



U Daily Plus

**A SYNERGISTIC FORMULATION
OF PROBIOTICS AND
PREBIOTICS AND ITS
APPLICATION IN
GASTROINTESTINAL HEALTH
BASED ON MICROBIOME
MODULATION**

Novel and Systematic Way for Development

Preliminary screening:
genomics+microbiology
functions



Contrast & fine
screen



In vitro validation



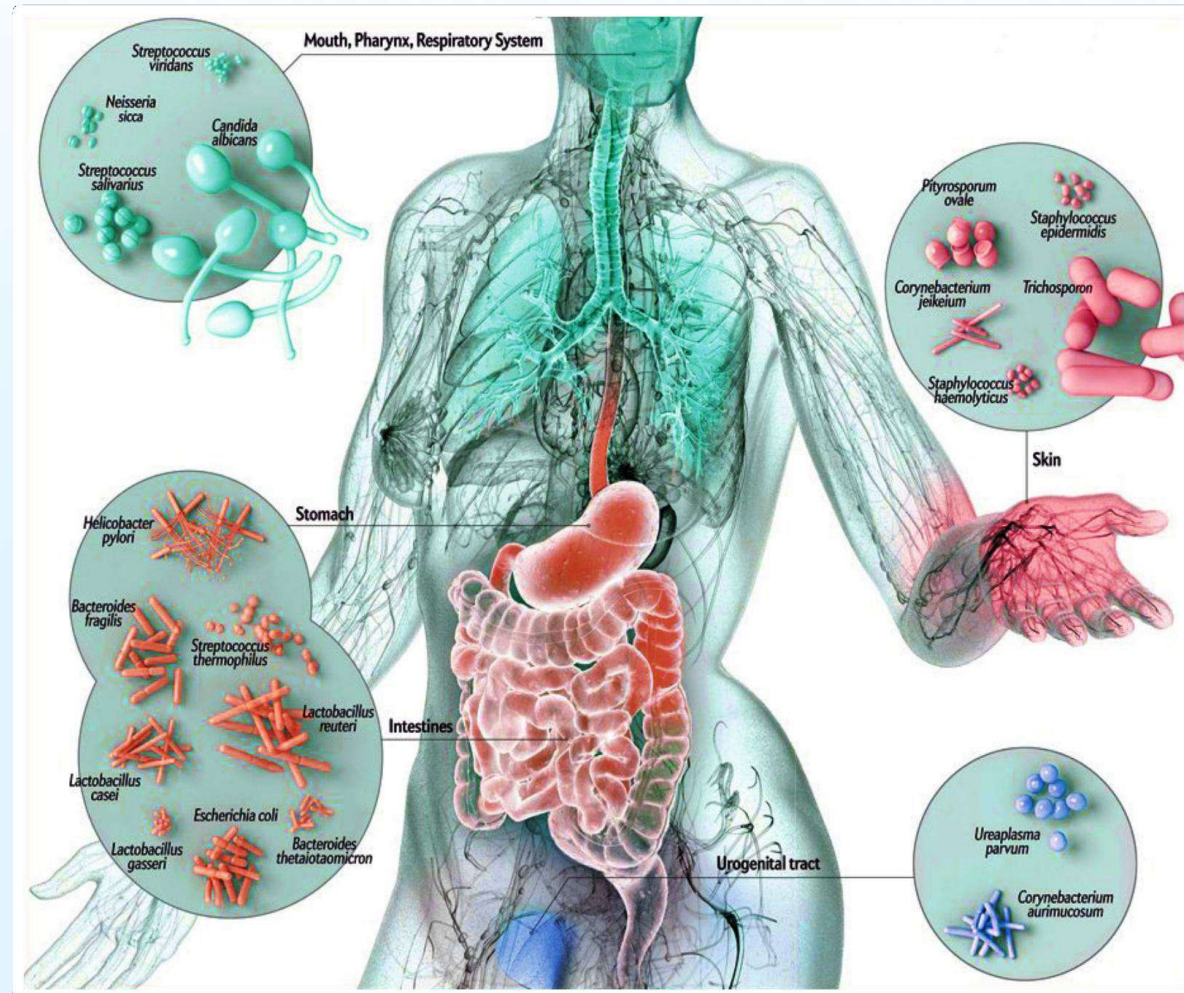
Animal testing



Population testing



Process &
production



Our invention manifests a synergistic formulation of probiotics and prebiotics for gastrointestinal health by a novel and systematically way from initial screening to industrial production by targeting gut microbiota.

Preliminary Screening: Genomics+Microbiology Functions

nature
biotechnology

RESOURCE

<https://doi.org/10.1038/s41587-018-0008-8>

OPEN

1,520 reference genomes from cultivated human gut bacteria enable functional microbiome analyses

Phylum annotation

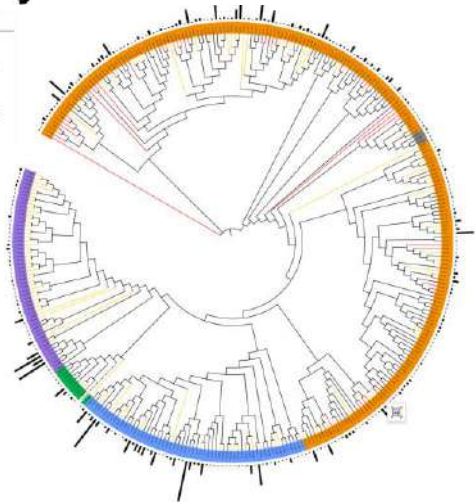
Bacteroidetes

Actinobacteria

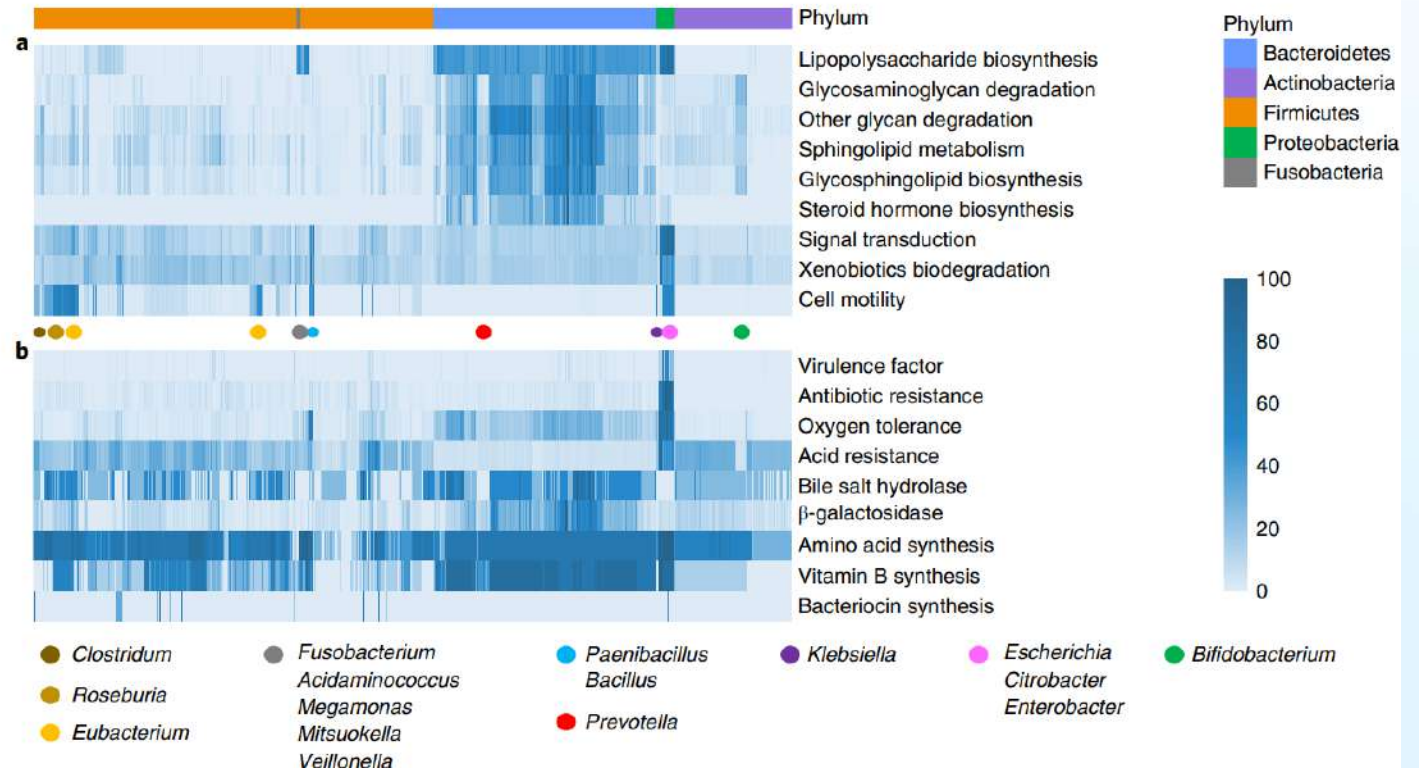
Firmicutes

Proteobacteria

Fusocacteria



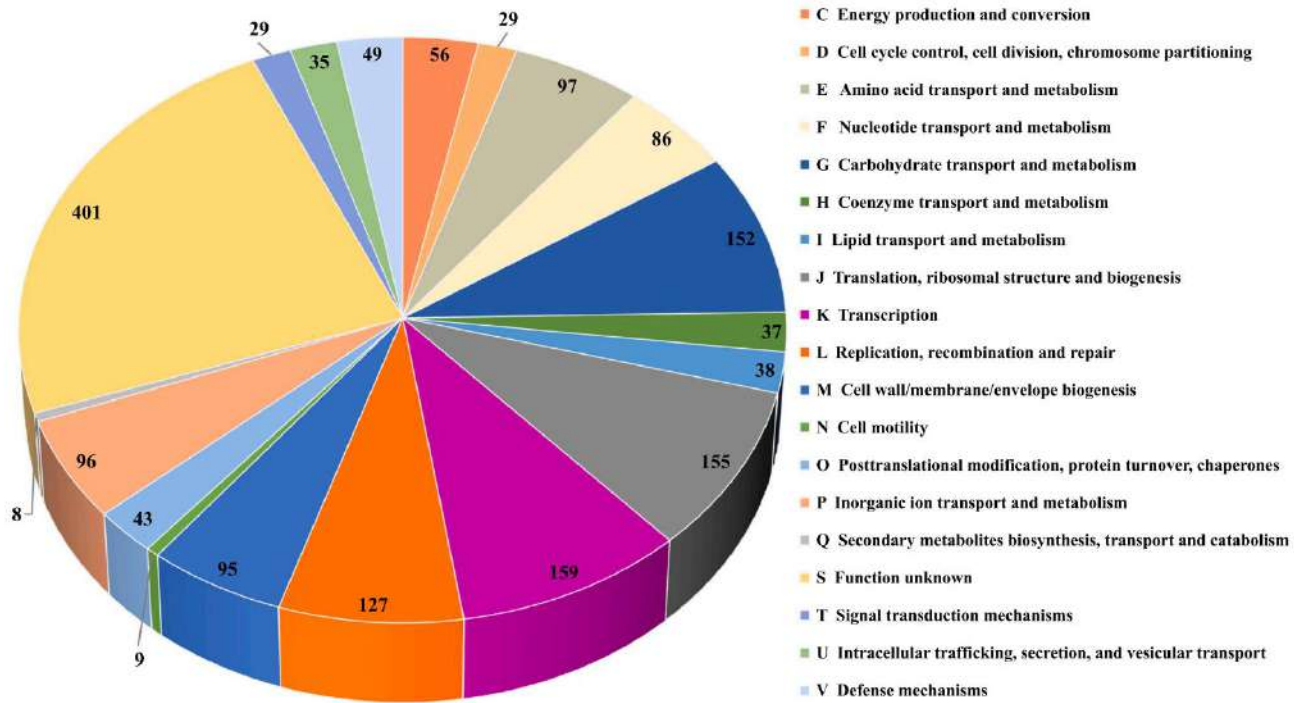
Phylogenetic tree of 1,520 isolated gut bacteria based on whole-genome sequences.



Functional landscape of gut microbiota

First, we presented the a novel Culturable Genome Reference (CGR), a collection of 1,520 high-quality draft genomes generated from >6,000 bacteria cultivated from fecal samples of healthy humans, which covered all major bacterial phyla and genera in the human gut. The novel method was published in the Journal of Nature Biotechnology.

Contrast and Fine Screening

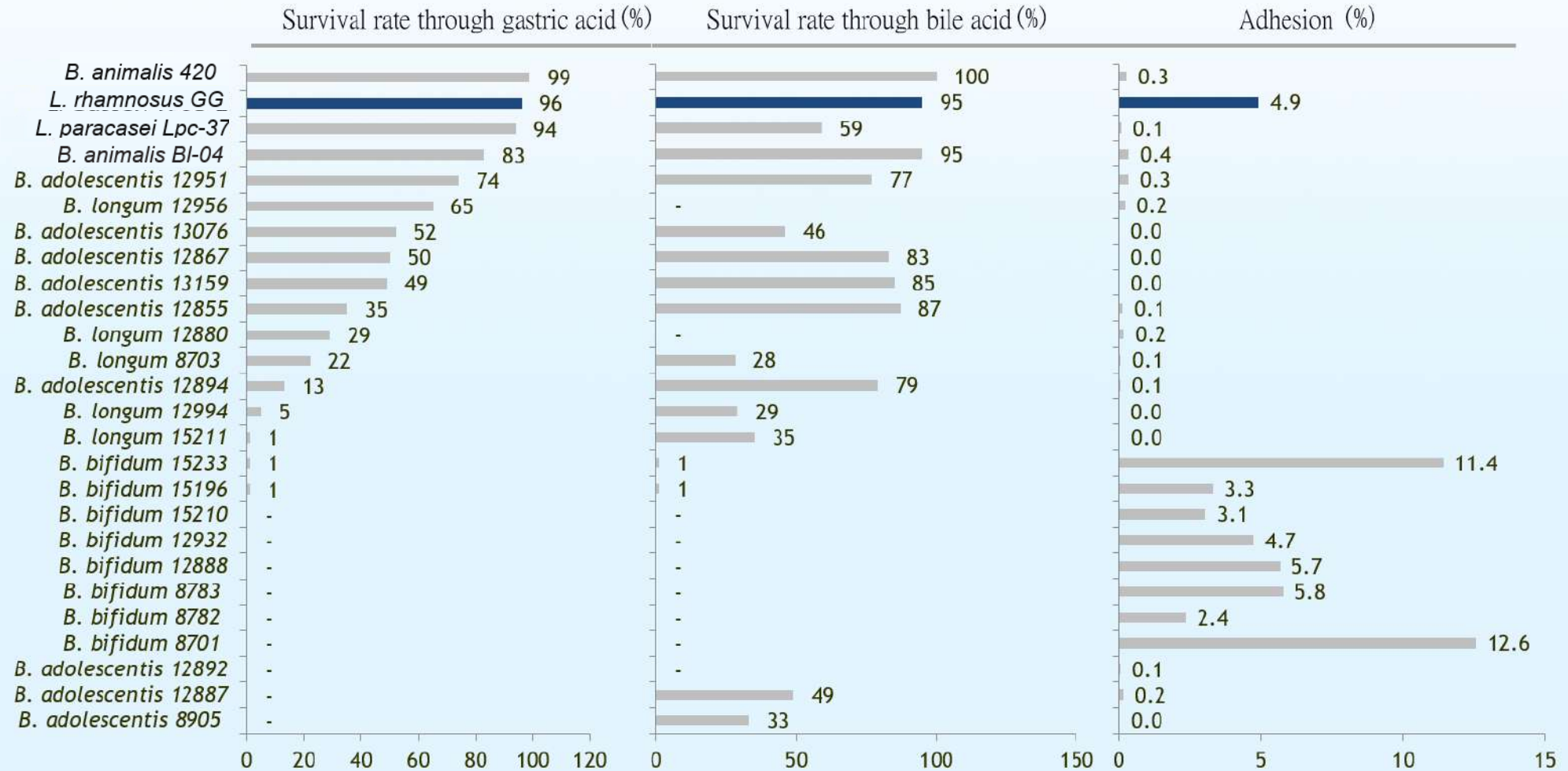


Important genes encoding probiotic-related genes in *Lactobacillus gasseri* TP08-1.

Stress response	query	Preferred name	Length (bp)	KEGG	EC	PFAMs
Bile resistance	gene_945	<i>cbh</i>	978	K01442	3.5.1.24	CBAH
	gene_292	<i>atpB</i>	717	K02108	-	ATP-synt_A
	gene_293	<i>atpE</i>	213	K02110	-	ATP-synt_C
	gene_294	<i>atpF</i>	501	K02109	-	ATP-synt_B
	gene_295	<i>atpH</i>	549	K02113	-	OSCP
Acid resistance	gene_296	<i>atpA</i>	1,512	K02111	3.6.3.14	ATP-synt_ab,ATP-synt_ab_C,ATP-synt_ab_N
	gene_297	<i>atpG</i>	957	K02115	-	ATP-synt
	gene_298	<i>atpD</i>	1,443	K02112	3.6.3.14	ATP-synt_ab, ATP-synt_ab_N
	gene_299	<i>atpC</i>	441	K02114	-	ATP-synt_DE, ATP-synt_DE_N
	gene_1656	<i>dlzB</i>	1,224	K03739	-	MBOAT
Immunomodulation	gene_1658	<i>dlzD</i>	1,302	K03740	-	DltD
	gene_841	<i>dnaJ</i>	1,167	K03686	-	DnaJ,DnaJ_C,DnaJ_CXXCXGXG
Heat stress	gene_842	<i>dnaK</i>	1,872	K04043	-	HSP70
	gene_843	<i>grpE</i>	579	K03687	-	GrpE
Oxidative stress	gene_938	<i>tpx</i>	489	K11065	1.11.1.15	AhpC-TSA,Redoxin
	gene_1301	<i>trxB</i>	936	K00384	1.8.1.9	Pyr_redox_2
	gene_481	<i>spxB</i>	1,737	K00156,K00158,K01577,K01652	1.2.3.3,1.2.5.1,2.2.1.6,4.1.1.8	TPP_enzyme_C,TPP_enzyme_M,TPP_enzyme_N
	gene_1739	<i>pox1</i>	1,809	K00158	1.2.3.3	TPP_enzyme_C,TPP_enzyme_M,TPP_enzyme_N
Adhesion	gene_958	<i>npr</i>	1,365	K05910	1.11.1.1	Pyr_redox_2,Pyr_redox_dim
	gene_368	<i>tuf</i>	1,191	K02358,K15771	-	GTP_EFTU,GTP_EFTU_D2,GTP_EFTU_D3
DNA and protein protection and repair	gene_1067	<i>FbpA</i>	1,692	-	-	DUF814,FbpA
	gene_1473	<i>epsU</i>	1,437	K03328	-	Polysacc_synt,Polysacc_synt_3,Polysacc_synt_C
DNA and protein protection and repair	gene_398	<i>mrsA</i>	558	K07304,K12267	1.8.4.11,1.8.4.12	PMSR,SelR
	gene_396	<i>mrsB</i>	432	K07305	1.8.4.12	SelR
	gene_514	<i>clpC</i>	2,469	K03696	-	AAA,AAA_2,ClpB_D2-small,Clp_N,UVR
	gene_1262	<i>clpE</i>	2,169	K03697	-	AAA,AAA_2,ClpB_D2-small,UVR
	gene_1664	<i>clpE</i>	2,118	K03697,K04086	-	AAA,AAA_2,ClpB_D2-small,UVR
	gene_1677	<i>clpP</i>	588	K01358	3.4.21.92	CLP_protease
DNA and protein protection and repair	gene_370	<i>clpX</i>	1,266	K03544	-	AAA_2,ClpB_D2-small,zf-C4,ClpX
	gene_1140	<i>luxS</i>	477	K07173	4.4.1.21	LuxS

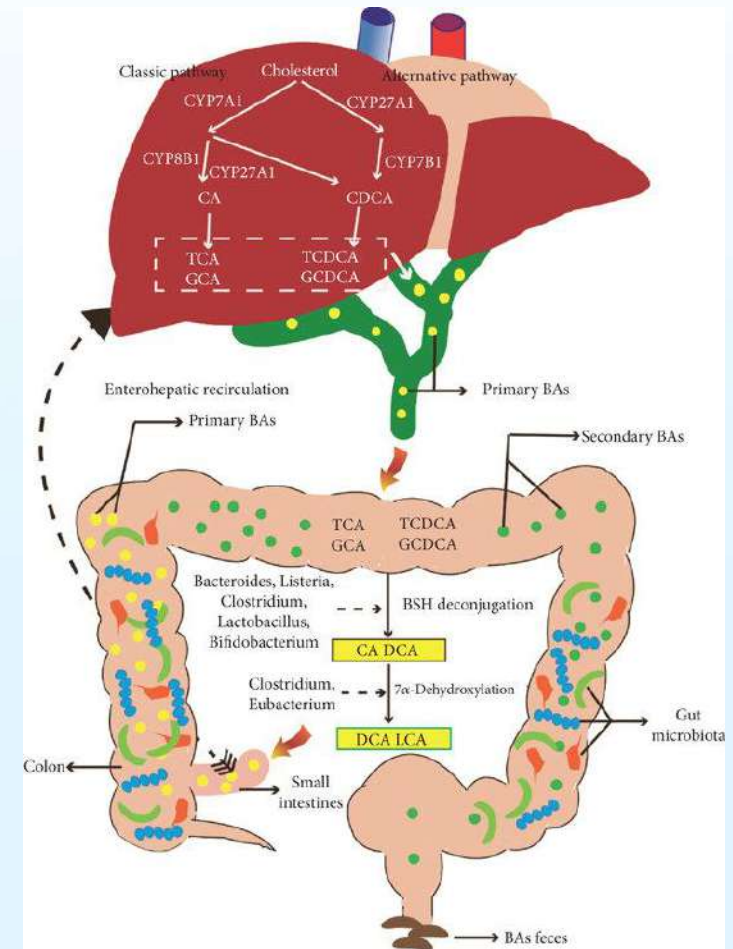
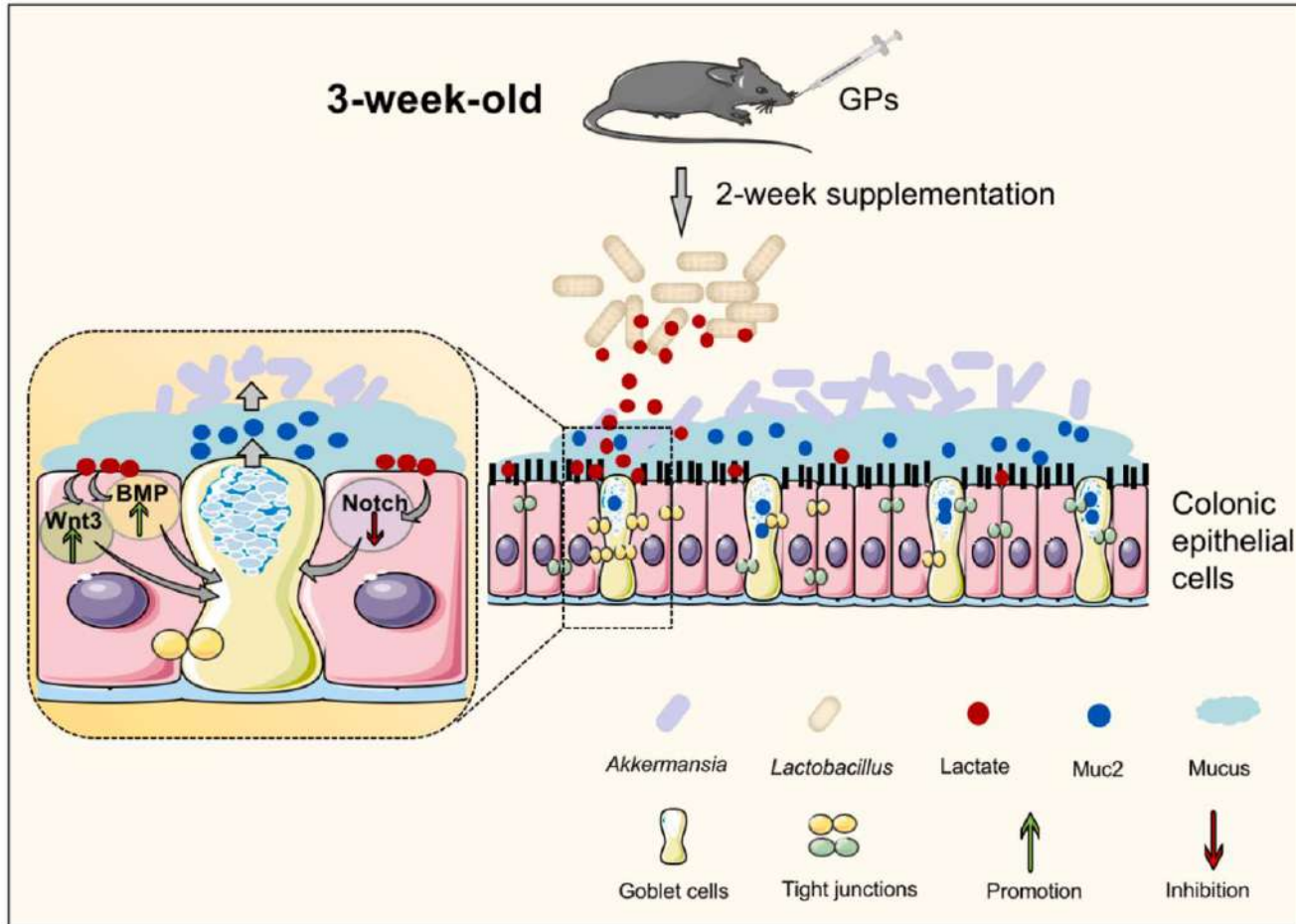
Then we identified and separated four probiotics *Bifidobacterium animalis ssp. lactis* B420, *Lactocaseibacillus rhamnosus* GG, *Lactocaseibacillus paracasei* Lpc-37, *Bifidobacterium animalis ssp. Lactis* Bl-04 by extracting and analyzing abundant categories of probiotic related genes from CGR regarding bile/acid resistance, immune-modulation, heat stress, oxidative stress, adhesion, DNA/protein repair and protection.

Survival and Adhesion Testings



All the four probiotics outperformed other probiotics in terms of gastric acid/bile salt survival and adhesion to intestine cells, which are crucial prerequisite for a probiotic.

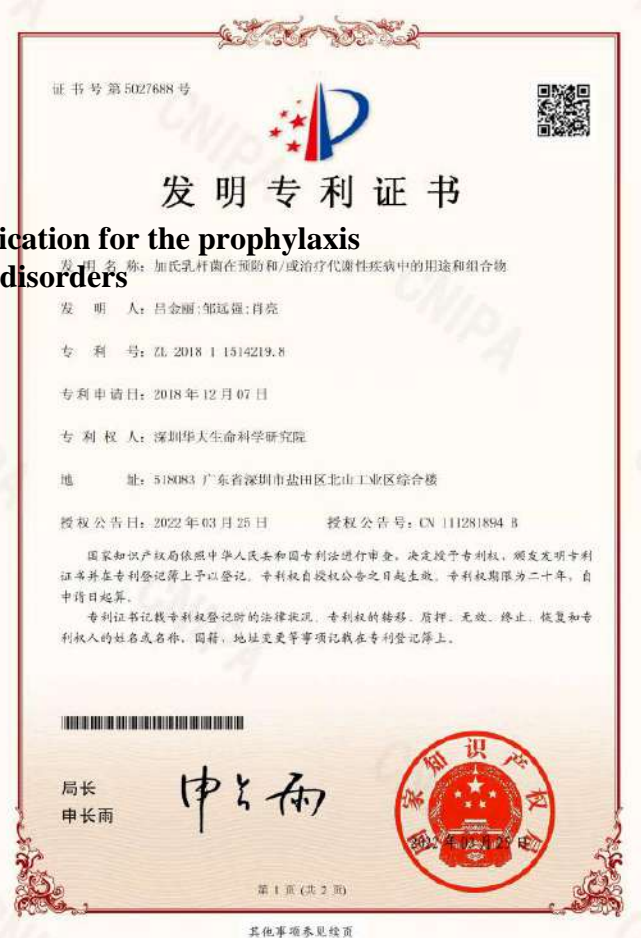
Unique Probiotic Genes



KEGG metabolic pathways showed all the four probiotics harbored unique genes enhancing epithelial cells junction tightness, producing short fatty acids and so on.

Intellectual Property

L. gasseri TF08 and its application for the prophylaxis and treatment of metabolic disorders ZL 2018 1 1514219.8



Regarding its ability for the prophylaxis and treatment of gastrointestinal disorders, the solid evidence of all four probiotics including 52 international patents and more than 600 high-quality journal articles.



Probiotic characteristics of *Lactobacillus gasseri* TF08-1: A cholesterol-lowering bacterium, isolated from human gut

Mengmeng Wang^{a,b}, Tongyuan Hu^a, Xiaojian Lin^{a,c}, Hewei Liang^a, Wenxi Li^{a,c}, Shaowei Zhao^a, Yiyi Zhong^{a,d}, Haifeng Zhang^{a,d}, Lan Ge^{a,d}, Xin Jin^a, Liang Xiao^{a,c,e,f,g,h}, Yuanqiang Zou^{a,c,e,f,g,h}

^a BGI-Shenzhen, Shenzhen 518083, China
^b College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
^c School of Business and Biotechnology, South China University of Technology, Guangzhou 520006, China
^d BGI Precious Materials (Shenzhen) Technology Co., Ltd, Shenzhen, China
^e Qinghai Europe Advanced Institute for Life Sciences, BGI Shenzhen, Qinghai 206535, China
^f Shenzhen Engineering Laboratory of Detection and Intervention of Human Internal Microbiome, BGI-Shenzhen, Shenzhen, China
^g BGI College of Hainan Institute of Medical and Pharmaceutical Sciences, Zhanjiang University, Zhanjiang 530032, China
^h BGI Research-Wuhan, BGI Wuhan, 430074, China

ARTICLE INFO

Keywords:
Lactobacillus gasseri
Cholesterol-lowering
Probiotics

ABSTRACT

Lactobacillus contribute to maintain the human healthy and use for nutritional additives as probiotics. In this study, a cholesterol-lowering bacterium, *Lactobacillus gasseri* TF08-1, was isolated from the feces of a healthy adolescent, and its probiotic potentials were evaluated through genomic mining and *in vitro* test. The assembled draft genome comprised of 1,974,590 bp and was predicted total of 1,940 CDSs. The annotation of the genome revealed that *L. gasseri* TF08-1 harbored abundant categories of functional genes in metabolic and information processing. Moreover, strain TF08-1 has capacity to utilize D-Glucose, Sucrose, D-Maltose, Salicin, D-Xylose, D-Cellobiose, D-Mannose, and D-Trehalose, as the carbon source. The safety assessment showed strain TF08-1 contained few antibiotic resistance genes and virulence factors and was only resistant to 2 antibiotics detected by antimicrobial susceptibility test. A high bile salt hydrolase activity was found and a cholesterol-reducing effect was determined *in vitro*, which the result showed a remarkable cholesterol removal capability of *L. gasseri* TF08-1 with an efficiency of 84.40%. This study demonstrated that the strain showed great capability of esopolysaccharide production, and tolerance to acid and bile salts. Therefore, these results indicate that *L. gasseri* TF08-1 can be considered as a safe candidate for probiotic, especially its potential in biotherapeutic for metabolic diseases.

1. Introduction

Probiotics are defined as "live microorganisms, which when administered in adequate amounts, confer a health effect on the host" [1]. The species of the genus *Lactobacillus*, such as *L. acidophilus*, *L. rhamnosus*, *L. gasseri*, and *L. plantarum*, are deemed to be important probiotics because of their specific properties that are beneficial to the health of the host [2,3]. As a probiotic, bacterial strain that can enter the small intestine to perform function must have the ability to resist bile and the acidity of the gastrointestinal tract. Another functional property that distinguishes probiotics is their potential to produce antibacterial

substances and lower cholesterol levels [3,4]. Cholesterol is an important basic substance in human metabolism activities. Studies have revealed that high-level blood cholesterol is recognized as a major risk factor for atherosclerosis, coronary heart disease (CHD), and other cardiovascular diseases (CVD) [5,6], which remains the principal cause of death in the world [7]. The traditional drug treatment for cholesterol-lowering has some undesirable side effects, such as neurocognitive disorders [8], myopathy, and autoimmune diseases [9], so probiotics have received more attention to reduce serum cholesterol levels [10]. The investigators verified the mechanism of probiotics on cholesterol-lowering, including increasing bile acid excretion,

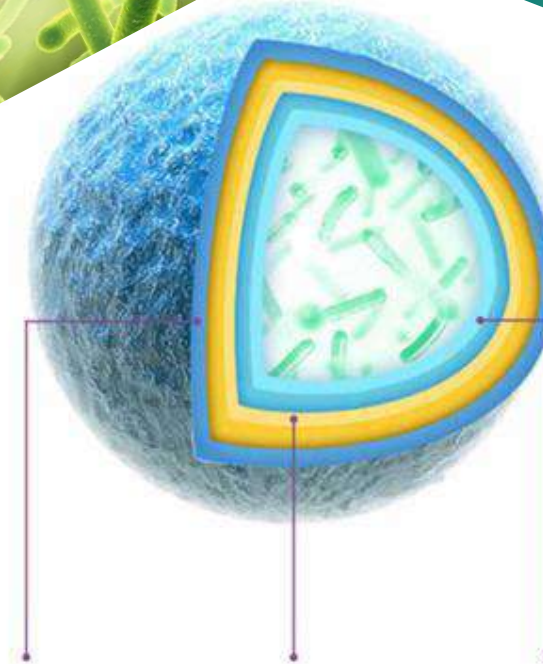
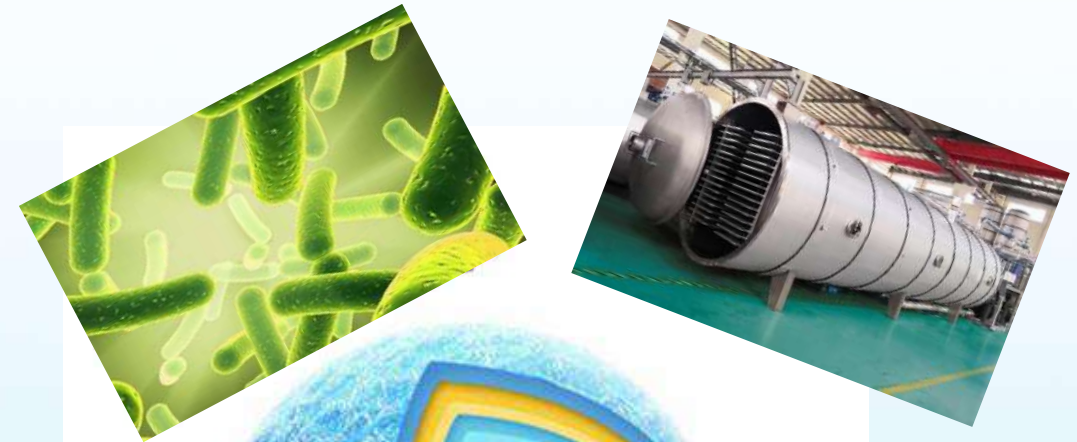
* Correspondence to: BGI-Shenzhen, Beishan Industrial Zone, Shenzhen 518083, China.
E-mail address: xiaoyuanqiang@genomics.cn (Y. Zou).

<https://doi.org/10.1016/j.enzmictec.2023.110276>
Received 28 March 2023; Received in revised form 4 June 2023; Accepted 5 June 2023
Available online 7 June 2023
0141-0229/© 2023 Elsevier Inc. All rights reserved.

Patented Protection Technology



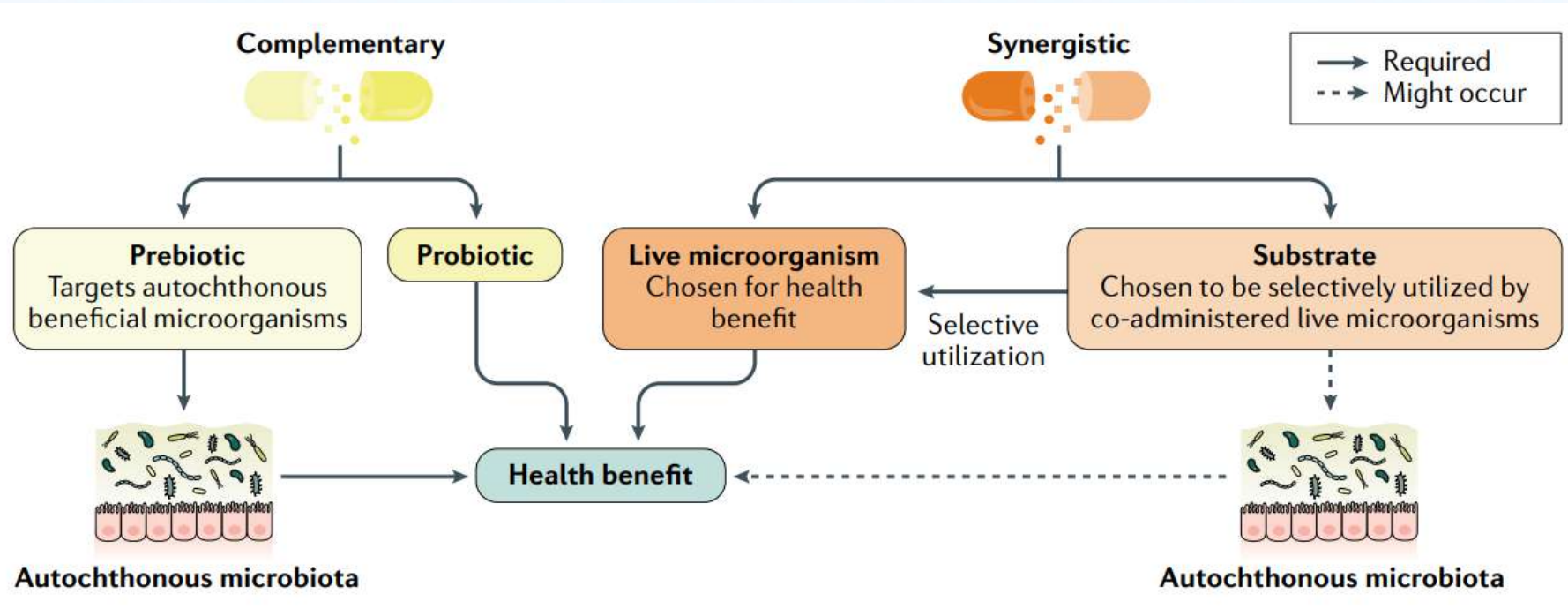
**A freeze dried technology for probiotic protection
ZL 2016 1 1031344.4**



Three Layer Protection

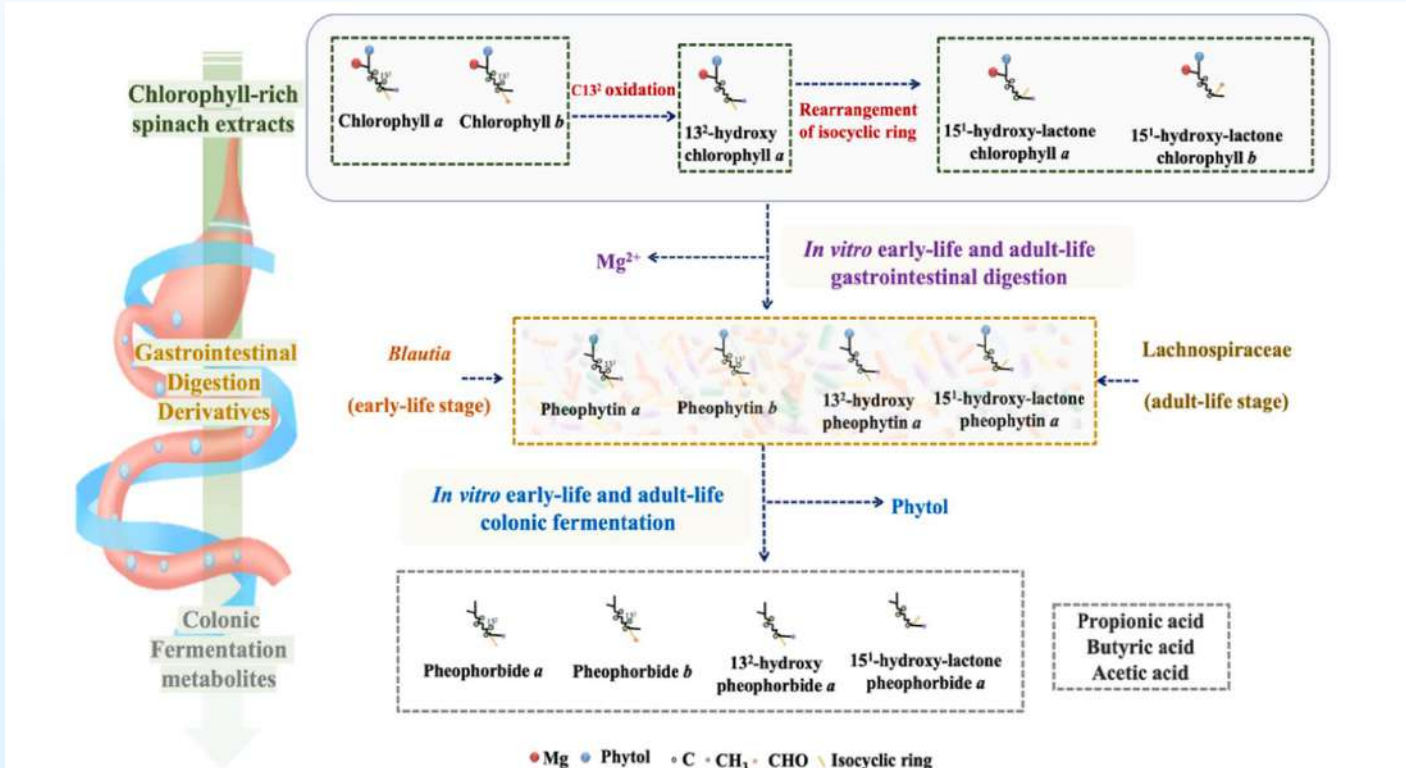
To further protect the probiotic during production process and maintain stable performance during storage, a patented freeze dried and three-layer protection technology was applied to enhance their resistance to adverse environmental factors such as temperature, pH, gas composition.

Formulation of Probiotics and Chlorophyll-rich Spinach Extract



Then we formulate a synergistic combination of all four probiotics and chlorophyll enriched spinach extract in which the spinach extract is designed to be selectively utilized by the co-administered by the probiotics.

Formulation of Probiotics and Chlorophyll-rich Spinach Extract



国家知识产权局

200032
上海市小水塘路681号外经大厦21楼 上海领信律师事务所
发文(021-61258043)新办(021-61258018)

发文日:
2023年07月04日

申请号: 202310801277.3 发文序号: 2023070400503230

专利 申请 受理 通知书

根据专利法第28条及其实施细则第38条、第39条的规定, 申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日等信息通知如下:
申请号: 202310801277.3
申请日: 2023年06月30日
申请人: 华大精微(深圳)科技有限公司, 中国农业大学
发明人: 钟一林, 罗凯, 张燕, 朱海峰, 李斌, 申素霞, 张杰
发明创造名称: 益生菌组合在改善肠道菌群及制备含其的产品中的应用
经核实, 国家知识产权局确认收到文件如下:
权利要求书 1份 2页, 权利要求项数: 30项
说明书 1份 10页
说明书附图 1份 2页
说明书摘要 1份 1页
生物材料存活证明 1份 2页
生物材料存活证明 1份 2页
生物材料保藏证明 1份 2页
发明专利请求书 1份 5页
实质审查请求书 文件份数: 1份
申请方案卷号: P23014231C

提示:
1. 申请人收到专利申请受理通知书之日起, 认为其记载的内容与申请人所提交的相应内容不一致时, 可以向国家知识产权局请求更正。
2. 申请人收到专利申请受理通知书之日起, 应向国家知识产权局办理各种手续, 逾期不办, 视为撤回专利申请。

审查员: 肖娟娟
联系电话: 010-62356655

审查员: 肖娟娟
联系电话: 010-62356655

2023.07.04

国家知识产权局

200032
上海市小水塘路681号外经大厦21楼 上海领信律师事务所
发文(021-61258043)新办(021-61258018)

发文日:
2023年04月04日

申请号: 20231035437.2 发文序号: 2023040400199450

专利 申请 受理 通知书

根据专利法第28条及其实施细则第38条、第39条的规定, 申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日等信息通知如下:
申请号: 20231035437.2
申请日: 2023年04月04日
申请人: 华大精微(深圳)科技有限公司, 中国农业大学
发明人: 钟一林, 罗凯, 张燕, 朱海峰, 李斌, 申素霞, 张杰
发明创造名称: 一种组合及其在改善肠道菌群中的应用
经核实, 国家知识产权局确认收到文件如下:
权利要求书 1份 3页, 权利要求项数: 10项
说明书 1份 13页
说明书附图 1份 6页
说明书摘要 1份 1页
发明专利请求书 1份 5页
实质审查请求书 文件份数: 1份
申请方案卷号: P23013379C

提示:
1. 申请人收到专利申请受理通知书之日起, 认为其记载的内容与申请人所提交的相应内容不一致时, 可以向国家知识产权局请求更正。
2. 申请人收到专利申请受理通知书之日起, 应向国家知识产权局办理各种手续, 逾期不办, 视为撤回专利申请。

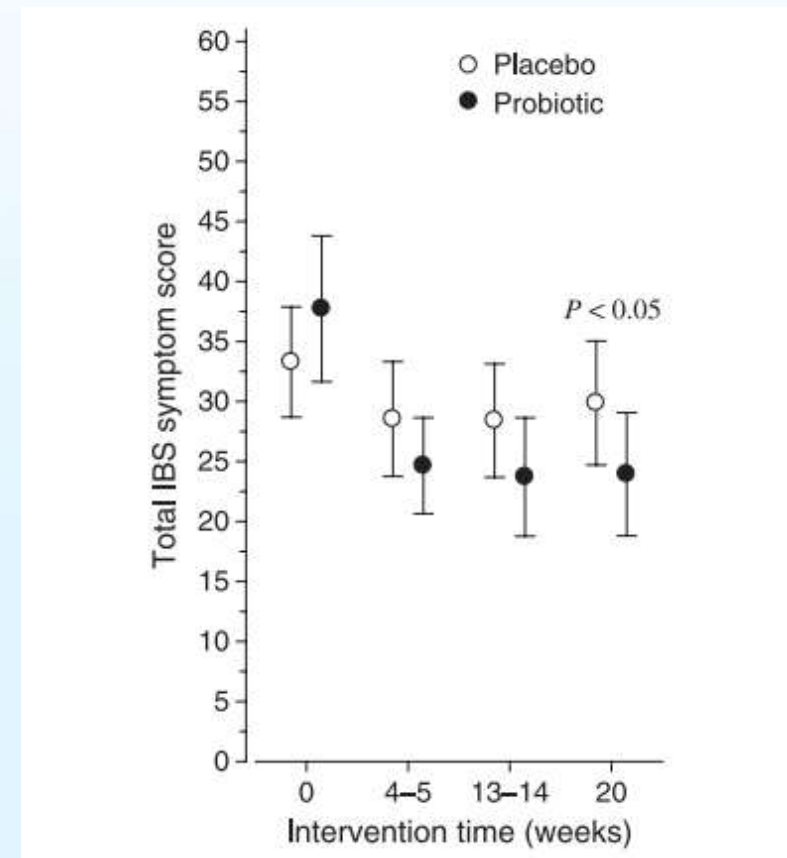
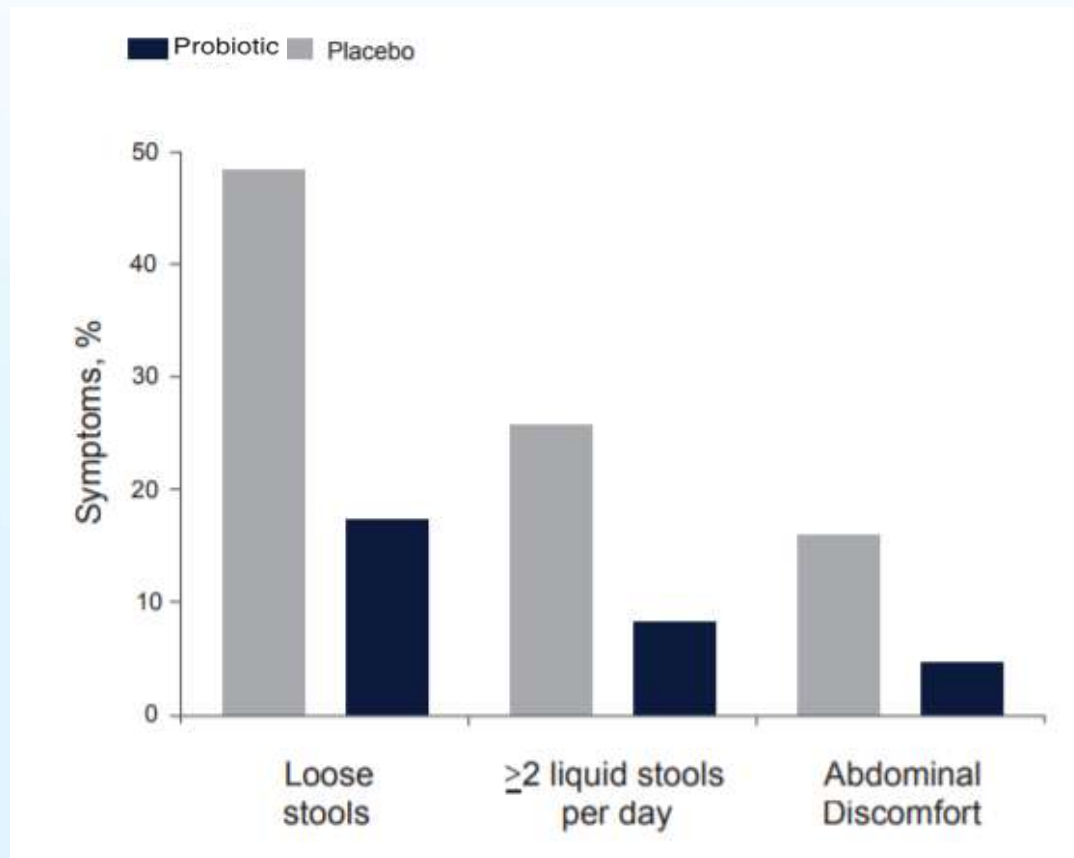
审查员: 肖娟娟
联系电话: 010-62356655

审查员: 肖娟娟
联系电话: 010-62356655

2023.04.04

The chlorophyll metabolites (pheophorbides) by the probiotics could participate in the activation of the nuclear retinoid X receptor involved in fatty acid metabolism, which can alleviate metabolism disorders. We filed two intentional patents their synergistic effects.

Clinical Study



Our clinical trials including a total number of 400 IBS related subjects orally supplemented with our combination of probiotics and plant-based prebiotics had a significant improvement regarding bloating, diarrhea, constipation, abdominal pain with statistical significance.

Sale Progress



**100
million RMB**

By 2023. 12

The product has gained outstanding market performance, including a total sale of 50 million bags and revenue of over 100 million Chinese Yuan.

我们从来不是“一个人”，
而是一个“人菌共生”的生态系统。

尹焯

We were never alone,
but living in a “human bacterial symbiosis” ecosystem.



THANKS