Lactobacillus rhamnosus Gorbach-Goldin (GG): A Top Well-Researched Probiotic Strain

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ABSTRACT

Probiotics, living microorganisms which when used in adequate amounts, have health benefits on the host, have attracted a considerable deal of interest in the biotechnology market. Various prokaryotic and eukaryotic microbes have been studied for probiotic purposes in human and animals. *Lactobacillus* and *Bifidobacterium* are the main probiotic genera; however, there are reports on the probiotic potential of strains belonging to *Bacillus, Lactococcus, Pediococcus* and also some yeast species. *Lactobacillus* species are regarded as the most investigated beneficial microbes for human and either animal probiotic development due to: a) anti-inflammatory effects, b) anti-allergic features, c) secretion of antimicrobial compounds, d) develops in aerobic and anaerobic conditions, and e) many Lactobacilli have been Generally Recognized As Safe (GRAS) by the Food and Drug Administration (FDA). Among Lactobacilli, *Lactobacillus rhamnosus* Gorbach-Goldin (GG) is known among the most in-detail studied strains and is regarded as a top well-researched and commercialized probiotic strain in the industrial biotechnology. Strain GG is a commercially significant probiotic which was originally isolated from the healthy human gastrointestinal tract in 1983. The documents studied this strain were reviewed here and it is shown that such successful strains in the probiotic market have been extensively studied from the probiotic traits and safety point of views and a minor part of the documents concerned the bioprocessing issues. Also, we showed that the beneficial and negative effects of this strain has been investigated in various clinical trial systems when it became commercialized. Various aspects of this strain are here reviewed, comparatively.

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Introduction

The clear association between diet and health is well-established nowadays. More recent discoveries indicated that our diet may modulate various functions in the body (1). Functional foods, of which probiotic containing foods are a subset, are now known vital for improving the safety level of the community (2, 3). Probiotics are 'living microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (4). Hundreds of probiotic strains of a varied range of species can be found in normal microflora of human beings (5). The concept of probiotics was first reported by Metchnikoff in 1907 (6). He postulated that the consumption of fermented dairy products was associated with longevity of certain ethnic groups of people (7). Thus, Metchnikoff suggested that such fermented products can govern the intestinal microflora to maintain the normal balance between pathogenic and nonpathogenic bacteria (8). Then, probiotics became a potential therapeutical approach in human and veterinary medicine (9). Appealing properties of probiotics include the application to solve the global problem of antibiotic resistance, the high index of safety, and the public's positive perception about "natural" or "alternative" therapies. Probiotics are classified, and generally regarded as safe, as opposed to antibiotics, which have a number of recognized adverse effects (10).

Due to the fact that probiotic microorganisms can be presented in various forms of products from dietary foods to supplements, the probiotic market in developing countries tend to be a potent sector. Further, from an international point of view, the global market of probiotic ingredients, supplements and food was estimated around 14.9 billion USD in 2007 (11). The probiotic market was estimated to be valued at $33.19 Billion in 2015 and it is projected to reach $46.55 Billion by 2020. Also, estimates show that the market was dominated by Asia-Pacific, followed by Europe, in 2014 (12). The growing demand for dietary supplements is also driving the probiotic ingredients market.

Nowadays, commercial probiotic supplements are available for human and animal use; however, many of these strains have not been exhaustively studied (13). Microbiologists have derived a varied range of supplementation of various probiotic strains which can confer different types of benefits (14). However, based on the above mentioned definition of probiotics, adequate numbers of viable cells of a probiotic, which is itself strain- and host-dependent, must reach the intestinal tract (15). Hence, probiotic organisms must be able to survive transit through the highly acidic environment of stomach and resist digestive bile (16). Besides, microorganisms that survive acid and bile must also possess a variety of characteristics which proved to be critical for a given probiotic strain to become stabilized in the gastrointestinal tract: adhesion to the intestinal epithelial cells, colonization in the intestinal tract, antimicrobial factors production, and inhibition of entero-pathogens (16-19). Additionally, other properties such as immunomodulation, modulation of metabolic activities, and interfering with risk factors (and procarcinogens), are also desirable (18, 20). An organism can only be considered to be a probiotic after most of these properties have been identified and a positive health effect has been documented (11, 21, 22).

Most of the commercialized probiotic strains fall in to a metabolic group of prokaryotes known as Lactic acid bacteria (LAB), which is a varied group of Gram-positive bacteria, are found in various habitats (22). LAB was traditionally considered as nonsporulating, catalase-negative microorganisms, but *Bacillus coagulans* as a Gram-positive, spore-forming, catalase-positive bacterial species is also a lactic acid producing prokaryote and is commercialized as a probiotic organism for animal and human usages (23). Further, *Sporolactobacillus vineae* is a species which is regarded as a new LAB (24). This metabolic group (LAB) comprise multiple genera within the order Lactobacilliales that are generally acid tolerant, of

which *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, and *Streptococcus* spp are among the most well characterized genera. Various members of these genera are detected in the normal microflora of the human gut (25) and also occur widely in dairy, meat, plants, and traditional and commercial fermented products (22). As a result of their ancient anthropological use in food processing and their fermentative nature which lead to lactic acid accumulation, they have become industrially important bacteria and are used in a very broad range of food and agricultural fermentation bioprocesses, worldwide (26).

The predominant and the most important subgroup of LAB that reside in the small intestine are *Lactobacillus* species. Some members of this genus of LAB are responsible for producing the enzyme lactase. They also collectively ferment carbohydrates in the gastrointestinal tract, producing lactic acid (27). Lactic acid helps establishment of an acidic environment in the digestive tract, which together with nicins: the antibacterial small peptides of lactobacilli, discourages many unwanted microorganisms that thrive in a neutral-alkaline environments. Lactic acid derived acidification also increases absorption of some significant minerals such as calcium, copper, magnesium, and iron (11, 17, 18, 19, 28).

Among the hundreds of LAB strains which have been documented as probiotic, *L. rhamnosus* (strains ATCC 53103, GG, LGG, GR-1, LB21, and 271), *L. acidophilus* (strains ATCC 700396, LA-1/LA-5, NCFM, DDS-1, and SBT-2062), *L. helveticus* (strains ATCC 15019, CECT 4305, LMG 13555, and B02), *Bifidobacterium infantis* (strains Shirota and Immunitas), *Bifidobacterium lactis* (strains Bb-02, LaftiTM, and B94) are known as the top well-researched probiotics (29). Also, among these five probiotics, *L. rhamnosus* Goldin Gorbach (30) and *L. acidophilus* have been studied more exhaustively. *L. rhamnosus* GG, a short Gram-positive, facultative anaerobic lactobacilli that often appears in chains, was originally considered as a subspecies of *L. casei*, but soon, it was found to be a distinct species thank to molecular data (31). Nowadays, *L. rhamnosus* strains are being used in commercial probiotic products. Interestingly, *L. rhamnosus* is regarded as the common flora of female genito-urinary tract and it sometimes is used as a starter culture in yogurt and dairy products (32). Also, *L. rhamnosus* GG has been studied extensively on its physiology, and either health benefits (33). This commercialized probiotic strain has been used worldwide since 1990 as an ingredient in food and dietary supplements, with no reported consumer illness or injury. This strain has been tested in clinical studies from newborn preterm infants to elderly populations in dosages up to at least 100 billion CFU/day, with no reported serious adverse events.

Detection and Characterization of L. rhamnosus GG

L. rhamnosus GG (ATCC 53103), a *L. rhamnosus* strain which was originally isolated in 1983 from the intestinal tract of a healthy human being; filed for patent on 17 April 1985, by Sherwood Gorbach and Barry Goldin, and the 'GG' refers to the first letters of their last names (30, 31). The story of this strain originates from a microbiological study in 1987. Goldin, Gorbach and his colleague Chang published the results of their study on treatment of relapsing *Clostridium difficile* colitis with the *L.* strain GG which they already isolated (34-36).

This new therapeutical approach was soon patented (34). The patent which Sherwood Gorbach and Barry Goldin submitted refers to a strain of "*L. acidophilus* GG" with American Type Culture Collection (ATCC) accession number 53103; soon later this strain was revisited and was identified as a member of *L. rhamnosus* species. The patent claimed that the *L. rhamnosus* GG (ATCC 53103) strain is acid- and bile-stable which is known as a compulsory property of functionally probiotic strains (15). Also, it has a great avidity for human intestinal mucosal cells, and produces lactic acid (36). Strain GG has been applied as a probiotic-therapy agent in mice and human systems and its probiotic properties have been

confirmed to be useful for treatment of various forms of gastrointestinal (37-46) and respiratory (47-49) disorders and diseases, as numerous clinical trial studies showed. Additionally, various forms of disorders and diseases have been also studied in which *L. rhamnosus* GG was applied as a probiotic therapy agent (50-53). Further, multiple studies reviewed the potential of *L. rhamnosus* as a helpful approach to improve the vaginal and urinary health and to decrease the vaginal irritations (54, 55). The general history of this strain, from isolation in 1983 till now, is summarized in figure 1 (56-59).

Potentials and Applicability of L. rhamnosus GG as a probiotic

In 1987, Silva et al. indicated that *Lactobacillus* sp. strain GG produces a substance with potent inhibitory activity against a wide range of bacterial species (57). This substance showed inhibitory activity against anaerobic bacteria (*Bacteroides* spp., *Bifidobacterium* spp. and *Clostridium* spp.), Enterobacteriaceae members, *Pseudomonas* spp. *Staphylococcus* spp., and Streptococci. Silva et al. also showed that strain GG did not inhibit other lactobacilli (58). The reported inhibitory activity occurred between pH 3 and 5 and was thermostable (59-60). Also, Conway et al. studied the survival of lactic acid bacteria (including strain GG) in the human stomach and their adhesion to some of the intestinal cells (14). Additionally, in 1991, a method of treating gastrointestinal side effects of antibiotic therapy was invented which was based on application of a defined dosage of strain GG (30, 31). Also, this strain was soon cultivated in industrial scale and became commercialized in food and dietary supplements. In this period, the helpful effects of probiotics in the treatment of diarrheal diseases in mice and either human systems was evaluated (60, 61, 63-65). Vanderhoof et al. indicated the efficacy of *Lactobacillus* GG in reducing the incidence of antibiotic-associated diarrhea when co-administered with an oral antibiotic in children with acute infectious disorders (40). Besides, Hatakka et al. conducted a statistical trial and investigated the effects of long term consumption of a probiotic milk on the reduction of gastrointestinal and respiratory infections in children in day care centres (42). Then, Kalliomäki et al. showed that *Lactobacillus* GG was effective in prevention of early atopic disease in high risk children. Hence, Kalliomäki et al. concluded that gut microbiome can be assumed as a source of natural immunomodulators and probiotics, for prevention of atopic disease (63, 64). Further, a statistical survey in Finland indicated that increased probiotic use of *L. rhamnosus* GG has not led to an increase in *Lactobacillus* bacteremia (66-68). Collectively, the above researches made a fundamental knowledge on the therapeutical potential of strain GG which helped to make this strain acceptable in regulatory agencies, globally. Thus, the above mentioned researches were followed by an extensive investigation of probiotic features of *L. rhamnosus* GG in various supplementations and formula (56, 69-74). Also, the molecular features of this species have been exhaustively studied (74-76) (Figure 2). The whole genome of *L. rhamnosus* GG was sequenced by Morita et al. and can be accessed under the Genbank accession number NC_013198.1 (77).

Safety, Approves and Legal Issues

The minimum standards of a given dietary probiotic product is legalized by Food and Drug Administration of the Islamic Republic of Iran (IFDA) (Available at: http://www.fda.gov.ir). Such standards have been also well-reviewed, internationally (4, 20, 30, 78-81). Various official agencies all around the world have approved probiotic strains (including L. rhamnosus GG) for human uses. WHO summarized the needed specifications for application of such probiotic strains for human uses in report of a joint FAO/WHO expert consultation on evaluation of health and properties of probiotics in food including powder milk with live lactic acid bacteria (Córdoba, Argentina: 2001. Oct 1-4, Health and Nutritional Properties of Probiotics in Food

Including Powder Milk with Live Lactic Acid Bacteria). Also there is a similar report in WHO which concerned such application (Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-Treatment Agents, Acids and Bases. Ninth Report, FAO Nutrition Meetings Report Series, 1966, No. 40. World Health Organization Techn. Rep Ser No. 339. 1966). Additionally, European Food and Feed Cultures Association (EFFCA) reported such instructions (EFFCA Guidelines for Probiotics in Food and Dietary Supplements.

Functional foods, have recently justified the efforts of health authorities in many countries, especially Japan and the United States, to stimulate and support research on the physiological effects of food components and their health benefits and to authorize health claims. Thus, some regulations for administration of specific probiotic supplements regarding the age, gender, and medical records may be needed to consider (82). European Food and Feed Culture Association. 2008. Available at: http://bbs.bio668.com/simple/index.php?t35919.html). Similarly, different globally known official safety agencies reported similar instructions: FDA (Washington DC: U.S. Department of Health and Human Services. Food and Drug Administration; 2006. Dec, Guidance for Industry on Complementary and Alternative Medicine Products and their Regulation by the Food and Drug Administration. Draft Guidance., US Food and Drug Administration. Generally Recognized As Safe (GRAS). Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ default. htm), CDC (Venugopalan et al. 2010), International Scientific Association for Probiotics and Prebiotics 2005.

Available at: http://www.isapp.net/docs/ probiotic %20standards% 20justification .pdf.), Evidence for safety and efficacy of finished natural health products, Natural Health Products Directorate, Canada. 2006. Dec, Available at: http://dsp-psd.pwgsc.gc.ca/collection_2007/hc-sc/H164-9-2006E.pdf., IPA releases Guidelines for probiotic supplements (International Probiotics Association. 2008. Available from: http://www.naturalproductsinsider.com/hotnews/ipa-guidelinesprobiotic-supplements.html.), Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. (EFSA J. 2007; 587:1-16., and Codex Alimentarius Commission), Recommended International Code of Practice: General Principles of Food Hygiene. CAC/RCP 1-1969, Rev 4-2003. Available at: www.codexalimentarius.net/ download/standards/23/cxp_001e.pdf).

According to the surveys on the probiotic strains in the U.S. and Europe biotechnology market, it is found that a handful of well-researched probiotic strains have been developed in clinical research for regulatory board approval from the U.S. Food and Drug Administration (FDA) and European Food Safety Authority (EFSA).

Commercialization and Market

According to the researches concerning the market of probiotics, most of the commercialized probiotic products containing *L. rhamnosus* GG can be found in Europe (11, 83-85) (Table 1). *L. rhamnosus* GG has been used in multiple growth optimization, fermentation scale-up and medium design researches. Besides, exhaustive investigations are reported on the downstream processes, and biomass freeze- and spray- drying procedures at industrial level. According to Soccol et al., many companies, likely: Chr. Hansen, Essum AB, Probi AB, Valio, and Urex

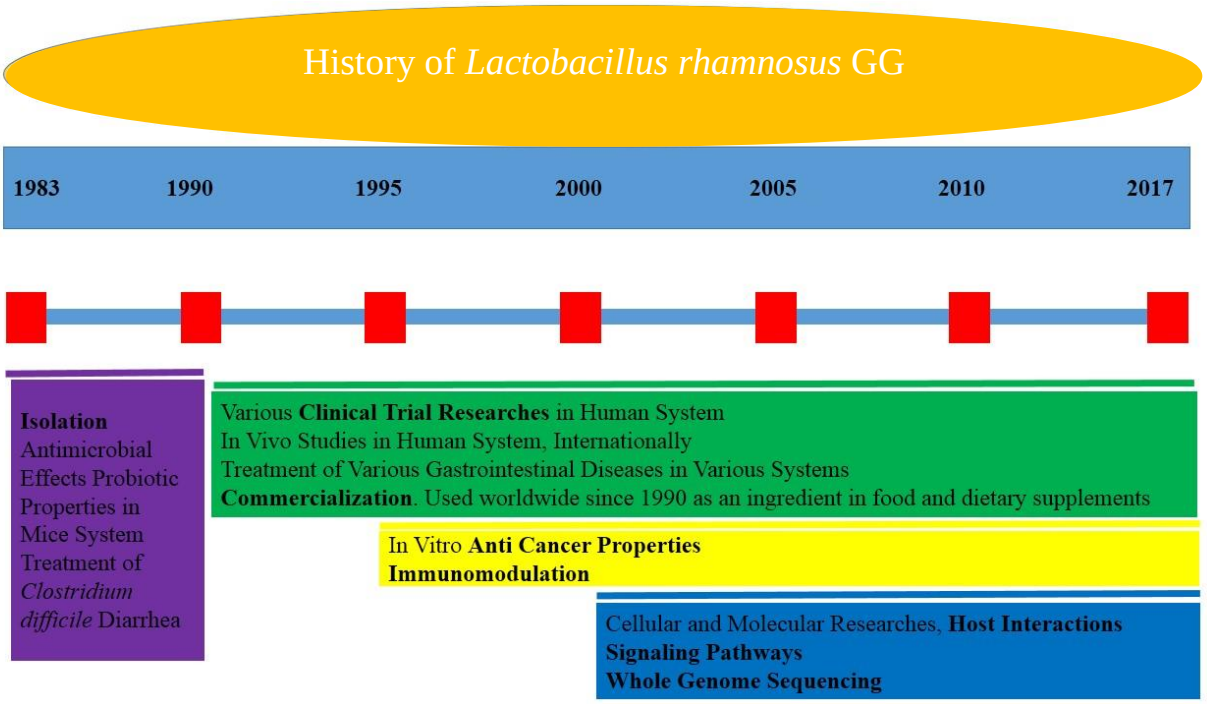

Fig 1. History of *L. rhamnosus* GG. Major milestones in the development of *L. rhamnosus* strain GG as a top well-research commercialized probiotic strain.

Biotech, manufacture the biomass of strain GG for human uses (11) (Table 2).

Understanding the benefits of various probiotic strains and how they affect digestive health is essential in order to properly evaluate which probiotic supplement is the best for one. The five most researched probiotic strains are reviewed comparatively (Table 3).

Multiple copies of the strain GG are available worldwide: ATCC 53103, BCRC 16000, CCM 7091, CCRC 16000, CCUG 34291, GG, LGG, LMG 17291, KCTC 5033, LMG 17291, LMG 18243, New England Med. Ctr. Hospitals strain GG, PRSF-L 175, PRSF-L 253, VTT E-96666.

L. rhamnosus is known for its ability to survive passage through the gastrointestinal tract and is thought to be among the best *Lactobacillus* strains for vaginal health (11).

"Pro-Flora Women's Probiotic", from Integrative Therapeutics Company, contains a formulated blend of *L. rhamnosus* GR-1® and *L. reuteri* RC-14® that supports vaginal colonization, healthy vaginal odor, and bowel regularity. The species information of *L. rhamnosus* is summarized in table 3.

Table 1. *L. rhamnosus* GG in commercial probiotic dairy products on the European market (11).

Products	**Trade names**
Commercial probiotic dairy products on the European market containing *L. rhamnosus* GG (LGG)	A-fil, Actimel, Aktifit, AB-piimä, Bella Vita, Bifidus, Bifisoft, BiofardePlus Biofit, Bioghurt, Biola, Biologic bifidus, Casilus, Cultura, Cultura Dofilus, Dujat Bio Aktiv, Ekologisk Jordgubbs Yoghurt, Emmifit, Everybody, Fit&Aktiv, Fjäll Yoghurt, Fundo, Gaio Dofilus, Gaio, LGG+, Gefilac, Gefilus, God Hälsa, Joghurt, Kaiku Actif, LC 1, LC 1 Go!, Le'Vive+, Milbona, , Onaka, Öresundsfil, Philura, Probiotic drink, Probiotisches, ProViva, Pro X, ProViva, RELA, Verum, Vifit Vitamel, Vitality, Weight Watchers, Yogosan Verum, ViktVäktarna, Vitality, Vivi Vivo Yakult, Yoco Acti-Vit.

Table 2. A review of the probiotic strains which are used in probiotic manufacturing companies (11, 83, 84, 85).

Probiotic Species	**Strain**	**Company**	**Ref.**
Bifidobacterium animalis	Bb-12	Chr. Hansen	86, 87, 88
Bifidobacterium bifidum	Bb-11	Chr. Hansen	89
Bifidobacterium infantis	Shirota Immunitas	Danone® Yakult	11
Bifidobacterium lactis	Bb-02 Lafti™ B94	DSM	2, 90
Bifidobacterium longum	BB536 SBT-2928 UCC 35624	Morinaga Milk Industry Snow Brand Milk Products UCCork	3, 11, 85
Bacillus lactis	DR10	Danisco (Howaru™)	91
L. acidophilus	LA-1/LA-5 NCFM DDS-1 SBT-2062	Chr. Hansen Nebraska Cultures Snow Brand Milk Products Rhodia	11, 92-95
L. casei	Shirota Immunitas	Chr. Hansen Danone Yakult (Yakult®)	11, 96
L. fermentum	RC-14	Chr. Hansen Urex Biotech	11
L. helveticus	CECT 4305 LMG 13555 B02		11

L. rhamnosus	GG LGG GR-1 LB21 271	Chr. Hansen Essum AB Probi AB Valio Urex Biotech	9, 76, 77, 11
L. lactis	L1A	Essum AB	11
L. paracasei	CRL 431	Chr. Hansen	11, 96-105
L. plantarum	299v Lp01	Probi AB	11, 97
L. reuteri	SD2112/ MM2	Chr. Hansen Biogaia	11, 104

Table 3. Species Information on *L. acidophilus*, *L. rhamnosus*, *L. helveticus*, *B. lactis* and *B. infantis* in global catalogue of microorganisms (GCM).

Probiotic species	Strains	Publications	Protein Sequence	Genome	Nucleotide Sequences	Patents
L. acidophilus	128	2755	282	1	73	505
L. rhamnosus	298	1496	3	2	219	171
L. helveticus	88	801	258	0	207	74
B. lactis	5	447	0	0	3	21
B. infantis	1	2	0	0	0	0

Conclusion

According to this review, most of the documents on *L. rhamnosus* GG have concerned the safety issues and so mainly various clinical trials focused on the applicability of the probiotic features in treating gastrointestinal disorders in human system. Studying the probiotic features of this strain started in 1987 and there is still an ongoing effort to increase the knowledge on application of this probiotic in human system. Interestingly, such researches have shown the beneficial effects of such probiotic strains in treatment of not only the gastrointestinal disorders and diseases, but also respiratory, and even neurological problems. This review shows that a minor part of the researches focused on the microbiology of the strain and bioprocess optimization itself. Thus, the huge amount of investigations which performed as clinical trials might underestimate the works highlighted the bioprocess fermentation point of view.

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Conflict of interest

None declared conflicts of interest.

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