, BJB ed, 1992) and other nutrients, for example, isoflavones from soy milk, as reported in a recent study (Pham TT, et al., 2007) Additionally, their cell wall components and metabolic products provide immune support and anti-inflammatory action (Bogdanov, IG, 1978; Hosono, A et al.; 1986; Reddy, GV et al; 1983; Shahani, KM et al; 1983).

The major metabolic activities of probiotics include proteolysis (breakdown of food proteins), lipolysis (breakdown of food fat) and the conversion of lactose (milk sugar) to lactic acid. These changes are effected through the respective bacterial enzymes. Probiotics in the gastrointestinal tract could therefore help humans suffering from impaired digestion due to lack or dysfunction of the inherent digestive enzymes, by pre-digesting ingested food components. This property of probiotics is particularly useful in infant, geriatric and convalescent nutrition. Additionally, people who suffer from "lactose intolerance" who cannot consume milk and dairy products without experiencing gastrointestinal disturbances, are benefited by the lactose-hydrolyzing enzymes supplied by lactic acid producing cultures (Alm, L.; 1982).

Probiotics are now clinically proven to have a number of health benefits including usefulness in irritable bowel syndrome (Saggioro A, 2004; Fan, YJ et al, 2006), allergic conditions (Saavedra, M., 2007, Abrahamsson TR, 2007), skin health maintenance (Thestrup-Pedersen K. 2003), dental health maintenance (Meurman JH, et al., 2007), in supporting healthy blood pressure levels (Aihara, K. et al.; 2005), immune functions (Liong, MT, 2007; Trois, L et al.; 2007) and liver functions (Bongaerts, G et al.; 2005), pain relief support (Gawronska, A et al.,2007), in the management of vaginal infections (Uehara, S et al; 2006), as anti-inflammatory agents (Tok, D et al, 2007), and in supporting cardiovascular health and wellness (Agerholm-LL et al., 2000; Naruszwicz, M et al.; 2002). Recent research also reveals potential benefits in obesity management (Ali, AA et al; 2005).

Prebiotics are food materials such as non-digestible oligosaccharides found in plant foods and whole grains that nourish the beneficial bacteria. Inulin (found naturally in chicory and Jerusalem artichoke) and fructooligosaccharides (FOS) (Mitsuoka, T; 1987) are commonly added to probiotic dietary supplements as prebiotics.

DEVELOPING EFFECTIVE PROBIOTICS FOR HUMAN AND ANIMAL HEALTH

The ideal probiotic microorganism should have the following characteristics:

1. Non pathogenicity to humans.
2. High tolerance to bile and gastric acidity.
3. Production of L-(+) lactic acid only during fermentation (since the D-(-) optical isomer of lactic acid has been associated with metabolic acidosis).
4. Capability for easy proliferation in vivo.
5. Capability for easy proliferation in vitro.
6. High survival rate through processing conditions (during harvesting, drying etc.)
7. High stability at room temperature separately or when mixed with other ingredients.
8. Lack of potential to develop virulence.

BACILLUS COAGULANS (FORMERLY KNOWN AS *LACTOBACILLUS SPOROGENES*)

Probiotic cultures in the form of vegetative cells that are not room temperature stable need to be freeze-dried or encapsulated by special processes to remain viable after processing, storage and exposure to acid and bile in the gastrointestinal tract (Gilliland, SE et al., 1990). Such probiotics are therefore "dead on arrival" if they are not adequately protected,

Bacillus coagulans, formerly known as *Lactobacillus sporogenes* (marketed as Lactospore®) is a **shelf-stable (at room temperature)** probiotic, with clinically documented efficacy in supporting health and wellness (Monograph, 2002; Gandhi, AB; 1988; Losada, MA et al, 2002). It was originally isolated from a food source (green malt). Being in sporulated form, the culture survives and proliferates in the gastrointestinal environment unlike vegetative cells that may be destroyed under these conditions. The culture produces only the beneficial L-(+) form of lactic acid in the gastrointestinal tract.

HISTORICAL: TAXONOMY AND NOMENCLATURE

Lactobacillus sporogenes was first described in 1932 by L.M. Horowitz-Wlassowa and N.W. Nowotelnow, the name was accepted in the fifth edition of "Bergey's Manual of Determinative Bacteriology" and also appeared in the sixth edition. However, it was transferred to genus *Bacillus* in the seventh edition of Bergey's manual as a result of an effort to simplify cataloging. Prof. O. Nakayama of Yamanashi University in Japan isolated Lactobacillus *sporogenes* from green malt in 1949. The characteristics of culture as cited in Bergey's Manual (Seventh Edition) and other sources

are: “Gram - positive spore-forming rods 0.9 by 3.0 to 5.0 micron size, aerobic to microaerophilic, producing L-(+)-(dextrorotatory) lactic acid homo-fermentatively.” Several scientific papers still use the original name, *Lactobacillus sporogenes* (Gandhi, AB; 1994). The culture was deposited in the ATCC by Japanese researchers as *Lactobacillus sporogenes*, and later reclassified as *Bacillus coagulans*. These researchers used the culture to prepare natto a fermented food product prepared from soybeans (Naruse, K and Naruse, W; 1978). The culture is used in functional foods in Japan.

CLINICAL EFFICACY AND SAFETY

Clinical efficacy has been shown in the management of gastrointestinal problems associated with infections or the use of antibiotics. Conditions include diarrheal diseases and constipation, as studied in populations of all ages, including infants (Abstracts, 1968; Chandra, RK et al, 2002; Dhongade, RK et al, 1977). A recent study revealed that prophylaxis with *Lactobacillus sporogenes*, in combination with FOS, significantly reduced the number of days and duration of events in children with antibiotic-induced diarrhea (La Rosa, M. et al.; 2003).

The culture was shown to support healthy cholesterol levels (Seok, EK et al, 1987; Mohan, JC et al; 1990a, 1990b) and demonstrated benefits in the management of non-specific vaginitis (Sankholkar, PC et al.). A recent study showed the efficacy of suppositories containing *Lactobacillus sporogenes* in the management of vaginal infections (Kale, VV et al., 2005).

In other studies, benefits in the management of aphthous stomatitis (recurrent ulcers in the mouth) and glossitis (inflammation of the tongue) (Mathur, SN et al, 1970), and promising results in the management of allergic skin diseases (eczema) in infants, were observed (Abstracts, 1968).

Some of these clinical data are summarized below. **No untoward side effects were reported in any of these studies.** In acute toxicity studies wherein *L. sporogenes* in the form of a preparation containing not less than 5×10^9 spores was fed at levels of 1, 3 or 5 g/kg for 7 days orally to mice, neither mortality nor abnormality such as diarrhea, occurred.

GASTROINTESTINAL HEALTH

Indicated for improvement of symptoms due to abnormalities in the intestinal flora.

A total of 567 cases in 19 institutions in Japan were evaluated[1].

Condition	% cases showing improvement	Number of patients showing improvement
Diarrhea due to acute or chronic gastroenteritis	93.7	118/126
Maldigestion accompanied with diarrhea	85.9	79/92
Infantile diarrhea	87.9	58/66
Constipation	65.4	17/26

[1] Abstracts of papers on the clinical study of Lacbon (Sporlac) compiled by the Sankyo Co. Ltd. Japan.

Other Clinical Studies: Healthy Serum Lipid Profiles

Short term hypolipidemic effects of oral L. sporogenes therapy

Dose: 360 million spores per day in tablet form

Subjects: 7 patients (15 men and 2 women in the 32-61 year age group) with type II hyperlipidemia in an open label fixed dose trial.

Duration: 12 weeks

Atherogenic Lipid Ratios were improved:	
Total/HDL cholesterol ratio:	Decreased by 24%
LDL/HDL ratio:	Decreased by 33.4%

J.C. Mohan, R. Arora & M. Khalilullah, Indian Journal of Medical Research [B], 92: 431-432 (1990).

Study 2: 112 newborn infants in rural India were randomized to receive a daily oral dose of 100 million L. sporogenes or a placebo for one year.
Morbidity was monitored each week for 12 months.

Effect of L. *sporogenes* feeding on rotavirus diarrhea

	L. *sporogenes*	Placebo	p
Number of infants	55	57	
Number of infants with episodes of rotavirus diarrhea	44	50	NS
Number of episodes	3.4 *(1.0)*	8.6 *(1.7)*	<0.02
Duration of each episode (days)	3.6 *(1.0)*	6.8 (1.1)	<0.05
Days ill/12 months	13 *(3)*	35 (5)	<0.01

Data are shown as mean *(SD). NS : statistically not significant*

Chandra, R.K. Nutrition Research 22 (2002) 65-69

Clinical Study: Non-specific vaginitis

Subjects: 44 patients with non-specific vaginitis [12 with leucorrhea after cervical surgery; 32 (26 of reproductive age and 6 post-menopausal, without previous therapy)]

Dose: 2 vaginal tablets/day (150 million spores/tab.)

Duration of treatment: 7-14 days

Results:

Complete Relief	91% cases
Partial relief:	9% cases

In a similar clinical trial using M.T.P. vaginal pessaries containing broxyquinoline and brobenzoxeldine where only 26.67% of cases studies were cured.

Conclusion: Lactobacillus sporogenes is effective in non-specific vaginitis by raising the vaginal acidity, and thereby checking the growth of pathogenic organisms.

Sankholkar, P.C and Sali, M.S. Clinical study report from B.J. Medical College, Pune, India.

Allergic Skin Diseases

Condition: Allergic skin diseases

- No. of subjects: 5
- Treatment: 200-450 million spores / day in divided doses for 4-12 days
- Effectiveness rate: 80.0%

Conclusion: Obvious eruptions of strophulus and eczema decreased from the third day (topical therapy employed concomitantly).

Condition: Miscellaneous symptoms

- No. of subjects: 10
- Treatment: 20-50 million spores / day in divided doses for 4-20 days
- Effectiveness rate: 80.0%

Conclusion: Favorable response seen in infants with anorexia nervosa and malnutrition.

Abstracts of papers on the clinical study of Lacbon (Sporlac) compiled by the Sankyo Co. Ltd. Japan.

MECHANISMS OF ACTION

Lactospore® is potentially helpful in restoring gastrointestinal microecological balance through:

- Competitive inhibition of pathogens
- L-(+) lactic acid and hydrogen peroxide production
- Production of bacteriocins

Lactospore® is potentially effective in reducing the symptoms of non-specific vaginitis through pH reduction and the consequent inhibition of causative pathogens. Cardiovascular benefits through maintenance of healthy blood lipid levels are probably through deconjugation of bile salts and inhibition of LDL formation.

USE AS VETERINARY PROBIOTIC

In pets and farm animals, administration of Lactospore® would facilitate the establishment of a healthy gastrointestinal microfloral profile, reduce digestive upsets, improve feed utilization and support a healthy immune system. In controlled studies with *L. sporogenes* fed to farm animals and birds, feed efficiency and healthy growth were enhanced. Fecal counts of lactobacilli increased while those of putrefactive cultures fell in animals and birds that received the probiotic (Gandhi, AB et al, 1990; Kim, YM, et al.1985; Kumar, ORM et al, 1989; Han, JK et al; 1984).

CONCLUSIONS

The role of probiotics has expanded from use in gut health maintenance and supplementation in the event of dysbiosis (microfloral imbalance) during antibiotic therapy, to a wide range of health applications. With the increasing body of knowledge and research on known and emerging probiotic strains, future trends envisage their increased inclusion in dietary supplements and functional foods targeting diverse preventive health maintenance needs.

Current innovations in functional foods with probiotics, including yogurts, beverages, bakery products, preserves, pickles, breakfast cereals, nutrition bars and other convenience products, present attractive delivery options for these healthful ingredients.

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