Bifidobacterium sp as Probiotic Agent - Roles and Applications

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Abstract

Probiotics are live microorganisms (mostly bacteria) that colonize the gut and supply helpful benefits. Probiotics are completely different from other varieties of microorganism in that they are considered “good” microorganism or non-pathogenic in healthy individuals. Genus Bifidobacterium is the primary microbe that colonizes human channel, and it has positive health effects on its host. Because of the acknowledged health benefits, it is included in several purposeful foods. Bifidobacteria generally occur in extremely varied ecological niches. In order to survive in these explicit ecological niches, they should be highly adaptive. This review helps researchers incorporate the utmost criteria for an acceptable probiotic strain as in the case of Bifidobacterium sp. Bifidobacteria can be considered as a probiotic and it plays an important role in anticholesterolemic, lactose intolerance, anticancerogenic, immunomodulatory effects etc.

Keywords: Probiotics, Bifidobacterium species, Beneficial microorganisms, Functional foods.

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INTRODUCTION

Bifidobacterium consists of about 45 species and belongs to phylum Actinobacteria. They are indigenous components of human and animal gastrointestinal micro flora. They are gram +ve, non-sporing, non-motile, rod-shaped and catalase-negative anaerobes, which produce acetic acid and lactic acids from carbohydrates without the generation of carbon-dioxide. Genus can be distinguished from other bacterial groups like Lactobacilli, Actinomyces and anaerobic Corynebacterium by Fructose-6-phosphate phosphoketolase (F6PPK ) assay. Tissier isolated Bifidobacterium from healthy breastfed infants in the year 1899. It is also isolated from infant feces, breast milk, cow’s milk, yogurts, sour cream, cheese desserts, ice cream and powdered milk. Bifidobacteria species is predominant in the guts of breastfed infants. Bifidobacterium turn out medicinal drug chemicals, enzymes, vitamins B and K and carboxylic acid which decreases the growth of fungus, also helps in digestion and facilitates absorption of nutrients. Probiotics are “live microorganisms that, once consumed in sufficient quantity, offer health benefits to their host”. Selection criteria for probiotics

While choosing the probiotics strain for human purpose, it ought to be isolated from the microflora that probably stick to the human intestinal wall. The strain ought to be properly isolated and known before use. There are a variety of standards to be followed throughout while choosing microorganism for probiotics. Strains of eubacterium and Bifidobacterium are usually used. Members of alternative genus like Bacillus licheniformis have conjointly been investigated to be used as probiotics. Selected strains should be non-pathogenic and non-toxic. Bifidobacterium is generally considered safe. Before choosing alternative probiotics, toxicologic studies should be performed.

Commercially important probiotics

The world population have become alert to the relation between nutrition and physiological state. Probiotic cultures are exploited extensively by the dairy farm business as tools for the production of novel purposeful products. Historically probiotics are incorporated in dairy products, milk and cheese; besides milk-based probiotic products, attempts are being made to use and develop nonmilk probiotic products. A variety of carriers of probiotics have been examined recently together with edible spreads and meat. New product like cereals, fruits and vegetables are in the developmental stages.

The Beneficial effects of probiotics

Probiotics offer familiar health benefits like prevention and amelioration of intestine diseases. They have hypocholesterolemic, anticancer and antioxidative effects. Probiotics are reported to be helpful in atopic dermatitis, wound and scar-healing, and possess skin-rejuvenating properties. The major health attributes of probiotics are shown in Fig.1.

Bifidobacterium

Bifidobacterium species are one of the most abundant microbes in natural microflora of colon. The bacteria in 25% of adult feces and 80% of infant feces are Bifidobacterium. Bifidobacterium species plays an important role in human health by prevention of intestinal infections, decreasing cholesterol, stimulating immune system therfor decreasing cancer risk.

Table 1. Beneficial effects of Bifidobacterium sp

<table>
<thead>
<tr>
<th>Beneficial Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of vitamins: B1 (Thiamine), B7 (Biotin), B11 (Folic acid), B12 (Cobalamine) and Riboflavin biosynthesis.</td>
<td>8</td>
</tr>
<tr>
<td>Alleviation of lactose intolerance</td>
<td>60,61,62</td>
</tr>
<tr>
<td>Prevention and treatment of inflammatory bowel disease</td>
<td>63,64</td>
</tr>
<tr>
<td>Reduction of serum cholesterol</td>
<td>65</td>
</tr>
<tr>
<td>Prevention of acute diarrhea</td>
<td>66</td>
</tr>
<tr>
<td>Prevention of colorectal cancer</td>
<td>67,25,68</td>
</tr>
</tbody>
</table>
of diarrhea\textsuperscript{22}. Antibiotic associated diarrhea in children and adult patients are also recommended to take probiotics along with antibiotics this have proven antibiotic donot interfere with the probiotics\textsuperscript{23}.

**Bifidobacteria in colorectal cancer**

Probiotics suppress the growth of bacteria which convert procarcinogens into carcinogens thereby reducing the amount of carcinogens in the intestine\textsuperscript{17}. Many species of Bifidobacterium have been found to reduce the incidence of tumour in the liver, colon, small intestine and mammary gland of rats\textsuperscript{24}. Bacterial enzymes like α-glucuronidase and nitroreductase play a vibrant role in the development of cancer by hydrolysing carcinogenic compounds. Studies have reported that probiotics, including species of *Bifidobacteria*, decrease the activity of these enzymes thereby reducing various cancers\textsuperscript{25}.

A recent study has reported that probiotic supplements containing *B. longum* and other species of *Bifidobacterium*, suppress the total number of colon cancer cells in rats\textsuperscript{26}. Many studies have concluded that certain strains of probiotics can be used as an adjuvant in cancer treatment\textsuperscript{22}.

**Bifidobacteria in allergic disorders**

The shift of Th1/Th2 cytokine balance towards Th2 response is associated with allergic disorders which cause the activation of Th2 cytokines and release of interleukin 4, 5 and 13 and also IgE production\textsuperscript{27}. Studies have reported that long term consumption of *Bifidobacterium lactis* significantly reduces the allergic symptoms in children. A study evaluated the clinical and

![Health attributes of Probiotics](https://example.com/probiotics-health-attributes.png)

**Fig. 1.** Health attributes of Probiotics
immunological changes of atopic dermatitis after consuming probiotics containing B. animalis and L. acidophilus found to play a major role in the treatment of atopic dermatitis28,29,30,31.

**Bifidobacterium in Hepatic Encephalopathy**

Probiotics found to be effective against the treatment of hepatic encephalopathy32. Intake of different Lactobacillus and Bifidobacterium strains in an acute liver injury rat model has shown various effects on bacterial translocation and hepatocellular damage33. In another study five days of probiotic therapy with Bifidobacterium strains in alcoholic patients have significantly lowered AST and ALT activity at the end of treatment34.

**Characterization of Strain**

**Bile and acid tolerance**

Gastric acid and bile play a significant role in the body’s defence against ingested microorganisms. In order to use bacteria as probiotics, it should be made to resist bile salts35. Bile salt plays a fundamental role in the defence mechanism of the gut.

**Production of antimicrobial substances**

Various of antimicrobial substances has been produced by probiotic bacteria. Some of these compounds includes short fatty acids, bacteriocins, hydrogen peroxides etc. These have the ability to compete against gastrointestinal microbes and thereby inhibit pathogen bacteria36,37,38. Bifidobacterium produce antimicrobial proteins such as bacteriocins, which are substances for the inhibition of food borne pathogens39. Bacteriocins promotes antimicrobial activity through pore formation which causes cell lysis and prevents biosynthesis of cell wall40.

**Adhesion properties of Bifidobacterium**

The adhesion onto mucin is used to evaluate the ability of the strains to colonise the intestine. This property is an important factor for the selection of probiotics. Mucin in the intestine of hosts provides protection from enteric pathogens via steric hindrance specific binding for viruses and bacteria. Certain Bifidobacterium strains like B. bifidum, B. longum has afcA and engBF genes which express mucin degrading glycosidase 41.

**Carbohydrate metabolism**

Bifidobacteria are saccharolytic and play a significant role in the fermentation of carbohydrates in colon. It can also ferment different sources of carbon such as xylo-oligosaccharides. Bifidobacteria degrade hexose sugars through “bifid shunt” a metabolic pathway. In this, Fructose 6 phosphoketolase is broken down to fructose 6 phosphate in the presence of inorganic phosphate42. F6PPK assay using different chemicals like CTAB and Triton X-100 (Sigma) is a reliable test for genus identification of bifidobacteria 35.

**Culture media for Bifidobacterium species**

Due to the non-availability of appropriate selective media, the isolation of Bifidobacterium sp from various niches has been a difficult task. Several media for the enumeration of Bifidobacterium sp are tested, and Wilkins Chalgren’s agar, containing 100 mg/L Mupirocin, is used as selective medium of Bifidobacterium species from milk and cheese 44. Reinforced Clostridial Agar and MRS Agar containing cysteine, Columbia agar medium containing lithium chloride and sodium propionate are used as selective media in dairy products and in quality control laboratories46. MTPY medium is used for isolating Bifidobacteria from hens’ gastrointestinal tract46,47.

A study by Wasilewska et al (2003)48 developed another selective medium from milk: Raffinose Propionate lithium Mupirocin. In the year 2003, Ewa isolated Bifidobacteria from infant feces by two newly-modified Gerche’s media:

1. One with stimulating Bifidobacteria growth maltodextrin instead of lactose.
2. Addition of lithium chloride (3g/L) and Penicillin G, Sodium salt (50 U/L).

Culture medium used in different samples for the enumeration of Bifidobacterium sp is shown in Table 2.

**Species of Bifidobacterium**

More than thirty two Bifidobacterium species have been identified by scientists49,50. Distribution of principal species of Bifidobacterium is represented in Table 3 and major findings on Bifidobacterium sp in Table 4.

**Bifidobacterium Sp in probiotic foods**

Bifidobacterium bifidum grew better in milk under anaerobic conditions than standard strains. This strain gave the best results when it was added at 5-10% to milk and when stored at pH 5.0-5.3, and storage at lower pH adversely affected growth and acid production. Kanbe et al (2007)51 reviewed the characteristics of
Bifidobacterium spp. growth factors and evidence for its beneficial effects on protein metabolism, vitamin metabolism, fecal excretion, kidney problems and the immune response. Fernandes et al (1987) and Guo et al (2010) mentioned that Lactobacillus spp. along with B. bifidum constituted health benefits. Hughes and Hoover (2011) discussed Bifidobacteria, a group of lactose-utilizing bacteria, with emphasis on classification. Recent research has focused on these organisms and the benefits obtained from the ingestion of bifid-containing food products. Misra and Kuila (2001) summarized the biological activities, the role of Bifidobacteria, preparation and the antimicrobial activity and therapeutic properties of bifidus milk. Consumption of bifidus milk with a high number (108 cfu/g) of this organism will provide L (+)-lactic acid, antibiotic factor and live Bifidobacteria in addition to other nutritional components. The combined action of these factors will create favorable conditions for the proliferation of intestinal Bifidobacteria, and discourage the growth of harmful organisms.

**Bifidobacteria and functional foods**

Functional foods are “food containing some health promoting components, also called as super food, designer foods etc., and functional by means of adding probiotics. Certain Lactic acid bacteria including Lactobacillus and Bifidobacterium genus are used as functional ingredients in probiotic food products. Bifidobacterium sp, isolated from different food products, is shown in Table 5. The main challenge associated with the application of probiotic cultures in functional food is their viability during processing. B. animalis subsp. lactis BB-12 is a widely used probiotic strain in food manufacture because of its viability.

**Table 2.** Culture medium used in different samples for enumeration of Bifidobacterium sp

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Media Used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast milk</td>
<td>MRS supplemented with 0.5 g/L L-cysteine HCL</td>
<td>35</td>
</tr>
<tr>
<td>Infant feces</td>
<td>MRS + 0.25 g/L L-cysteine, Bifidobacterium Agar</td>
<td>1,7,6</td>
</tr>
<tr>
<td>Dairy products</td>
<td>TPY agar</td>
<td>2</td>
</tr>
<tr>
<td>Milk</td>
<td>MRS broth, Bifido selective medium</td>
<td>71</td>
</tr>
<tr>
<td>Animal feed</td>
<td>BSM agar, MRS supplemented with cysteine HCL and Mupirocin</td>
<td>72</td>
</tr>
<tr>
<td>Yogurts</td>
<td>Galactose (GL) Agar</td>
<td>1</td>
</tr>
<tr>
<td>Infant gut</td>
<td>Garches broth</td>
<td>73</td>
</tr>
</tbody>
</table>

**Table 3.** Distribution of species of Bifidobacterium

<table>
<thead>
<tr>
<th>Human Groups</th>
<th>Rabbit Species</th>
<th>Cow</th>
<th>Swine Faeces</th>
<th>Honeybee Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. adolescentis</td>
<td>B. magnus</td>
<td>B. bifidum</td>
<td>B. pseudocatenulatum</td>
<td>B. indicum</td>
</tr>
<tr>
<td>B. bifidum</td>
<td>B. saeculare</td>
<td>B. breve</td>
<td>B. choerinum</td>
<td>B. coryneforme</td>
</tr>
<tr>
<td>B. breve</td>
<td>B. cumiculi</td>
<td>B. boum</td>
<td>B. aerophilum</td>
<td></td>
</tr>
<tr>
<td>B. angulatum</td>
<td>B. magnus</td>
<td>B. ruminantium</td>
<td>B. thermoacidophilum</td>
<td></td>
</tr>
<tr>
<td>B. catenulatum</td>
<td>B. cumiculi</td>
<td>B. thermophilum</td>
<td>B. suis</td>
<td></td>
</tr>
<tr>
<td>B. gallicum</td>
<td>B. thermophilum</td>
<td>B. infantis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. pseudocatenulatum</td>
<td>B. suis</td>
<td>B. longum</td>
<td>B. pseudolongum</td>
<td></td>
</tr>
</tbody>
</table>
General features of Bifidobacterial genomes

The NCBI knowledge base presently holds 254 publicly-out Bifidobacteria ordering sequences, of which 114 represents a complete ordering sequence. 3 or a lot of complete ordering sequences are Bifidobacterial species, like B. animalis, B. adolescentis, B. breve, B. bifidum, B. longum and B. angulatum. The typical size of a Bifidobacterial ordering is 2.2 Mb, with appreciable differences in size. For instance, B. indicum LMG11587 has an ordering size 1.73 Mb, whereas B. scardovii CM12489 has 3.16 Mb. Bifidobacterium orderings generally have 52–58 RNA genes per genome, except the ordering of B. longum subsp. infantis ATCC15697, which has seventy nine tRNA genes. The amount of rRNA

<table>
<thead>
<tr>
<th>Bifidobacterium species</th>
<th>Major findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. longum</td>
<td>• One amongst the foremost common strains within the gut with excellent antioxidative activity.</td>
<td>74,75,76,77,78,79</td>
</tr>
<tr>
<td></td>
<td>• Consumption of B. longum plus fructo-oligosaccharides (FOS) helps in the treatment of Minimal Hepatic Encephalopathy (MHE).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Anti-obesity and immunomodulatory effects.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Continuous intake of BB536 decreases the incidence of influenza and fever, probably by potentiating innate immunity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Has vital anti-aging benefits, fewer issues with regularity, higher memory recall and improved bone health.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• BB536 ingestion modulates the intestinal environment and also improves health care of early patients receiving enteral feeding.</td>
<td></td>
</tr>
<tr>
<td>B. breve</td>
<td>• B. breve plays a significant role in mucosal host defense and helps in preventing infectious diseases.</td>
<td>80,81</td>
</tr>
<tr>
<td></td>
<td>• Antiallergic strain.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Administration of live B. breve strain Yakult and GOS (galactose oligosaccharides) improves the clinical condition of patients with ulcerative colitis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Oral administration of B. breve alleviates UV induced barrier changes and oxidative stress in skin.</td>
<td></td>
</tr>
<tr>
<td>B. lactis</td>
<td>• Intestinal anti-inflammatory activity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Daily intake of ice-cream which contains B. lactis reduces the salivary levels of mutants streptococci in young adults.</td>
<td>82,83,84</td>
</tr>
<tr>
<td></td>
<td>• Partially inhibits the gluten/gliadin-induced damage in small intestinal mucosa.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Utilizes Resistant Starch (RS) as substrate and up regulate the acute apoptotic response to a carcinogen in colon.</td>
<td></td>
</tr>
<tr>
<td>B. bifidum</td>
<td>• Inhibit H. pylori both invitro and invivo.</td>
<td>21,86</td>
</tr>
<tr>
<td></td>
<td>• B. bifidum G9-1 is useful for prophylactic treatment in IgE-dependent allergic disease.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Invitro study, B. bifidum S17, highly adherent strain with potent anti-inflammatory capacity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Combination of both L. acidophilus and B. bifidum reduces body weight among 53 obese people.</td>
<td></td>
</tr>
<tr>
<td>B. coagulans</td>
<td>• Invitro B. coagulans has antioxidant and free radical scavenging properties.</td>
<td>87</td>
</tr>
<tr>
<td>B. infantis</td>
<td>• B. infantis 35624 a probiotic strain relieves many of IBS symptoms.</td>
<td>88</td>
</tr>
</tbody>
</table>
operons among Bifidobacterial genomes generally ranges from 2 to 5, and it has been recommended that the amount of rRNA operons present on a genome correlate to the variation of a specific species to environmental conditions.  

**CONCLUSION**

The growing attention in each basic analysis and industrial importance of probiotics and the role of Bifidobacterium species is highlighted during this work. Bifidobacteria, as probiotics, may become an important means of reduction of serum cholesterol, alleviation of lactose intolerance, treatment of inflammatory bowel diseases, acute diarrhea, colorectal cancer and other intestinal infections. And these days there is a growing trend in the market for food supported with probiotics. And also it is applied in a variety of products such as powdery health food and medicines, tablets, capsules etc. Most of the Bifidobacterium species currently being studied and in use appear to be safe, with no adverse effects on health. However, it is safe to conclude that Bifidobacterium species holds a great potential as probiotics.

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**CONFLICT OF INTEREST**

The authors declares that there is no conflict of interest.

**AUTHORS’ CONTRIBUTION**

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

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**DATA AVAILABILITY**

All datasets generated or analysed during this study are included in the manuscript.

**ETHICAL STATEMENT**

This article does not contain any studies with human participants or animals performed by any of the authors.

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