LACTOSPORE®

(A Lactic Acid Bacillus Preparation)
OVERVIEW

Probiotic therapy in its rudimentary form originated in the Near and Middle East, where ancient physicians prescribed fermented milks for the treatment of various diseases, including tuberculosis and gastrointestinal disorders. Clinical interest in fermented milks and their role in human health was triggered by a Russian physician, E. Metchnikoff, who attributed the longevity of people in the Balkan countries to the regular consumption of fermented milk. The healthful properties of fermented milk are provided by the indigenous lactobacilli, which are also normal inhabitants of the human gastrointestinal tract, skin and vaginal mucosa.

Lactobacilli through their metabolic processes prevent the growth of putrefactive organisms by competitive inhibition, the generation of a non-conducive acidic environment and the production of bacteriocins. They improve the digestibility of ingested food constituents and the bioavailability of nutrients. They also synthesize vitamins B, possess hypocholesterolemic activity and enhance the immune system response.

*Lactobacillus* therapy has been shown to be effective in the treatment of a variety of disorders, including gastrointestinal disorders, vaginal infections, hepatic encephalopathy, hypercholesterolemia and conditions related to deficiency of vitamins B. Lactobacilli have proved to be useful in delaying the induction of tumors and have found use in the prevention of colon cancer. Lactobacilli are thus valuable therapeutically and are often used as adjuvants to antibiotic therapy. They also find use as growth enhancers for domestic animals and poultry.

The therapeutically used species of *Lactobacillus* include *L. acidophilus, L. brevis, L. casei, L. bulgaricus* and *L. bifidus*. However, the evidence for effectiveness of implantation, survival and proliferation of these organisms in the gut are not impressive.

A superior species, which is effectively implanted in the gut, is semi-resident and can survive the gastric acidity and bile is *Lactobacillus sporogenes*. This organism forms spores which are protected by nature’s own microencapsulation system and germinate into viable cells in the intestine, which can then proliferate extensively. Clinical trials with *L. sporogenes* have proven successful in the treatment of gastrointestinal disorders, non-specific vaginitis, aphthous stomatitis, hepatic encephalopathy and in growth improvement of farm animals. *L. sporogenes* is therefore the probiotic of choice in clinical applications. LACTOSPORE® a preparation of *L. sporogenes*, is a registered trade mark of Sabinsa Corporation.
INTRODUCTION

LACTOSPORE is a lactic acid bacillus preparation manufactured and distributed by the SABINSA CORPORATION. This booklet reviews the background, nutritional and therapeutic aspects and current status of the use of lactic acid bacillus preparations, and presents arguments for the superiority of LACTOSPORE over other such products in the market, known as “probiotics”, used in microbiotherapy.

The foundations of probiotic (meaning “in favor of life”) microbiotherapy lie in the postulate of Metchnikoff, a Russian physician, that the growth of toxin-producing putrefactive organisms in the gastrointestinal tract could be controlled by the implantation of beneficial lactobacilli in the gut. The clinical application of preparations containing lactobacilli was initiated on the basis of Metchnikoff’s THEORY OF LONGEVITY, which associated prolonged youth and a healthy old age with the continuous ingestion of lactobacilli. Metchnikoff attributed the longevity of the residents of the Balkan countries to the regular consumption of Bulgarian buttermilk. In the early 1900’s, he claimed to have successfully cured many of his patients who suffered from a wide variety of organic illnesses, ranging from dryness of skin and gastrointestinal disorders to atherosclerosis, through the therapeutic use of lactobacilli. Metchnikoff suggested that aging is the process of chronic putrefactive intoxication caused by certain intestinal bacteria and that these harmful effects could be mitigated through regular ingestion of live Lactobacillus cultures - a postulate that created a sensation in those early days. The enthusiasm shown then by eminent doctors of those times, advocating the therapeutic use of Lactobacillus, laid the foundations of LACTOBACILLUS THERAPY or MICROBIOThERAPY.

Fermented milks have been a part of the human diet since ancient times. Their efficacy in alleviating gastrointestinal disorders has been exploited in systems of traditional medicine the world over. Lactic acid bacteria, the indigenous microbial flora in fermented milks and natural inhabitants of the human gastrointestinal tract were thought to be responsible for the longevity of their hosts through their curative and prophylactic actions.

The role of lactic acid bacteria in gastrointestinal microecology has been the subject of extensive research. It is widely believed that these bacteria prevent the growth of putrefactive microorganisms responsible for ill health by competitive inhibition, the generation of a non-conducive acidic environment and/or by the production of antibiotic-like substances (bacteriocins). Their metabolites may include B group vitamins. Their proteolytic, lipolytic and β-galactosidase activities improve the digestibility and assimilation of ingested nutrients, thereby rendering them valuable in convalescent/geriatric nutrition and as adjuncts to antibiotic therapy. Lactic acid bacteria also colonize the skin and mucus membranes and play an important role in preventing bacterial and fungal infections of the skin and genito-urinary tract. Lactobacilli have a protective role against vaginal infections. They utilize glycogen in the vaginal epithelial cells to produce lactic acid which helps to maintain the pH of this environment at 4.0-4.5.
This environment is non-conducive for the growth of pathogens like *Candida albicans*, *Trichonomas vaginalis* and non-specific bacteria, which are responsible for vaginal infections.

An adverse balance among intestinal bacteria with marked reduction in lactic acid bacteria and an increase in putrefactive pathogens in the fecal flora has been observed in conditions like food allergy and eczema. The beneficial role of lactic acid organisms in preserving intestinal integrity and health has been extensively documented. However, the nutritional and therapeutic value of these organisms is still somewhat controversial.

In recent years, there has been an increasing interest in the relationship between intestinal microflora and their effects on the health of the human host. The ecosystem of the human gastrointestinal tract is extremely complex, colonized by more than 500 species of bacteria. Although lactobacilli in general represent a smaller percentage of the intestinal flora, their metabolic functions make them important. On colonization of the germ-free gastrointestinal tract in the human infant, shortly after birth, with normal gut flora, 2.4% are lactobacilli. The species of lactobacilli normally present include *L. bifidus* (*Bifidobacterium bifidum*), *L. acidophilus*, *L. casei*, *L. fermentum*, *L. salivarius*, *L. brevis*, *L. leichmanii*, *L. plantarum* and *L. cellobiosus*. About a third of the fecal dry weight consists of bacteria.

Populations at high risk for colon cancer have been found to harbor gut flora which efficiently metabolize steroids and hydrolyze glucuronides. A diet containing large amounts of viable lactobacilli significantly lowered these activities in such individuals. The normal fecal flora in humans include the following organisms:

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Viable colonies (per gm. of fecal matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>1 million - 1 billion</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>0-1 million</td>
</tr>
<tr>
<td>Streptococci</td>
<td>1000-1 billion</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>1 million - 1 billion</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>10 million - 10 billion</td>
</tr>
</tbody>
</table>

In the process of performing their metabolic activities in the human gastrointestinal tract, these microflora convert complex ingested food constituents into easily digestible forms, perform detoxification functions, and produce metabolites of nutritional and therapeutic significance to the host. A delicate balance exists in the symbiotic relationship between these microflora and the human host.

The composition of the intestinal microflora is constantly changing, being influenced by factors such as diet, emotional stress, age and treatment with antibiotics or other medications.
In general, lactobacilli are acid tolerant and can survive and proliferate at the low pH in the stomach. An optimal “balance” in the gastrointestinal microbial population is associated with good health in humans. This balance between beneficial bacteria and potentially harmful bacteria is referred to as EUBIOSIS.

In view of the pressures of modern existence, the maintenance of a normal, healthy, balanced microbial population (EUBIOSIS) in the gastro-intestinal tract is a difficult task. Humans are often subjected to various stress conditions such as changes in food consumption patterns, vagaries of the weather, extensive travel and somatic diseases that necessitate treatment with antibiotics and immunosuppressive drugs. Under such adverse circumstances, the harmful bacteria may become predominant (a condition referred to as bacterial overgrowth) and create an imbalance which may in turn impair normal gut functions and lead to various problems, ranging from inefficient digestion, diarrhea, constipation, and flatulence to severe gastro-intestinal disorders.

A logical approach to restoring the balance of intestinal flora is the use of probiotics. However, reports on the survival and effectiveness of these microorganisms in the gastrointestinal tract are inconsistent. Ingested as viable organisms, these microbes often do not survive the rigors of the gastric environment.

In order to provide the beneficial effects of lactic acid bacteria, many manufacturers have been marketing various lactobacillus preparations. The reported health effects of these preparations include effectiveness in the treatment of a variety of disorders such as colitis, constipation, diarrhea, flatulence, gastric acidity, gastroenteritis, gingivitis, hypercholesterolemia, hepatic encephalopathy and tumorigenesis, and in recolonization of the intestine with beneficial flora after treatment with antibiotics. However, the reports are controversial owing to the differences in viability of the implanted flora in the gastrointestinal tract. Successful implantation depends upon the following factors:

1. A high count of viable lactobacilli retaining their viability during manufacturing into dosage forms and subsequent storage.
2. Survival of lactobacilli, once ingested, in the acidic gastric secretions and their safe passage to the intestine.
3. The production of a sufficient quantity of metabolites antagonistic to pathogens. These include L (+) (dextrorotatory) lactic acid and bacteriocins.

Various species of lactobacilli have been examined including *L. bulgaricus*, *L. bifidus*, *L. acidophilus*, *L. casei*, *L. brevis* and *S. thermophiles*. *L. acidophilus*, long regarded as the best candidate for therapeutic use, has been shown to be ineffective in alleviating certain gastrointestinal disorders. Besides, it produces D(-) (levorotatory) lactic acid, which is not an effective antagonistic agent and may introduce metabolic disturbances.

A superior and convenient species of *Lactobacillus* is *L. sporogenes*. This species, also named *Bacillus coagulans*, forms spores, which on activation in the acidic environment of the stomach, can germinate and proliferate in the intestine, produce the favored L (+) form of lactic acid and effectively prevent the growth of pathogens. In effect, the process can be equated to the slow release of viable cells, leading to prolonged
and effective beneficial microbial activity. *L. sporogenes* spores are slowly excreted out of the human system, long after the termination of therapy. In view of the fact that the World Health Organization (W.H.O.) has recommended restricted intake of D(-) lactic acid for adults and total avoidance of the use of this form of lactic acid in infant nutritional products, *L. sporogenes* is the *Lactobacillus* favored in infant nutritional programs.

LACTOSPORE®, a preparation containing viable spores of *L. sporogenes*, is a registered trade mark of SABINSA CORPORATION. *L. sporogenes* preparations in powder, tablet and capsule forms have been used in successful clinical trials in the treatment of gastrointestinal disorders, vaginal infections, hypercholesterolemia, lactose intolerance, hepatic coma and precoma and as an adjuvant to antibiotic therapy.

Some commercially available preparations are as follows:

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Composition</th>
<th>Dosage form</th>
<th>Therapeutic use</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPORLAC®, LACTOSPORE®</td>
<td><em>L. sporogenes</em></td>
<td>tablet, powder</td>
<td>Lactose intolerance, gastro-intestinal infections, hypercholesterolemia, hepatic coma and precoma.</td>
</tr>
<tr>
<td>SANVITA</td>
<td><em>L. sporogenes</em>, Vitamins B, L-lysine monochloride</td>
<td>granules</td>
<td>Adjuvant to antibiotic therapy, convalescence therapy, enhancing immune response</td>
</tr>
<tr>
<td>SANVITONE</td>
<td><em>L. sporogenes</em>, vitamins B, minerals antioxidants, fungal diastase.</td>
<td>capsules</td>
<td>Geriatric and convalescence therapy, enhancing immune response, improves digestion.</td>
</tr>
<tr>
<td>MYCONID®</td>
<td><em>L. sporogenes</em></td>
<td>vaginal tablets</td>
<td>Non-specific vaginitis, leucorrhoea and after antifungal /antiprotozoal treatment.</td>
</tr>
<tr>
<td>BACTOLYTE</td>
<td><em>L. sporogenes</em> isotonic salts</td>
<td>powder</td>
<td>Oral rehydration therapy. Antibiotic with adjuvant for nutritional status enhancement and maintenance of gastrointestinal ecological balance.</td>
</tr>
<tr>
<td>AMPILAC</td>
<td><em>L. sporogenes</em>, ampicillin</td>
<td>capsules</td>
<td>Antibiotic with adjuvant for nutritional status enhancement and maintenance of gastrointestinal ecological balance.</td>
</tr>
<tr>
<td>LACBON</td>
<td><em>L. sporogenes</em></td>
<td>tablets, powder</td>
<td>Diarrhea, dyspepsia, urticaria, eczema, streptococcus, flora modifier after chemotherapy.</td>
</tr>
<tr>
<td>BECOPLUS</td>
<td><em>L. sporogenes</em>, B complex, zinc</td>
<td>capsule, powder</td>
<td>B complex deficiency adjuvant with antibiotics</td>
</tr>
<tr>
<td>NUTROLIN-B</td>
<td><em>L. sporogenes</em>, B complex</td>
<td>capsule, powder</td>
<td>B complex deficiency adjuvant with antibiotics</td>
</tr>
<tr>
<td>GUTFLOR</td>
<td><em>L. sporogenes</em>, B complex</td>
<td>tablet, capsule</td>
<td>Adjuvant with antibiotics</td>
</tr>
<tr>
<td>VIZYLAC</td>
<td><em>L. sporogenes</em>, B complex</td>
<td>tablet, capsule</td>
<td>Adjuvant with antibiotics and chemotherapeutic agents</td>
</tr>
</tbody>
</table>
This review details the role of L. sporogenes in microbiotherapy.

**BACKGROUND INFORMATION : THE LACTIC ACID BACTERIA**

Lactic acid bacteria have the property of producing lactic acid from fermentable sugars. The genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* are important members of this group. The taxonomy of lactic acid bacteria has been based on the gram reaction and the production of lactic acid from various fermentable carbohydrates.

Lactobacilli are gram positive and vary in morphology from long, slender rods to short coccobacilli, which frequently form chains. Their metabolism is fermentative; some species are aerotolerant and may utilize oxygen through the enzyme flavoprotein oxidase. While spore bearing lactobacilli are facultative anaerobes, the rest are strictly anaerobic. The growth is optimum at pH 5.5-5.8 and the organisms have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates. The genus is divided into three groups based on fermentation patterns:

1. homofermentative: produce more than 85% lactic acid from glucose.
2. heterofermentative: produce roughly 50% lactic acid and considerable amounts of ethanol, acetic acid and carbon dioxide.
3. other heterofermentative species: produce DL-lactic acid, acetic acid and carbon dioxide.

The species which have been used therapeutically include:

- *L. sporogenes*
- *L. acidophilus*
- *L. plantarum*
- *L. casei*
- *L. brevis*
- *L. delbruckii*
- *L. lactis*

The metabolic activities of lactobacilli are responsible for their therapeutic benefits.

Lactobacilli cultured in a milk medium perform the following activities:

1. **Proteolysis:**

Proteins are broken down into easily assimilable components.

\[
\text{Protein} + \text{H}_2\text{O} \xrightarrow{\text{proteinases from lactobacilli}} \text{Polypeptide: Polypeptides} + \text{H}_2\text{O} \xrightarrow{\text{polypeptidases from lactobacilli}} \text{Amino acids}
\]
These activities of lactobacilli in the gastrointestinal tract make protein ingested by the host easily digestible, a property of great value in infant, convalescent and geriatric nutrition.

2. **Lipolysis**

Complex fat is broken down into easily assimilable components.

\[
\text{Triglycerides (fat)} \xrightarrow{\text{lipases}} \text{Fatty acids + glycerol from lactobacilli}
\]

This property finds use in the preparation of dietetic formulations for infants, geriatrics and convalescents.

Evidence from preclinical and clinical trials has revealed that lactobacilli can break down cholesterol in serum lipids\(^{10,11}\). Lactobacilli also assist in the deconjugation of bile salts\(^{12}\). Both of these findings have clinical significance.

3. **Lactose metabolism**

Lactic acid bacteria have the enzymes \(\beta\)-galactosidase, glycolase and lactic dehydrogenase (LDH) which produce lactic acid from lactose. Lactic acid is reported to have some physiological benefits\(^{13}\) such as:

a) Enhancing the digestibility of milk proteins by precipitating them in fine curd particles.

b) Improving the utilization of calcium, phosphorus and iron.

c) Stimulating the secretion of gastric juices.

d) Accelerating the onward movement of stomach contents.

e) Serving as a source of energy in the process of respiration.

The levels of optical isomeric forms of lactic acid produced depend upon the nature of the culture. The structural configurations of these isomers are as follows:

\[
\begin{align*}
\text{COOH} & \quad \text{COOH} \\
\text{H} & \quad \text{HO} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{C} & \quad \text{C} \\
\text{OH} & \quad \text{H}
\end{align*}
\]

\(\text{D(-) levorotatory lactic acid} \quad \text{L(+)} \text{ dextrorotatory lactic acid}\)

In humans, both isomers are absorbed from the intestinal tract. Whereas L(+)-lactic acid is completely and rapidly metabolized in glycogen synthesis, D(-)-lactic acid is metabolized at a lesser rate, and the unmetabolized acid is excreted in the urine. The presence of unmetabolized lactic acid results in metabolic acidosis in infants. *L. acidophilus* produces the D(-) form and is therefore of disputable clinical benefit,
although it has earlier been the probiotic of choice in various therapeutic formulations\textsuperscript{75}. \textit{L. sporogenes} on the other hand produces only L(\(+\)-) lactic acid and hence is preferred.

The ability of lactobacilli to convert lactose to lactic acid is used in the successful treatment of lactose intolerance. The people suffering from this condition cannot metabolize lactose due to lack or dysfunction of essential enzyme systems. Lactic acid, by lowering the pH of the intestinal environment to 4 to 5, inhibits the growth of putrefactive organisms and \textit{E. coli}, which require a higher optimum pH of 6 to 7. Some of the volatile acids produced during fermentation also possess some antimicrobial activity under conditions of low oxidation-reduction potential.

**Production of bacteriocins**

Bacteriocins\textsuperscript{3,14} are proteins or protein complexes with bactericidal activities directed against species which are closely related to the producer bacterium. The inhibitory activity of lactobacilli towards putrefactive organisms is thought to be partially due to the production of bacteriocins.

Some of the bacteriocins isolated from lactobacilli are listed in Table 2.1:

**Table 2.1 : Bacteriocins isolated from** Lactobacilli

<table>
<thead>
<tr>
<th>Substance</th>
<th>Producing species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidolin</td>
<td>\textit{L. acidophilus}</td>
</tr>
<tr>
<td>Acidophilin</td>
<td>\textit{L. acidophilus}</td>
</tr>
<tr>
<td>Lactacin B</td>
<td>\textit{L. acidophilus}</td>
</tr>
<tr>
<td>Lactacin F</td>
<td>\textit{L. acidophilus}</td>
</tr>
<tr>
<td>Bulgarin</td>
<td>\textit{L. bulgaricus}</td>
</tr>
<tr>
<td>Plantaricin SIK-83</td>
<td>\textit{L. plantarum}</td>
</tr>
<tr>
<td>Plantaricin A</td>
<td>\textit{L. plantarum}</td>
</tr>
<tr>
<td>Lactolin</td>
<td>\textit{L. plantarum}</td>
</tr>
<tr>
<td>Plantaricin B</td>
<td>\textit{L. plantarum}</td>
</tr>
<tr>
<td>Lactolin 27</td>
<td>\textit{L. helveticus}</td>
</tr>
<tr>
<td>Helveticin J</td>
<td>\textit{L. helveticus}</td>
</tr>
<tr>
<td>Reuterin</td>
<td>\textit{L. reuteri}</td>
</tr>
<tr>
<td>Lactobrevin</td>
<td>\textit{L. brevis}</td>
</tr>
<tr>
<td>Lactobacillin</td>
<td>\textit{L. brevis}</td>
</tr>
</tbody>
</table>

**Production of other antagonistic substances**

Lactic acid bacteria also inhibit the growth of harmful putrefactive microorganisms through other metabolic products such as hydrogen peroxide, carbon dioxide and diacetyl.
The metabolites of lactic acid bacteria that exert antagonistic action against putrefactive microorganisms and their mode of action are summarized in Table 2.2:

Table 2.2: Antagonistic activities caused by lactic acid bacteria

<table>
<thead>
<tr>
<th>Metabolic product</th>
<th>Mode of antagonistic action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Carbon dioxide</td>
<td>Inhibits decarboxylation ?</td>
</tr>
<tr>
<td></td>
<td>Reduces membrane permeability ?</td>
</tr>
<tr>
<td>2. Diacetyl</td>
<td>Interacts with arginine-binding proteins.</td>
</tr>
<tr>
<td>3. Hydrogen peroxide / Lactoperoxidase</td>
<td>Oxidizes basic proteins.</td>
</tr>
<tr>
<td>4. Lactic acid</td>
<td>Undissociated lactic acid penetrates the membranes, lowering the intracellular pH.</td>
</tr>
<tr>
<td></td>
<td>It also interferes with metabolic processes such as oxidative phosphorylation.</td>
</tr>
<tr>
<td>5. Bacteriocins</td>
<td>Affect membranes, DNA-synthesis and protein synthesis.</td>
</tr>
</tbody>
</table>

**B-vitamins synthesis:**

Experiments on fermented milk products have revealed that lactic cultures require vitamins B for their metabolic activities. However, some lactic cultures synthesize B-vitamins. Friend et al. reported that the vitamins B content of fermented milk products was a function of species as well as the strain of lactic acid bacteria used in their manufacture. Similarly, vitamins B are synthesized by the lactic cultures in the gut microflora, in symbiosis with other flora.

It has been observed that the diet of the host influences the nature and levels of beneficial intestinal microflora, such as lactobacilli. The presence of dietary fructooligosaccharides was found to enhance the healthful effects of intestinal lactic acid bacteria. These compounds, found naturally in foods such as onions, edible burdock and wheat, are effectively employed as non-nutritive sweeteners (Neosugar, Meiologo®). They have the advantage of being indigestible by humans and farm animals, rendering them valuable in dietetic products. They are, however, selectively utilized by intestinal lactic acid bacteria, especially bifidobacteria, thereby enhancing the healthful effects of these beneficial intestinal flora.

**NUTRITIONAL AND THERAPEUTIC ASPECTS OF LACTOBACILLI**

Fermented milk products have been used therapeutically in the ancient systems of medicine in the Near and Middle East for centuries. However, the nutritional and therapeutic value of lactic acid organisms is still controversial. Several preclinical and
clinical studies have been performed, showing that fermentation of food with lactobacilli increases the quantity, availability, digestibility and assimilability of nutrients. A number of studies also show that ingestion of preparations containing lactobacilli lowers the serum cholesterol level in humans and animals\textsuperscript{4,8-11,21}. Fermented dairy products have also been claimed to inhibit tumor proliferation and to enhance the immune functions\textsuperscript{3,4,22,23}. Fermented dairy products and lyophilized lactobacilli preparations have also proved to be useful in the prevention and treatment of gastrointestinal disorders such as constipation and infections such as salmonellosis, shigellosis and antibiotic-induced diarrhea. \textit{Lactobacillus GG} has been useful in the treatment of recurring diarrhea caused by toxins produced by \textit{Clostridium difficile} \textsuperscript{24}. A preparation of \textit{Lactobacillus brevis} has been found to be effective in the treatment of recurrent headache\textsuperscript{25}. Lactobacilli have also been successfully used as adjuvants in the treatment of fungal and protozoal vaginitis and in the treatment of non-specific bacterial vaginitis\textsuperscript{26}. The nutritional and therapeutic benefits of lactobacilli are summarized in Figure 2.1.

### Figure 2.1

**Nutritional and Therapeutic Benefits of Lactobacilli**

- **Therapeutic benefits**
  - Restoration of the ecological balance of intestinal microflora
  - Alleviation of lactose intolerance
  - Enhancement of immunity
  - Detoxification of harmful products

- **Nutritional benefits**
  - Vitamins B production
  - Improved digestibility of food components and enhanced bioavailability of nutrients
  - Elimination of carcinogenic end products
  - Suppression of food-borne pathogens

**Nutritional Benefits**

Studies on rats have shown improved growth rate and increased feed efficiency when the rats were fed with yogurt containing lactobacilli\textsuperscript{22}. Improved feed efficiency in rabbits fed diets supplemented with \textit{L. sporogenes} has been reported.\textsuperscript{27} Although several lactobacilli require B-vitamins for growth, some of these organisms are capable of synthesizing B-vitamins\textsuperscript{16}. The levels of some of the B-vitamins in yogurt are shown in the Figures 2.2 (a,b).\textsuperscript{28} Similarly, bioavailability of copper, iron, calcium, zinc, manganese and phosphorus was increased in yogurt-fed rats\textsuperscript{4}. 
**Vitamin Content of Milk and Yogurt**

![Bar chart showing vitamin content comparison between milk and yogurt](image)

**Figure 2.2(a)**

**Figure 2.2(b)**

**Therapeutic Benefits**

Earlier research on indigenous microflora in animals and humans has shown their host-specificity and location-specificity, complexity in composition and their beneficial effects on the hosts. The important effects of probiotics as described in recent literature are summarized in Table 2.3:
Table 2.3

<table>
<thead>
<tr>
<th>CLAIMED APPLICATIONS OF PROBIOTICS IN ANIMALS AND HUMANS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APPLICATIONS IN ANIMALS</strong></td>
</tr>
<tr>
<td>Disturbance of indigenous microflora due to non-infectious disbacteriosis, antibiotic therapy and stress.</td>
</tr>
<tr>
<td>In neo-nates, with deficient development of intestinal microflora due to restricted access to the mother.</td>
</tr>
<tr>
<td><strong>Growth promotion:</strong></td>
</tr>
<tr>
<td>Higher feed conversion</td>
</tr>
<tr>
<td>Destruction of anti-nutritional factors</td>
</tr>
<tr>
<td>Synthesis of vitamins</td>
</tr>
<tr>
<td>Pre-digestion of proteins</td>
</tr>
</tbody>
</table>

| **APPLICATIONS IN HUMANS**                             |
| Disturbance of indigenous microflora due to non-infectious disbacteriosis, antibiotic therapy and radiation therapy. |
| In neo-nates, with deficient development of intestinal microflora due to confinement in neonate intensive care unit. |
| **Health promotion:**                                  |
| Inhibition of carcinogenesis                            |
| Anticholesterolemic effects                             |
| Increased calcium resorption                            |
| Decrease of lactose intolerance                        |
| Destruction of anti-nutritional factors                 |
| Synthesis of vitamins                                   |
| Pre-digestion of proteins                               |

Preparations containing lactobacilli have been shown to be effective in the treatment of a variety of disorders and infections including colitis, constipation, diarrhea, recolonization of the intestine with pathogens after treatment with antibiotics, flatulence, acidity, hepatic encephalopathy, tumorigenesis, hypercholesterolemia, headache and vaginitis.

1. **Hypercholesterolemia**

Coronary heart disease is often related to elevated serum cholesterol levels. Preclinical studies with laboratory rats fed fermented milk mixed with animal feed showed lower serum cholesterol levels as compared to rats fed with skim milk-supplemented feed. Pulusani and Rao\(^{30}\) showed that this difference could not be attributed to simple redistribution of cholesterol between the plasma, liver and other body pools. Another study showed that the consumption of *L. acidophilus* fermented milk supplemented with a commercial feed lowered serum cholesterol levels in weanling rats. In a study by Gilliland et al.\(^{31}\), pigs were fed a high cholesterol diet which increased their serum cholesterol levels. When these animals were simultaneously fed *L. acidophilus*, the rise in serum cholesterol levels was inhibited. Since *in vitro* studies showed that the organism assimilated cholesterol from the culture medium, the authors concluded that the *Lactobacillus* bound cholesterol to the intestinal lumen, thereby reducing its absorption.
into the blood stream. Compounds such as orotic acid, lactose, casein, and hydroxymethyl glutaric acid have been suggested to be hypocholesterolemic factors. Yogurt was also found to reduce dietary cholesterol-induced hypercholesterolemia in rabbits. Administration of *L. sporogenes* to rabbits fed high cholesterol diets to increase the serum cholesterol level resulted in 90% inhibition of rise in serum cholesterol. *L. sporogenes* in *in vitro* studies was found to assimilate cholesterol from the culture medium, suggesting that this organism could assimilate cholesterol directly from the gastrointestinal tract.

Studies by Hepner et al. on healthy human volunteers (with no history of cardiovascular disease) showed that dietary supplementation with yogurt decreased serum cholesterol. Mann and Spoerig surmised from clinical trials that the lower serum cholesterol levels in individuals from the Masai tribe of Africa could be attributed to high consumption levels of fermented milk.

**Mechanism of action:**

Emulsification of dietary fat is an intermediate process in fat absorption. Bile salts, together with phospholipids and cholesterol form micelles which helps in the absorption of cholesterol. Lactobacilli deconjugate the bile salts in the intestine to form bile acids and thereby inhibit micelle formation. This leads to decreased absorption of cholesterol. Cholesterol entering the intestine through the enterohepatic circulation is similarly treated. Lactobacilli elaborate the enzyme conjugated bile acid hydrolase (CBH), which hydrolyzes bile salts, and hydroxy steroid dehydrogenase (HSDH) which degrades bile acids and interrupts the enterohepatic circulation of bile acids. Another factor thought to be elaborated by lactobacilli is hydroxy methyl glutarate CoA (HMG CoA) which inhibits HMG CoA reductase, the rate limiting enzyme in endogenous cholesterol synthesis. All these factors collectively contribute to the hypocholesterolemic effects of lactobacilli as illustrated in Figure 2.3.
2. Lactose intolerance

Individuals with deficiency of the enzyme β-galactosidase (lactase) suffer from abdominal distress when they consume milk or dairy products. These individuals can, however, tolerate yogurt, as more than 50% of the lactose in yogurt is converted into lactic acid by the starter cultures during fermentation. Additionally, when yogurt is eaten, the inherent lactic acid bacteria release lactase in the gastrointestinal tract of the consumer.\(^{37}\)

Alm\(^{36}\) monitored the increase in serum glucose (derived from lactose) in control subjects and in lactose intolerant subjects who were given a 500 ml dose of milk or yogurt. When given milk, the lactose-intolerant subjects had a much lower rise in serum glucose as compared to the controls. This difference was marginal in the case of yogurt. In a separate study\(^{38}\), it was observed that administration of fermented *acidophilus* milk markedly decreased the breath hydrogen level in lactose-intolerant subjects when compared with the high breath hydrogen levels when taking unfermented milk. Another researcher noted that lactose-intolerant subjects given 18 grams of lactose in yogurt had only about one-third as much hydrogen excretion as in the case of the same amount of lactose in milk or water.\(^{39}\) There was significant lactase activity in the intestine one hour after ingestion of yogurt. *Lactobacillus sporogenes* was found to possess considerable β−galactosidase activity when tested *in vitro*.\(^{40}\) *In vivo* studies on the effect of yogurt and fermented milks on lactose digestion have been performed.\(^{41}\) The results revealed that all samples of yogurt tested dramatically and similarly improved lactose digestion regardless of their β-galactosidase activity. The response to fermented milks varied from marginal...
improvement with *B. bifidus* milk to nearly complete lactose digestion with *L. bulgaricus* milk.

*Mechanism of action:*

Lactobacilli provide the enzyme β–galactosidase which hydrolyzes lactose. The hydrolyzed lactose is converted to lactic acid as explained in an earlier section describing the metabolism of lactose in lactic acid bacteria.

3. **Hepatic encephalopathy**

Hepatic encephalopathy is a neurologic disorder associated with liver failure and elevated blood ammonia levels. The enzyme urease from intestinal proteolytic bacteria acts on amino acids, urea and other nitrogenous compounds leading to the production of ammonia. Under normal circumstances ammonia is absorbed and detoxified in the liver. However, in patients suffering from liver failure, the detoxification mechanism is impaired and the ammonia levels rise in the circulating blood. This depresses the nerve functions leading to hepatic coma and precoma. *L. acidophilus* was found to be effective in decreasing fecal urease level\(^4^2\). The use of lactobacilli in patients on long term treatment with neosporin produced an improvement in EEG and clinical status of 71% of these patients and a fall in blood ammonia in 60% of the patients tested\(^4^3\).

*Mechanism of action:*

Lactobacilli produce lactic acid and other substances creating a gastrointestinal environment which is not conducive for the growth of putrefactive organisms. This results in lower intestinal urease levels and consequently lower blood ammonia levels. In addition, the low pH due to lactic acid production disfavors the absorption of ammonia from the gut into the tissues and facilitates excretion of ammonia from the blood into the gut. This explains the usefulness of lactobacilli in the treatment of hepatic encephalopathy.

4. **Carcinogenesis**

Several preclinical and clinical trials have shown that fermented dairy products or the starter cultures used in their manufacture inhibit transplantable animal and human tumor lines.

These anti-tumor properties are based on\(^2^9\):

1. Inactivation or inhibition of carcinogenic compounds produced in the gastrointestinal tract by specific microorganisms.

2. Stimulation or enhancement of the immune response.

3. Reduction of intestinal bacterial enzyme activities- Some of these enzymes may convert procarcinogens into carcinogens.

Friend et al.\(^4^5\) investigated the inhibitory effect of yogurt on the proliferation of Ehrlich ascites tumor cells in male Swiss mice. They observed that feeding yogurt resulted in a 28% to 35% reduction of tumor cells when compared to control groups fed milk. DNA
synthesis in the tumor line of animals receiving yogurt was only 75% of that found in animals fed a commercial diet. Subsequently, Reddy et al.\textsuperscript{44} determined the antitumor effects of various yogurt components. Shahani et al.\textsuperscript{19} reported that feeding milk and colostrum fermented with \textit{L. acidophilus} resulted in 16% to 41% reduction in tumor proliferation.

In earlier studies, Bogdanov et al.\textsuperscript{23} observed that \textit{L. bulgaricus} possessed potent antitumor activity. They isolated three glycopeptides which showed biological activity against sarcoma-180 and solid Ehrlich ascites tumor. In the etiology of colon cancer, the conversion of procarcinogens to carcinogenic compounds by intestinal bacterial enzymes plays an important role, a phenomenon observed in predominantly meat-eating populations\textsuperscript{47}. These enzymes are β−glucuronidase, azoreductase and nitroreductase. One group of researchers\textsuperscript{48} found a marked decrease in the levels of these enzymes in rats fed diets orally supplemented with \textit{L. acidophilus}. These studies were extended in an animal model of colon cancer induced by the chemical carcinogen 1,2 dimethyl hydrazine (DMH). This compound is activated into a proximate carcinogen in the large bowel by the β−glucuronidase produced by intestinal flora\textsuperscript{50}. DMH-treated animals were given \textit{L. acidophilus} in powdered form and compared with controls. At 20 weeks, 40% of the \textit{L. acidophilus}-treated animals had tumors compared to 77% of the control animals (p < 0.2) while at 36 weeks, 73% of the \textit{L. acidophilus} animals and 83% of the control animals had tumors\textsuperscript{4}.

These results show that \textit{L. acidophilus} can prolong the induction of colon tumors. In a more recent study\textsuperscript{4}, the same authors found that oral \textit{L. acidophilus} supplementation to the diet in rats lowered the amount of carcinogenic amines excreted in the feces after feeding procarcinogen precursors to these animals. The evidence to date suggests that lactobacilli may slow tumor development in laboratory animals. However, there is no conclusive evidence to suggest that lactobacilli or their fermented products can prevent cancer in humans.

\textbf{Mechanism of action:}

Putrefactive colonic microflora produce the enzymes β−glucuronidase, azoreductase and nitroreductase which convert procarcinogens to carcinogens. Lactobacilli, by competitive inhibition and the production of non-conducive acidic environment, suppress the metabolic activity of colonic microflora and in this manner may reduce the formation of carcinogens in the large intestine.

Lactic acid bacteria suppress carcinogen-induced mutations. \textit{In vitro} studies with mutagens such as 4-nitroquinoline-N-oxide\textsuperscript{51} revealed that the bacterial cells themselves as well as their metabolites possessed anti-mutagenic action. It has been suggested that some bacterial cell wall fractions possess anti-mutagenic activity\textsuperscript{53}.

\section*{5. Intestinal Infection}

A variety of studies have examined the proposition that lactobacilli and dairy products fermented with lactobacilli can alleviate gastrointestinal disorders. The results however, are inconsistent, probably due to differences in \textit{Lactobacillus} strains used as well as variation in conditions of preparation and storage of cultures or fermented products.
Results showing significant amelioration of diarrheal symptoms of salmonellosis in weanling rats\textsuperscript{49}, salmonellosis\textsuperscript{54} and shigellosis\textsuperscript{55,56} in children have been reported. Antibiotic-induced diarrhea was prevented by use of \textit{Lactobacillus}\textsuperscript{57}. The \textit{Lactobacillus} species used in these studies were \textit{L. acidophilus} and/or \textit{L. bulgaricus}. Administration of another strain of \textit{Lactobacillus}, which produces a broad spectrum bacteriocin, \textit{Lactobacillus} GG, helped relieve symptoms of relapsing \textit{Clostridium difficile} colitis\textsuperscript{24} resulting from antibiotic treatment for an infection.

Lactobacilli, particularly \textit{L. acidophilus}\textsuperscript{58} and \textit{L. sporogenes}\textsuperscript{59,60} have also been used in the treatment of chronic constipation and flatulence.

\textit{Mechanism of action:}

Lactobacilli, through the production of lactic acid and bacteriocins create an intestinal environment which is not conducive for the growth of pathogens. Lactic acid also helps relieve constipation by improving the bowel movement.

6. \textbf{Immune response system}

Antibodies against intestinal bacteria are commonly detected in healthy humans. These antibodies are produced when the host is stimulated by the antigens of the intestinal bacteria. Defense tissues such as the thymus, lymph nodes, spleen and bone marrow are well developed in conventional mice, but poorly developed in germ free mice, indicating the influence of intestinal flora on the host’s immune response. In more detailed studies with germ-free animals that were fed yogurt, an increase in the levels of immunoglobulins, IgG\textsubscript{1}, IgG\textsubscript{2a}, IgG\textsubscript{2b} and IgM were detected in the serum\textsuperscript{61}.

Recent studies have shown that \textit{L. brevis} sub species \textit{coagulans}, may enhance the body’s capacity to produce alpha interferon, natural killer (NK) cell activity and 2-5A-synthase enzyme activity, each important aspects of the body’s natural defenses. Scientists at the Institut Pasteur de Kyoto\textsuperscript{62} showed that when 10 healthy adults consumed this bacterial supplement, their average producing capacity of alpha interferon increased 65\% after two weeks and 59\% after four weeks. In the same time frame, natural killer cell activity increased 68\% and 47\%, as shown in Figure 2.4.

![Figure 2.4](image-url)
The US Food and Drug Administration has approved alpha interferon for use in treating
certain types of cancer, hepatitis and genital warts.

7. **Treatment of recurring headaches:**

In many patients suffering from various forms of migraine headache, prophylaxis by
antiserotonin agents, although successful in 60-70% of the cases, may result in the
serious complication of retroperitoneal fibrosis. The use of orally administered capsules
containing *L. acidophilus* was tried by one group of researchers in a series of 20 patients.
Of 16 patients followed up over a period of 1-2 months, nine reported complete relief or
considerable reduction in frequency of attacks. In three others, in whom the frequency
did not decrease, there was definite reduction in the severity of the headaches. No
adverse symptoms were noted.

8. **Treatment of aphthous stomatitis and glossitis**

Lactobacilli have been effectively employed in the treatment of aphthous stomatitis and
glossitis. These conditions arise due to imbalance in the intestinal flora resulting in B
vitamins deficiency. The administration of lactobacilli to colonize the gut is helpful in
this condition.

9. **Treatment of vaginitis**

Vaginal infections can be caused by a variety of organisms of which *Trichomonas
vaginalis*, a protozoan parasite, and *Candida albicans*, a yeast-like fungus are the chief
non-bacterial organisms responsible. No single bacterial species is responsible for
vaginal infection and hence this type of infection is called non-specific vaginitis (NSV).
Symptoms include a grey vaginal discharge, an unpleasant amine smell and “clue” cells,
*viz.*, vaginal epithelial cells coated with Gram-variable bacteria. NSV is the most
frequent type of vaginal infection. A notable symptom is a significant reduction in the
numbers of lactobacilli present and a proliferation of other bacteria in the vaginal
environment, including *Gardrenella vaginalis* and anaerobes.

Lactobacilli are natural inhabitants of the vaginal mucosa. The predominant species is *L.
acidophilus*. Lactobacilli maintain the vaginal pH in the range 4.0-4.5 through glycogen
fermentation to lactic acid. This establishes an environment unfavorable for the growth of
pathogens. The level of glycogen in the epithelial cells of the vagina is controlled by
circulating estrogens.

Prophylaxis by oral administration of *L. acidophilus* as well as treatment by intravaginal
application of lactobacilli have proved to be effective in the treatment of vaginitis. A
commercial formulation using *L. sporogenes*, trade marked MYCONIP®, is successfully
marketed for this indication.

10. **As adjuvant to antibiotic treatment**

The microecological balance of the gut flora is disturbed by treatment with antibiotics.
Some of the beneficial flora are killed and on stopping treatment, pathogens begin to
reestablish themselves in the intestine. Overgrowth of these organisms and the
subsequent invasion of the system by yeast like *Candida albicans* cause inflammatory, immunologic, neurologic and endocrinologic problems. This occurs due to proliferation and toxin production by these organisms in the host tissues. Administering lactobacilli along with antibiotics helps to prevent this syndrome.\textsuperscript{65,66} The lactobacilli through their metabolic activities establish themselves in the gut, vaginal or oral environment and provide conditions which are non-conducive to the growth of pathogens. *Lactobacillus* therapy is essential after treatment with anti-amoebic drugs.

Drugs such as estrogens and oral contraceptives, if administered during antibiotic treatment, have a significant failure rate. If lactobacilli are administered concurrently, they provide essential intestinal microflora which can correct this situation by deconjugating drug complexes and keeping the drug in circulation.\textsuperscript{47}

11. **Growth-promoting effect of probiotics**

Lactobacilli have been recommended for veterinary use, being effective in restoring the gastrointestinal microecological balance and helping in the establishment of healthy rumen flora.\textsuperscript{67} This in turn results in improved health and growth of farm animals.

*Mechanism of action:*

By reducing the intestinal ammonia concentration and by preventing intestinal infections caused by putrefactive organisms, lactobacilli, particularly *L. sporogenes* are effective growth promoters for chicks and domestic animals. This explains the use of probiotics in animal and poultry feeds.\textsuperscript{67} In experimental trials with probiotics, it has been found that the effectiveness of treatment depends upon factors such as type, viability and composition of the implanted lactobacilli; type of dosing; and type and age of the recipient animal.\textsuperscript{29}

12. **Anti-HIV activity of lactic acid bacteria**

A journal article hypothesizes that live *Lactobacillus* cultures may be used therapeutically in patients suffering from AIDS. This hypothesis is based on the enhancement of antimicrobial resistance, immunomodulatory action and the anabolic effect caused by the consumption of live lactobacteria, as described in earlier sections of this review.
**Lactobacillus sporogenes**: A SUPERIOR PROBIOTIC

*L. sporogenes* was first isolated and described in 1933 by L.M. Horowitz-Wlassowa and N.W. Nowotelnnow and the name was accepted in the fifth edition of “Bergey’s Manual of Determinative Bacteriology”. Later, it was transferred to *Bacillus coagulans* in the seventh edition of Bergey’s manual due to simplification in cataloging. However, in honor of the original discoverers, the name *Lactobacillus sporogenes* is used widely, except for taxonomical purposes. According to the Eighth Edition of Bergey’s Manual of Determinative Bacteriology, “Various spore-bearing rods which produce lactic acid, are facultative or aerobic and catalase positive, have generally and correctly been assigned to the genus *Bacillus*.”

The characteristics of *L. sporogenes* 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 homofermentatively.” Since *L. sporogenes* exhibits characteristics typical of both genera *Lactobacillus* and *Bacillus*, its taxonomic position between the families *Lactobacillaceae* and *Bacillaceae* has often been discussed. This, along with the fact that there is no universally accepted official classification leaves room for controversy in the nomenclature. Some authors refer to *L. sporogenes* as *Bacillus coagulans*, although there seems to be no documented similarity between these organisms. The differentiation characteristics of *L. sporogenes* are indicated in Table 2.4:

<table>
<thead>
<tr>
<th>Property</th>
<th>Bacillus</th>
<th>Lactobacillus</th>
<th>Sporolactobacillus</th>
<th>L. sporogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Benzidine</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NA&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nitrate red.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NA&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gram-reaction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endospores</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>-&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>m-A&lt;sub&gt;2&lt;/sub&gt;PM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>-&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fatty acid</td>
<td><strong>Bacillus-type</strong></td>
<td><strong>Lactobacillus-type</strong></td>
<td><strong>Bacillus-type</strong></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> *L. plantarum* may be motile and contains m-A<sub>2</sub>PM<sup>c</sup> in its cell wall

<sup>b</sup> Some species including *B. coagulans* can produce lactic acid

<sup>c</sup> meso-diaminopimelic acid, <sup>d</sup> data not available

*L. sporogenes* grows in the temperature range of 35° C to 50° C; the optimum pH range is 5.5-6.5. Unlike other lactobacilli currently in clinical use, *L. sporogenes* can form spores. Sporulation is the development in microorganisms of bodies each wrapped in a protective coat (a natural process of microencapsulation in a calcium-dipicolinic acid-peptidoglycan complex). Under favorable conditions, the spores germinate into viable bacilli and carry on their life activities. The spores of *L. sporogenes* are ellipsoidal bodies.
measuring 0.9 to 1.2 by 1.0 to 1.7 microns. Their morphology and formation are schematically represented in Figures 2.5 and 2.6.

Figure 2.5: Schematic representation of the bacterial spore
This property of spore formation by *L. sporogenes* is the main characteristic that makes it the probiotic of choice in clinical applications. On oral administration, these spores survive the acidic gastric environment and are activated due to the low pH, mechanical churning action of the stomach and the water in the gastric environment. The spore coats imbibe water, swell, and the increased water content causes a rise in the metabolic rate of the sporulated bacilli. Outgrowths begin to protrude from the spore-coats. The spores pass on to the duodenum where the outgrown cells germinate and transform into viable vegetative cells. They begin to proliferate in the small intestine, multiplying rapidly. Usually, germination takes place about four hours after ingestion. A large supply of viable *L. sporogenes* is thereby ensured in the small intestine. These cells settle in the intestinal tract and continue their metabolic activities, producing lactic acid and probably bacteriocins, which render the intestinal environment non-conducive for the growth of harmful pathogenic bacteria. The maintenance of a low, constant level of lactic acid on the inner surface of the intestinal tract helps restore the microecological balance after antibiotic therapy. Antibiotic therapy may kill beneficial microbes which help in the synthesis of vitamins B and digestive enzymes. Since *L. sporogenes* produces only L(+)lactic acid, it does not cause metabolic acidosis.

**Morphological and physiological characteristics of *L. sporogenes***

The vegetative cells are rods occurring singly, rarely in short chains, the filaments varying with cultural conditions. The cells are motile by means of flagella.
Carbohydrate fermentation:

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Acid production</th>
<th>Gas production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Properties of the spores

The spores of *L. sporogenes* are resistant to heat and other adverse environmental conditions, surviving even under a temperature of 100° C for twenty minutes in phosphate buffer at pH 7. The spores germinate in malt broth even in the presence of dilute hydrochloric acid (at pH 4.6 to 5.6), caustic soda solution (pH 7.6-9.6), saline solution (5%, 10%, 20% concentration), a 2.5% solution of boric acid as well as distilled water. The spores are two to eight times more resistant to antibiotics than the vegetative cells.
LIMITATIONS OF *L. acidophilus* AS THE SPECIES OF CHOICE IN *Lactobacillus* THERAPY

To be effective in the therapeutic situations described above, the *Lactobacillus* species should be efficiently implanted in the intestine following oral administration. This requires that the cells survive the rigors of preparation in dosage forms, storage and passage through the acidic gastric environment. On reaching the intestine, these cells should be able to establish themselves, remain viable, carry on their normal metabolic activities and proliferate extensively to perform their antagonistic functions against pathogens, for prolonged periods of time.

*L. acidophilus* has been the probiotic of choice for several years. However, the effectiveness of treatment with this species is uncertain. Although standards for *L. acidophilus* are established in the US, it has been reported that such preparations contain less than 1000 cells of viable bacteria against claims of billions. Besides, some probiotic supplements claiming to have viable cells of *L. acidophilus* present in large numbers have only very low numbers and others that claim to have one species of *Lactobacillus*, have a totally different species.

The evidence for the effectiveness of *L. acidophilus* probiotics as antidiarrheal agents is not convincing. Attempts at administration of *L. acidophilus* preparations as a prophylactic against infantile diarrhea have proven unsuccessful.

Quoting from a US Expert Panel report:

“...In the past 60 years, well over 200 papers have reported on the use of *Lactobacillus acidophilus* and other *Lactobacillus* organisms in the treatment of diarrhea. Despite the proliferation of studies, the very few controlled studies often show lack of effectiveness.”

The unsuccessful record of *L. acidophilus* and other lactobacilli in this context could be due to their failure to implant themselves in the intestine and proliferate sufficiently to perform their healthful activities. Another important factor against *L. acidophilus* as a probiotic is that its cells do not survive lyophilization. The freeze-dried cultures have to be stored under refrigeration and do not retain viability under normal conditions. According to Black, the survivability in low pH and bile of the gastrointestinal tract, moisture, high temperature and oxygen, are all parameters with lethal effects on lyophilized *L. acidophilus*.

The search for an effective L(+) lactic acid providing bacterial species for use as a probiotic has included clinical trials with the commonly used lactobacilli in addition to *L. acidophilus*. These include: *L. bifidus*, *L. casei*, *L. brevis* sub species *coagulans*, *L. bulgaricus* and *S. faecalis*. However, none of these cultures have provided consistently successful results.

The alternatives are:

1. To use a genetically engineered *Lactobacillus* capable of producing L(+) lactic acid with the required characteristics.
2. To use an effective spore-bearing species which can survive the gastric acidity and proliferate in the intestine, with viable cells being observed in the feces of the host, long after probiotic treatment.

Alternative 1 is as yet unavailable. The second alternative favors the use of *L. sporogenes* as a probiotic. *L. sporogenes* spores are stable to heat, acids, bile and other deleterious chemicals. Negligible falls in viable counts were observed experimentally at 40 degrees C and a relative humidity of 80% for two months, when heated with water at 60 to 90 degrees C for 30 minutes and when tested at pH of 2 for varying durations.
BENEFITS OF *Lactobacillus sporogenes* AS A PROBIOTIC

Clinical studies have revealed that *L. sporogenes* can be successfully implanted in the intestine. As explained in an earlier section, *L. sporogenes* satisfies the essential requirements of an efficient probiotic. Preparations of *L. sporogenes* in pharmaceutical dosage forms such as tablets, capsules, dried granules or powder have the following characteristics:

1. Contain a large number of viable lactobacilli that retain viability during preparation in pharmaceutical dosage forms and during storage before consumption. The spores are thermostable as against viable *L. acidophilus* cells which may not withstand lyophilization.
2. Survive in gastric secretions and bile of the upper digestive tract and reach the intestine safely.
3. Settle in the digestive tract and produce enough lactic acid and other antagonistic substances to inhibit the growth of pathogenic bacteria.

Being sporulated, they germinate under favorable conditions and produce sufficient viable cells which proliferate and perform vital healthful functions as described earlier. In addition, *L. sporogenes* spores are semi-resident and are slowly excreted out of the body (7 days after discontinuation of administration).

*L. sporogenes* is effective in the form of dietary supplements as well as when added to food products. *Natto* is a traditional fermented product from soya bean, consumed widely in Japan as a rich source of protein. Its flavor is improved by the incorporation of lactic acid bacteria such as *L. sporogenes*, *L. acidophilus* or *Pediococcus acidilactiti* to the starter culture (*Bacillus natto*), to yield a product called *yogurunatto* having superior flavor and storage characteristics as well as improved nutritional and therapeutic properties. A nutritive medium (homogenized mushroom, *Lentinus edodus*) is mixed with soya beans and fermented to yield this health food product. The requirement of availability of a number of viable lactic acid bacteria is fulfilled by using *L. sporogenes*.

CLINICAL STUDIES

1. GASTRO-INTESTINAL AND ASSOCIATED EFFECTS:

*Clinical trials performed in Japan with LACBON*[^60][L. sporogenes]*

Reports from various hospitals that performed clinical trials on groups of patients suffering from a variety of intestinal disorders and allergic skin diseases are summarized in Table 5.1.

It is evident that administration of *L. sporogenes* markedly improved the general clinical condition of the subjects and provided relief from intestinal disorders and allergic skin
conditions. Allergic skin conditions can be related to imbalance of intestinal flora in the subject. *L. sporogenes* therapy was helpful in such conditions.
### TABLE 5.1: SUMMARY OF SELECTED CLINICAL REPORTS FROM JAPAN: TRIALS WITH LACBON® (*L. sporogenes*)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of subjects</th>
<th>Treatment: <em>Lactobacillus</em> spores (millions)</th>
<th>Effectiveness Rates</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute and chronic intestinal catarrh</td>
<td>38</td>
<td>100-600 / day in divided doses for 2-12 days</td>
<td>86.8%</td>
<td>Recovery from diarrhea to regular normal stools; general symptoms including anorexia improved.</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>15</td>
<td>75-600 / day in divided doses for 3-12 days.</td>
<td>100.0%</td>
<td>Recovery from diarrhea to regular, normal stools from third to fourth day.</td>
</tr>
<tr>
<td>Constipation</td>
<td>10</td>
<td>300-750 / day in divided doses for 2-10 days</td>
<td>70.0%</td>
<td>Recovery to normal stools and disappearance of abdominal distention.</td>
</tr>
<tr>
<td>Abnormal intestinal fermentation</td>
<td>9</td>
<td>300-600 / day in divided doses for 3-14 days</td>
<td>100.0%</td>
<td>Vomiting and nausea disappeared; appetite improved; stools became normal and regular; diarrhea and stomach ache cured.</td>
</tr>
<tr>
<td>Dyspepsia infantum</td>
<td>26</td>
<td>100-200 / day in divided doses for 1-7 days</td>
<td>84.6%</td>
<td>General conditions and nature of stools improved. Frequency of stools decreased to half or less than that before medication.</td>
</tr>
<tr>
<td>Allergic skin diseases</td>
<td>5</td>
<td>200-450 / day in divided doses for 4-12 days</td>
<td>80.0%</td>
<td>Obvious eruptions of strophulus and eczema decreased from the third day (topical therapy employed concomitantly).</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>10</td>
<td>20-50 / day in divided doses for 4-20 days</td>
<td>80.0%</td>
<td>Response seen in anorexia of nervous type and malnutrition in infants.</td>
</tr>
</tbody>
</table>

The above data are cited from clinical reports by: Terumichi Kuniya, Pediatric Clinic of Shinko Hospital, Kobe; Jetsuo Nitta, Medical Clinic of Kugason Hospital; Goro Koide, Pediatric Clinic of Kanto Teishin Hospital; Michio Ogasawara, Medical Clinic of Kahoku Hospital; Susumu Nakazawa, Pediatric Clinic of Ebara Hospital.
**Studies in India with SPORLAC® (L. sporogenes)**

A total of 60 cases of neonatal diarrhea with watery stool frequency greater than 6 were examined for efficacy of SPORLAC treatment. Based on the suggested dosage level of SPORLAC at 5 million spores per kilogram body weight, each neonate was given a spore level of about 15 million spores per day. Some of the subjects had associated symptoms in addition to diarrhea:

- Jaundice: 3
- Septicemia: 3
- Cord Infection: 3
- Vomiting: 3

Most of the subjects (about 80%) had a history of breast-feeding. About 19% were both breast and bottle-fed and 1% were bottle-fed.

The average duration for recovery was 1.8 days and the results of the study are tabulated:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cases treated</th>
<th>Cases Cured</th>
<th>Success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>60</td>
<td>49</td>
<td>81.7%</td>
</tr>
<tr>
<td>Constipation</td>
<td>3</td>
<td>3</td>
<td>100%</td>
</tr>
<tr>
<td>Jaundice</td>
<td>3</td>
<td>3</td>
<td>100%</td>
</tr>
</tbody>
</table>

As compared to the normal practice of administration of antibiotic and antidiarrheal mixtures, the complicating side effects were not seen in the series of SPORLAC trials. The average recovery time of 1.8 days helped to reduce dehydration in the subjects to a great extent.

In a similar study in Japan (cited in 59) a comparable success rate of 78.4% with SPORLAC treatment for infantile diarrhea was obtained. An earlier study in India by Mathur et al. (cited in 59) found the average time for improvement in diarrheal conditions to be two to three days, with treatment.

### 2. Hypercholesterolemic Effects:

Short term hypolipidemic effects of oral *L. sporogenes* therapy (360 million spores per day in tablet form) were studied in 17 patients (15 men and 2 women in the 32-61 year age group) with type II hyperlipidemia in an open label fixed dose trial. Total serum cholesterol, LDL-cholesterol and total cholesterol to HDL-cholesterol and LDL-cholesterol to HDL-cholesterol ratios (p < 0.001) was reduced significantly over a period of three months. HDL-cholesterol was marginally increased (43.6±7 mg/dl vs 46.8± 8.9
mg/dl, p < 0.05). there was however no change in serum triglyceride levels. The results are shown in Figure 5.1.

![Figure 5.1](image)

**Hypocholesterolemic effects of *L. sporogenes*: clinical studies**

Atherogenic lipid ratios observed were as follows:

- Total / HDL-cholesterol 24.0% decrease
- LDL / HDL-cholesterol 33.4% decrease

No adverse effects of therapy were noted, except constipation in one patient.

3. USE OF *L. sporogenes* IN THE TREATMENT OF NON-SPECIFIC VAGINITIS:

Non-specific vaginitis is caused by a variety of pathogens including staphylococci, streptococci, pneumococci and *E. coli*. It may also be induced by a variety of causes including chemicals, drugs, surgical procedures, trauma and foreign bodies. *L. sporogenes* administration to increase the vaginal acidity by the action of the *Lactobacillus* on glycogen in the vaginal epithelial tissues was adopted in a clinical trial on 44 patients\(^\text{26}\). The patients were divided into two groups:

- **Group 1**: Twelve patients suffering from leucorrhea (white discharge) following cervical surgery.
- **Group 2**: Thirty two patients with nonspecific vaginitis without previous therapy. Of these, 26 were in the reproductive age and 6 were menopausal. The change in vaginal pH following treatment with MYCONIP® (*L. sporogenes* tablets) over a period of two weeks is shown in the Figure 5.2:
Figure 5.2

Most of the cases showing persistently alkaline pH were post-menopausal, where acid could not be produced in sufficient amounts due to low substrate glycogen levels. Glycogen levels depend upon circulating estrogen.

The response to treatment in both groups is tabulated below:

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Quick response and complete</td>
<td>8 (67%)</td>
<td>26 (81.25%)</td>
<td>34 (77.25%)</td>
</tr>
<tr>
<td>relief</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Delayed response but complete</td>
<td>2 (16.5%)</td>
<td>4 (12.5%)</td>
<td>6 (13.60%)</td>
</tr>
<tr>
<td>relief</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Improvement but not complete</td>
<td>2 (15.5%)</td>
<td>2 (6.25%)</td>
<td>2 (9.15%)</td>
</tr>
<tr>
<td>relief</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. No relief</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12 (100%)</td>
<td>32 (100%)</td>
<td>44 (100%)</td>
</tr>
</tbody>
</table>

In comparison with a clinical trial using M.T.P. vaginal pessaries containing broxyquinoline and brobenzoxeldine where only 26.67% of cases studied were cured, it can be seen that *L. sporogenes* therapy is the better alternative in the treatment of non-specific vaginitis. *L. sporogenes* therapy provided complete relief to 91% of the patients and partial relief to approximately 9%.

4. CLINICAL TRIALS IN THE TREATMENT OF APHTHOUS STOMATITIS AND GLOSSITIS

Sharma et al. noted that SPORLAC® therapy is one of the best available methods to treat recurrent oral ulcerations. At the dosage level of two tablets thrice daily for five days, (corresponding to 120 million spores of *L. sporogenes* per day), aphthous stomatitis was cured in two to three days.
**L. sporogenes AS A VETERINARY PROBIOTIC**

The effects of *L. sporogenes* on the growth performance and changes in microbial flora of the feces and intestinal contents of broiler chicks were investigated. It was found that body weight gain of chicks fed *L. sporogenes* at the level of 0.04% was significantly higher than that of the control. Feed efficiency was remarkably improved by the addition of *L. sporogenes* at the level of 0.04% and Zn-bacitracin at the 0.05% level. The *L. sporogenes* fed group showed better feed efficiency. Intestinal pH was reduced and fecal ammonia concentrations were slightly reduced. In feces, *L. sporogenes* and lactobacilli counts were increased and staphylococci and coliforms reduced by the addition of *L. sporogenes*.

The recommended dosages of probiotic *L. sporogenes* are as follows:

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Recommended dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicks</td>
<td>1 - 2 million spores (0.07-0.14 mg LACTOSPORE&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Growers</td>
<td>3 - 6 million spores (0.21-0.42 mg LACTOSPORE&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Layers/broilers</td>
<td>6 - 12 million spores (0.42-0.84 mg LACTOSPORE&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Pups</td>
<td>30 - 60 million spores (2.1-4.2 mg LACTOSPORE&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Dogs</td>
<td>100 - 200 million spores (7-14 mg LACTOSPORE&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Sheep, goats, calves</td>
<td>300 - 400 million spores (21-28 mg LACTOSPORE&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Cattle and horses</td>
<td>800-1000 million spores (56-70 mg LACTOSPORE&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>
TOXICOLOGICAL ASPECTS OF LACTOBACILLI

No toxic effects of Lactobacillus administration have been reported, except for metabolic acidosis in cases of ingestion of Lactobacillus preparations with the D (-) optical isomer of lactic acid, especially in association with unbalanced diets. A limit of 200 ml per 10 kg body weight of this isomer per day has therefore been recommended for young children.

No adverse reaction to L. sporogenes has been observed in either clinical or nonclinical studies.

1. Acute toxicity studies:

Acute toxicity studies with L. sporogenes were performed in male mice fed 1, 3 or 5 g/kg of LACBON powder containing not less than $5 \times 10^9$ spores of L. sporogenes for 7 days orally. No deaths occurred, nor was there any abnormality such as diarrhea.

2. Sub-acute toxicity studies:

Male rats were fed LACBON orally at the level of 0.3, 3 and 5 g/kg/day for 1.5 months. Body weight gains for treated groups were similar to those for the control group. Changes in organ weight showed no significant differences between treated and control groups.
**TESTING PROCEDURES AND STABILITY OF *L. sporogenes* CULTURES**

*L. sporogenes* cells are protected from destruction by environmental factors by the naturally-present microencapsulation system, the spore coat. In probiotic therapy, for successful implantation in the gastrointestinal tract, a very large number of viable lactobacilli is essential. In the case of non-sporulated species, attempts are made to retain the viability of the vegetative cells during prolonged storage by freeze-drying. These lyophilized cells have to be stored under adequate refrigeration and are susceptible to environmental damage. *L. sporogenes* spores can be stored at room temperature without loss of viability.

The methodology employed for testing the viability of these spores and conformance to specifications is outlined in the following pages:

The tests involved include:

Identification tests, viable lactobacillus spore count, estimation of the lactic acid producing capacity, determining loss on drying and ensuring the absence of contaminants.

1. **Identification tests:**
   a) **Description:**
   
   A white to grayish powder with characteristic odor and slightly sweet taste.
   
   b) **Microscopic examination:**
   
   Suspend a small quantity of *L. sporogenes* powder in about 5 ml sterile normal saline solution in a test tube and mix well.

   Prepare a wet mount on a glass slide by using one loopful of suspension as prepared above; cover with a coverslip.

   Examine the wet mount using a phase contrast microscope.

   The spores are seen as small, terminal, oval shaped refractile bodies within the dark vegetative cells.

   c) **Qualitative test for lactic acid production:**

   As described under “viable spore count” determination (see below), after incubation at 37°C for 48 hours, select the colonies with a platinum wire loop and transfer aseptically to a tube containing 20 ml of previously sterilized and cooled glucose yeast extract liquid medium. Incubate the tube at 37°C for 48 hours and then centrifuge at 2500-3000 rpm for 10 minutes. Transfer the clear supernatant liquid to a separatory funnel and extract by adding 5 ml of dilute sulfuric acid (10%) and 50 ml of ether. Collect the ether layer, evaporate in a water bath carefully to dryness and dissolve the residue in 5 ml of water. Add the solution drop-wise to Uffelman’s reagent, prepared by adding two drops of 1N
ferric chloride to 10 ml of 1% phenol solution. The color of the solution turns from bluish violet to yellow, indicating the presence of lactic acid.

2. Viable lactobacillus spore count:
The number of viable spores in a sample is determined by the following procedure:

(1) Dilution and heat-treatment
Weigh out 1 gm *L. sporogenes* powder sample into a surface-sterilized homogenizing container (jar), add 200 ml of sterile physiological saline and homogenize at about 12000 - 15000 rpm for 5 minutes.
Transfer 1 ml of the homogenized suspension into a 9.0 ml sterile physiological saline in a screw capped tube (25mm by 150mm size) and mix thoroughly.
Repeat this serial dilution until a dilution of 10^-6 dilutions are obtained. This is called the dilution factor.
Allow the final tube to stand in a water bath at 70° C for 30 minutes and then cool immediately to about 45° C.

(2) Plating
Liquify GYE (Glucose Yeast Extract) agar medium and cool to 45°C in a water bath. Set out sterile petri dishes, five per sample to be tested. Add 1 ml from the heat treated final dilution tube into each petri dish and then pour 15 ml of the molten medium into each of the petri dishes and mix thoroughly. When solidified, incubate the plates in an inverted position at 40°C for 48 hours.

(3) Counting
The plates showing 30 - 300 colonies are ideal for counting. Select and count up to six plates and average the count per plate. Calculate the number of viable cells per gram of sample by multiplying the average number of colonies counted per plate by the reciprocal of the dilution factor.
For example,
Average number of colonies per plate = 30
Final dilution factor = 200x10^-6,
The viable spore count is 30x200x10^-6 = 6000 million viable spores per gram.

Preparation of diluent:
Physiological saline is prepared by dissolving 8.5 gm sodium chloride and 15 mg sodium lauryl sulfate in 1000 ml of distilled water. The solution is sterilized with steam at 1.2 kg/cm² pressure at 120°C for 15 minutes and then cooled.

Preparation of GYE agar medium:
Glucose Yeast Extract agar medium has the following composition:

| Yeast Extract Powder (Difco) | 5.0 gm |
| Casitone (Peptone) (Difco)   | 5.0 gm |
| D-glucose (Difco) | 3.0 gm |
| K$_2$HPO$_4$ | 0.5 gm |
| KH$_2$PO$_4$ | 0.5 gm |
| MgSO$_4$ | 0.3 gm |
| Trace Mineral Solution* | 1.0 ml |
| Distilled water | 1000 ml |
| Agar (to be added after pH adjustment) | 15.0 gm |

Adjust pH of the medium to 6.3 using pH meter and sterilize medium with steam at 1.2 kg/cm$^2$ pressure at 120° C for 15 minutes.

**Preparation of trace mineral solution:**

| NaCl | 500mg |
| MnSO$_4$.5H$_2$O | 500mg |
| ZnSO$_4$.7H$_2$O | 80mg |
| CuSO$_4$.5H$_2$O | 80mg |
| CoSO$_4$.7H$_2$O | 80mg |
| Distilled water | 50ml |

Weigh accurately the required quantity of salts and add a small quantity of distilled water and dissolve well. Raise the volume to 50ml in a volumetric flask. The solution will attain a pink color. This solution can be kept in a refrigerator for 2 months.

**3. Lactic acid producing capacity**

To an accurately weighed 1.0 gm of *L. sporogenes* powder, add exactly 100 ml of sterile normal saline solution and homogenize at about 1200-1500 rpm. for 7-10 minutes. This makes a test solution of 1 : 100 dilution.

Transfer a small quantity of the test solution (about 10 ml) into a sterile test tube (25 mm x 150 mm) and allow to stand in a water bath at 75°C for 30 minutes. Cool immediately to 45-50°C. Pipette exactly 1.0 ml of the solution in 10 ml of GYE liquid medium previously sterilized and cooled to room temperature. Incubate the tubes at 37° C for 48 hours. Duplicate tubes are used for this purpose.

After incubation, titrate the lactic acid produced with 0.05N sodium hydroxide using bromothymol blue neutral red indicator. To meet specifications, not less than 10 ml of 0.05 N sodium hydroxide must be consumed in the titration.

**4. Loss on drying:**

A one gram sample of *L. sporogenes* powder, when dried at 100° for 3 hours should lose not more than 8 % of its weight.
5. Detection of *E. coli* and other coliform bacteria in Lactospore® powder

**Procedure**
Weigh 1.0 gm dry *L. sporogenes* powder and mix with 10 ml of sterile water in a 20 ml tube (25mm x 150 mm size), using a blender. Add 1 ml of this suspension into a petri dish and add about 10 ml of PC agar medium, mix well and allow to set. Then add 10 ml of PC agar medium to cover the earlier layer, spread evenly and allow to set and then incubate at 35°-37° C for 24 hours. Run the assay in triplicate.

**Results**
If there is *E. coli* contamination or if there are other coliform bacteria, they can be detected due to a change in color of the medium. *Proteus* and bacteria in intestinal flora produce pink colonies. *E. coli* produces dark red colonies.

**Preparation of P.C. medium:**
Prepare PC medium of following composition:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.00 gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.00 gm</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.00 gm</td>
</tr>
<tr>
<td>Na-deoxycholate</td>
<td>1.00 gm</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>2.00 gm</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>2.00 gm</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.03 gm</td>
</tr>
<tr>
<td>Agar</td>
<td>17.00 gm</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.1</td>
</tr>
</tbody>
</table>

Add peptone, NaCl, lactose, ferric ammonium citrate, KH$_2$PO$_4$ and neutral red to about 800 ml of water and dissolve them well. Separately dissolve Na-deoxycholate in an adequate amount of water and dissolve well. Combine both the solutions and raise the volume up to 1000 ml. Adjust the pH to 7.3± 0.1 and add agar. Dissolve by heating with constant stirring. Then pour 20ml of the medium into each test tube and sterilize at 100°C for 30 minutes, cool and store away from light (to avoid photo-reaction).

6. Accelerated stability studies at elevated temperature
To assess the viability of spores over prolonged storage periods, accelerated stability tests have been performed on samples of Lactospore®.

a) Sample used:

*Lactobacillus sporogenes*
(6000 million viable spores per gram)
b) Method:
Accelerated stability studies of *L. sporogenes* powder were carried out by incubating the powder in a brown bottle, sealed with a polyethylene plug cap, at 45°C ± 1°C for 90 days (considered to be equivalent to two years storage at room temperature).

*L. sporogenes* powder was used for this purpose (25 grams). The powder contained 7260 million viable spores per gram (claimed as not less than 6000 million viable spores per gram).

Glucose yeast extract agar medium was used, according to the method specified for *L. sporogenes* count by M/s Metchnikoff Biosystems Pvt. Ltd., Medchal, A.P., India. The details are described above.

The results obtained with a sample of Lactospore® are indicated in Figure 6.1. It is evident that the spores remain viable and conform to specifications even after a storage period equivalent to two years at room temperature.

**Viability of *Lactobacillus sporogenes* (by viable plate counts) in LACTOSPORE® powder at 45°C ± 1°C.**

![Figure 6.1 (Ref. 92)](image-url)
REFERENCES
26. Sankholkar, P.C and Sali, M.S. "Mycopip" (Sporlac) vaginal tablets in non-specific vaginitis. *Clinical study report from B.J. Medical College, Pune, India.*
60. *Abstracts of papers on the clinical study of Lacbon (Sporolac) compiled by the Sankyo Co. Ltd. Japan.*
68. Product Literature, Sabinsa Corporation, U.S.A.
90. Product Literature on Lacbon (1965)