肠道微生态与肠道疾病

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NANFANG HOSPITAL

内 容

1. 肠道微生态及相关概念
2. 肠道微生物在人体内的作用
3. 肠道微生态与肠道疾病
4. 益生菌的现状
5. 新型益生菌---*脆弱拟杆菌*
6. 本团队关于脆弱拟杆菌的工作
7. 小结

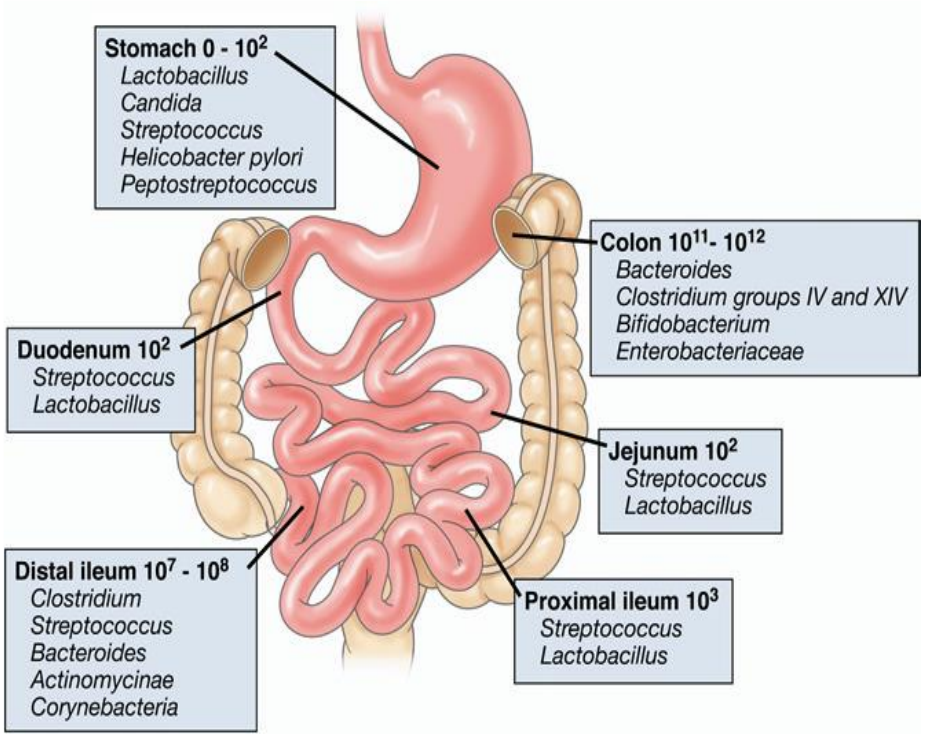
关于肠道微生态及相关概念

--*Intestinal microbiota*

➢胃肠道的微生态系统极为复杂，其内细菌共有500 多种，约10^{13}－10^{14}个不同的细菌，约是人体内真核细胞的10倍。

➢细菌浓度和多样性由胃、十二指肠（含**10^2–10^3CFU/g**细菌量）向空回肠、结肠（含**10^{11}–10^{12}CFU/g**细菌量）逐渐增加。

➢**99%**肠道微生物主要由**4**种菌门组成：厚壁菌门、拟杆菌门、变形菌门和放线菌门。



肠道不同部位的优势菌群及数量

微生物：与人类的环境、生活、健关系密切

肥料　杀虫剂　饲料添加剂

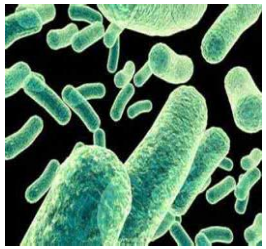
农业

食品

食用菌　微生态食品　食品添加剂

微生物发酵（沼气池）

能源



保健品

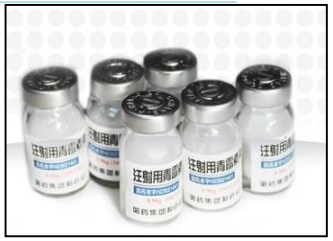
益生菌类保健品

环保

治理前　治理后

微生物治理水污染

医疗



微生物发酵制药

益生菌

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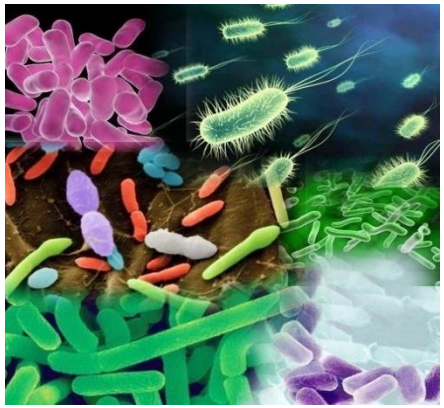
人体微生物群的作用和意义

- ✓ 人体=自身基因+Microbiome （微生物组）
- ✓ 人体微生物组复杂多样，是一个重要的“器官”
- ✓ Microbiota（微生物群）与人体健康密切相关
- ✓ 医学领域创新与革命的新机遇

什么是人体微生态系统?

生态系统 = 生物 + 生存环境

人体微生态系统 = 人体内微生物 + 生存环境



人体微生态系统： 是微生物与人体的组织细胞之间相互制约、相互群居相互平衡所构成的系统

中国微生态杂志

肠道菌群（Intestinal flora）

- 是微生物群的聚集，主要分布在肠粘膜表面和肠腔
- 肠道细菌包括：

原籍菌/常住菌—相对固定、伴随终生，密度高、免疫原性低

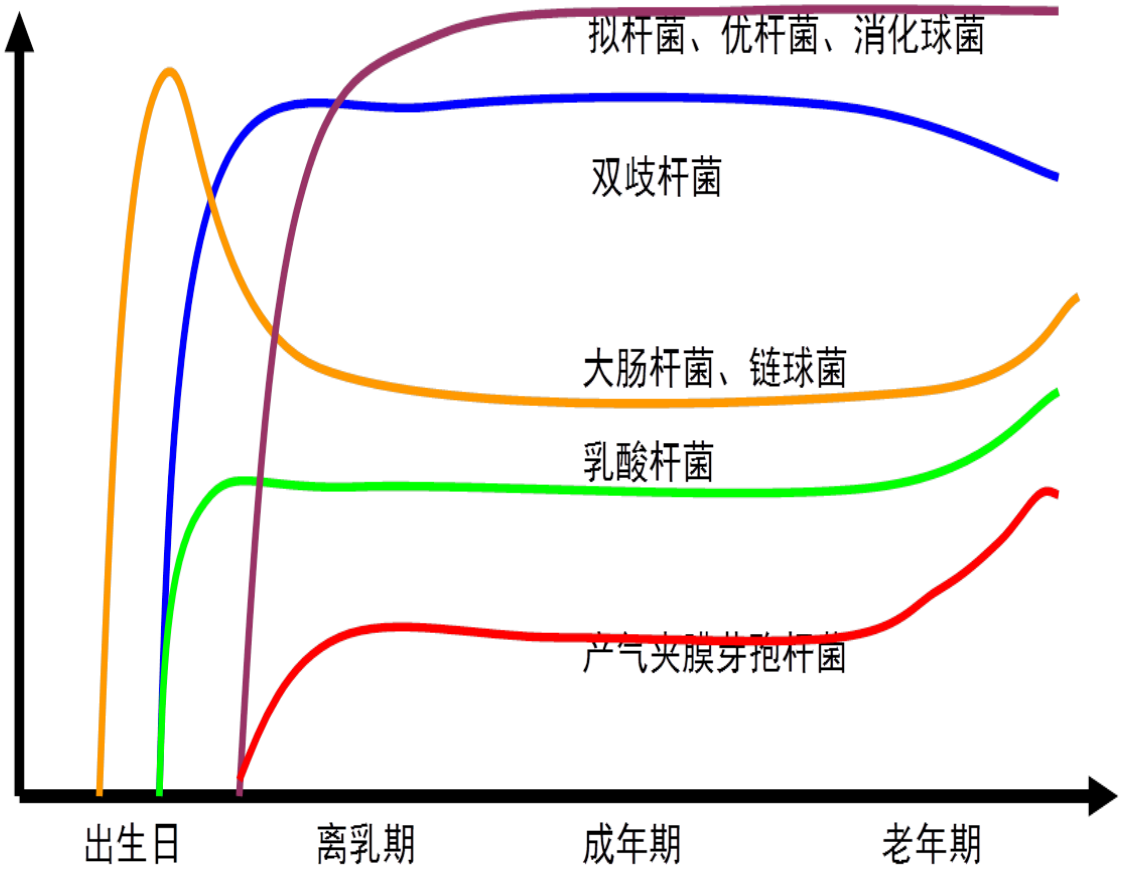
主要在出生时和生命的第1年内获得

外籍菌/暂住菌—匆匆过客、流动性大、有潜在致病性

持续从外界环境中摄入获得

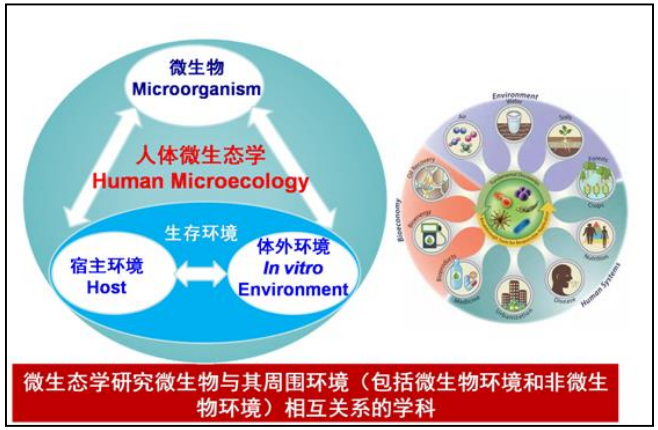
Falk et al, Microbiol Mol Biol Rev 1998

肠道菌群的建立及其影响因素

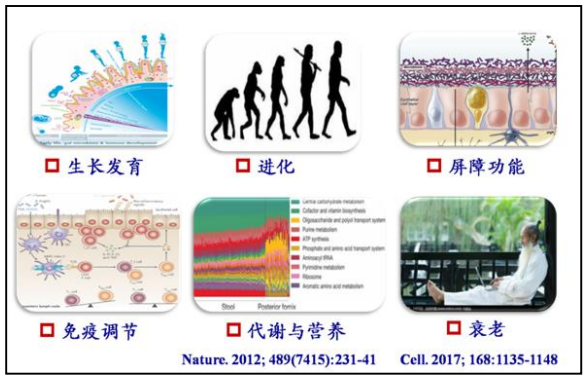


健康人肠道菌生理性演替（依光冈知足，1980）

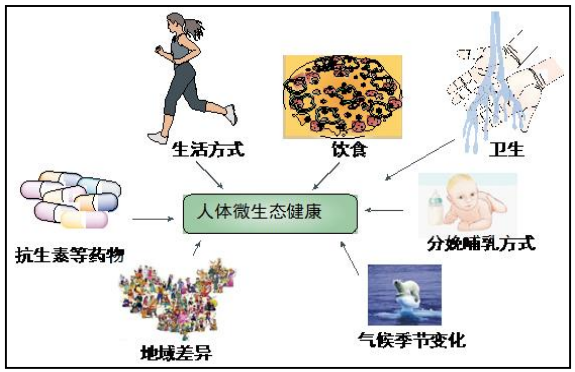
各国纷纷启动微生物组研究计划，我国正积极推进中



人体微生态研究内容



微生态是人体基本生命活动的参与执行者



影响人体微生态的诸多因素

人体微生态相关疾病

肠道微生物在人体内的作用

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正常菌群的生理作用

正常菌群是肠粘膜屏障的重要组成

抵御肠道潜在致病菌(外藉菌)

1.降低肠腔PH值,抑制外藉菌生长

2.专性厌氧菌形成一层生物膜活菌群，防止外藉菌的粘附定植

3.营养争夺作用

4.产生抗菌物质

免疫调节作用

1.全身免疫调节作用

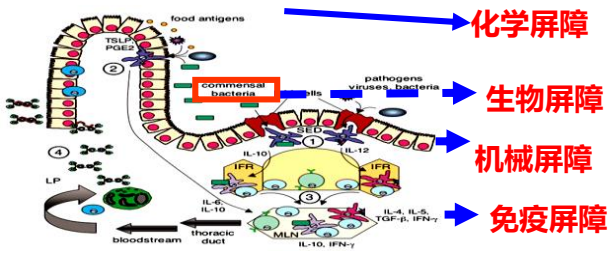
2.肠道局部免疫调节作用

合成维生素

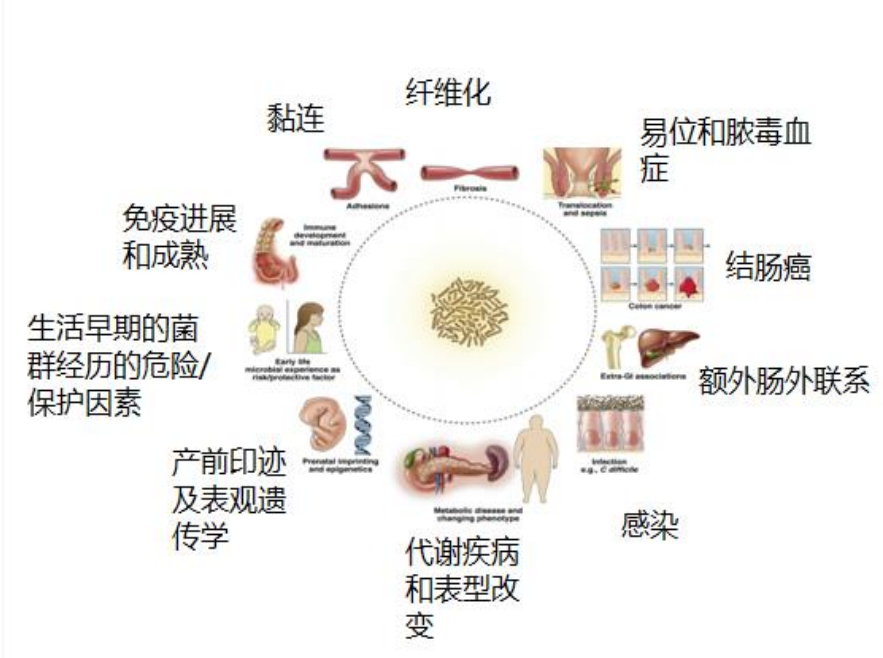
降解食物残渣

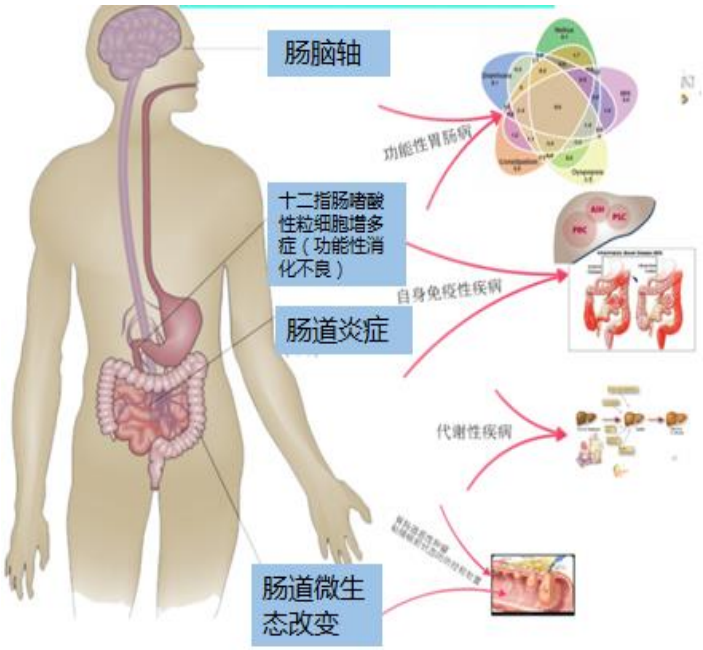
肠道微生态失衡的病理作用

- 致病菌增多,分泌肠毒素使肠上皮通透性增高
- 致病菌分泌免疫抑制蛋白，导致黏膜免疫失调
- 增多的致病菌直接侵袭、损伤肠上皮细胞，破坏肠黏膜屏障
- 产丁酸菌等数量下降，影响肠道上皮细胞的能量来源
- ---等



Nadine Cerf-Bensussan et al. Nature review.2010





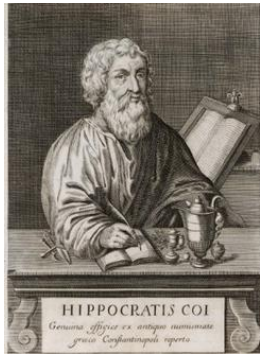
胃肠道微生态失衡可导致疾病

Gastroenterology 2014;146:1554–1563

可 见：

正常的肠道微生态在机体生理、代谢、营养等方面都发挥着重要作用

肠道菌群失调则会导致一系列慢性疾病---如IBD、癌症、糖尿病等



All diseases begin in the gut !

“一切疾病起源于肠道！”

“医学之父”——希波克拉底

Guarner F, Malagelada JR. Lancet 2003; 361: 512–19

Round JL, Mazmanian SK. Nat Rev Immunol, 2009, 9:313-324

Caitriona M. Guinane et al. Therapeutic Advances in Gastroenterology .2013

肠道微生态与肠道疾病

肠道微生态与

抗生素相关性腹泻
伪膜性肠炎
炎症性肠病
感染性腹泻
大肠癌
IBS
便秘

等肠道疾病密切相关

肠道微生态失衡

肠道菌群失衡（Imbalance of gut microbiota）定义：由于肠道菌群组成改变、细菌代谢活性变化或菌群在局部分布变化而引起的失衡状态，表现为肠道菌群在种类、数量、比例、定位转移（移位）和生物学特性上的变化。

	一度失衡	二度失衡	三度失衡
失衡特点	潜伏型，可逆	局限，不可逆失衡	不可逆，菌群交替或二重感染
临床表现	无或轻微胃肠道不适	慢性肠炎、慢性痢疾等	原籍菌大部分被抑制；病情急且重；多发于抗生素、免疫抑制剂、激素、肿瘤等后。

中华预防医学会微生态学分会.中国消化道微生态调节剂临床应用专家共识（2016版）[J].中华临床感染病杂志，2016，9（3）：193

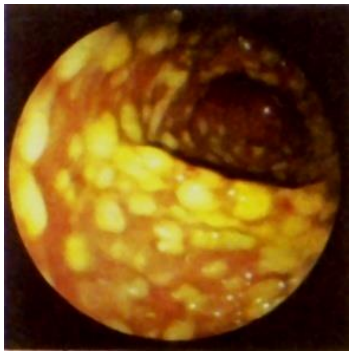
肠道菌群与伪膜性肠炎

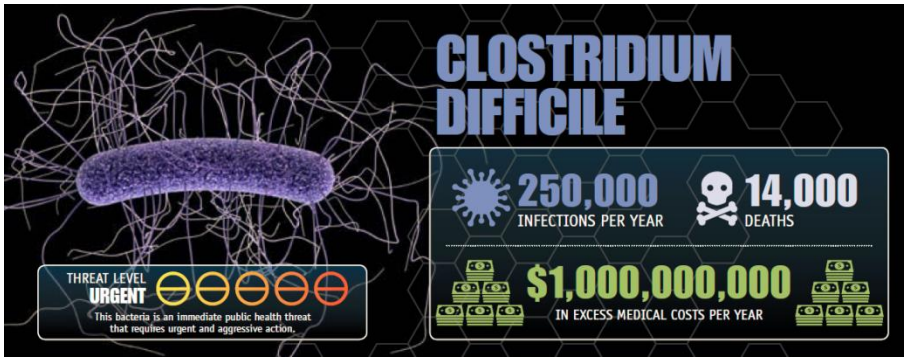
多为继发性感染

病原菌（难辨梭菌性肠炎）为条件致病菌

耐药菌株的感染增多、治疗困难

重症感染预后不良



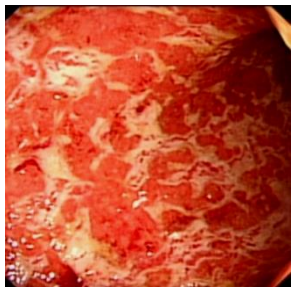
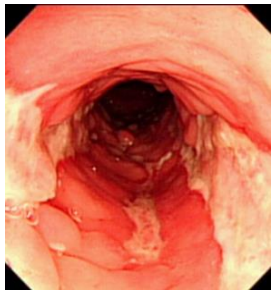


Antibiotic Resistance Threats in the United States, 2013， CDC

肠道菌群与IBD

IBD可能与: 遗　传　学
免　疫　学
肠道微生态紊乱
环 境 因 素
精 神 心 理

} 等因素相关

环境因素作用于遗传易感者，在肠道菌群的参与下，启动了肠道免疫及非免疫系统，最终导致免疫反应和炎症过程。

Cell Host & Microbe
Resource

The Treatment-Naive Microbiome in New-Onset Crohn's Disease

Dirk Gevers,[1] Subra Kugathasan,[4,24] Lee A. Denson,[5,24] Yoshiki Vázquez-Baeza,[6] Will Van Treuren,[7] Boyu Ren,[8] Emma Schwager,[8] Dan Knights,[9,10] Se Jin Song,[7] Moran Yassour,[1] Xochitl C. Morgan,[8] Aleksandar D. Kostic,[1] Chengwei Luo,[1] Antonio González,[7] Daniel McDonald,[7] Yael Haberman,[5] Thomas Walters,[11] Susan Baker,[12] Joel Rosh,[13]

SUMMARY

Inflammatory bowel diseases (IBDs), including Crohn's disease (CD), are genetically linked to host pathways that implicate an underlying role for aberrant immune responses to intestinal microbiota. However, patterns of gut microbiome dysbiosis in

biotic use amplifies the microbial dysbiosis associated with CD. Comparing the microbial signatures between the ileum, the rectum, and fecal samples indicates that at this early stage of disease, assessing the rectal mucosal-associated microbiome offers unique potential for convenient and early diagnosis of CD.

2014年，Gevers 等人进行了一项迄今最大规模，最系统的一项研究，此项研究排除了可能影响菌群的大部分因素，从多层面证实了IBD患者确实存在肠道菌群紊乱。

include Enterobacteriaceae, Pasteurellaceae, Veillonellaceae, and Fusobacteriaceae, and decreased abundance in Erysipelotrichales, Bacteroidales, and Clostridiales, correlates strongly with disease status. Microbiome comparison between CD patients with and without antibiotic exposure indicates that anti-

研究结果1	梭菌（IV 和 XIVa 群）、拟杆菌门明显下降
研究结果2	抗生素的使用导致以上菌群变化更大 抗生素可能加剧CD患者菌群失调

BioMed Research International
Volume 2016, Article ID 5828959, 9 pages
http://dx.doi.org/10.1155/2016/5828959

Hindawi

Review Article

Lower Level of *Bacteroides* in the Gut Microbiota Is Associated with Inflammatory Bowel Disease: A Meta-Analysis

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结论：肠道中拟杆菌的减少与IBD 发病相关，尤其是活动期病人

microbiota from 1990 to 2016. Quality of all eligible studies was assessed using the Newcastle-Ottawa Quality Assessment Scale (NOS). We compared the level of *Bacteroides* in IBD patients with that in a control group without IBD, different types of IBD patients, and IBD patients with active phase and in remission. *Results.* We identified 63 articles, 9 of which contained sufficient data for evaluation. The mean level of *Bacteroides* was significantly lower in Crohn's disease (CD) and ulcerative colitis (UC) patients in active phase than in normal controls. The level of *Bacteroides* in remission CD and UC patients was much lower than patients in the control group. *Bacteroides* level was even lower in patients with CD and UC in active phase than in remission. *Conclusions.* This analysis suggests that lower levels of *Bacteroides* are associated with IBD, especially in active phase.

H. pylori attenuates TNBS-induced colitis via increasing mucosal Th2 cells in mice

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Keywords: *H. pylori, crohn's disease, mucosal immunology, Th cells*

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Hp能够减轻TNBS诱导小鼠肠炎，Hp不但能够下调Th17和Th1细胞因子表达，还能上调Th2细胞因子表达，提高结肠黏膜CD4+T细胞Th2:T17比例。结果显示：Hp通过主要通过增加鼠结肠黏膜的Th2细胞而减轻TNBS诱导的肠炎。将来有可能通过使用Hp 的无毒株来治疗CD

Our results indicate that *H. pylori* attenuates TNBS-induced colitis mainly through increasing Th2 cells in murine colonic mucosa. Our finding offers a novel view on the role of *H. pylori* in regulating gastrointestinal immunity, and may open a new avenue for development of therapeutic strategies in CD by making use of asymptomatic *H. pylori* colonization.

Science
AAAS

MICROBIOTA

Ectopic colonization of oral bacteria in the intestine drives T_H1 cell induction and inflammation

Koji Atarashi,[1,2] Wataru Suda,[1,3,4] Chengwei Luo,[5,6] Takaaki Kawaguchi,[1,2] Iori Motoo,[2] Seiko Narushima,[2] Yuya Kiguchi,[3] Keiko Yasuma,[1] Eiichiro Watanabe,[2] Takeshi Tanoue,[1,2] Christoph A. Thaiss,[7] Mayuko Sato,[8] Kiminori Toyooka,[8] Heba S. Said,[4,9] Hirokazu Yamagami,[10] Scott A. Rice,[11] Dirk Gevers,[5] Ryan C. Johnson,[12] Julia A. Segre,[12] Kong Chen,[13] Jay K. Kolls,[13] Eran Elinav,[7] Hidetoshi Morita,[14] Ramnik J. Xavier,[5,6] Masahira Hattori,[3,4]* Kenya Honda[1,2]*

biota of the mice had been min
of the salivary microbiota (Fig. 1
indicate that bacterial species that constitute a small fraction of the oral microbiota can expand and colonize the gut, and a subset of these oral species can induce the accumulation of intestinal T_H1 cells.

To isolate T_H1 cell–inducing bacteria, we anaerobically cultured cecal contents from GF+CD#2 mice using several culture media and picked 224 colonies with different colony appearances. Sequencing of the 16*S* rRNA genes revealed that these colonies contained eight strains from diverse genera—including *Gemella*, *Bifidobacterium*, *Streptococcus*, *Escherichia*, *Fusobacterium*, *Veillonella*, *Anaerococcus*, and *Klebsiella*—and broadly rep-
resented the major members of the gut microbiota

1. 在肠道某些正常菌群被抑制的情况下，口腔来源的Klebsiella spp.能定植于肠道，并激起机体异常的免疫反应

2.在基因易感性宿主中， Klebsiella spp.的异位定植可引起严重的慢性肠道炎症

3. Klebsiella spp高度耐药且促炎，但其异位定植能力与自身肠道状况相关，保护好肠道原籍菌能够抵抗其异位定植

4.针对口腔细菌，尤其是.Klebsiella spp的治疗有望为缓解IBD提供新的治疗策略

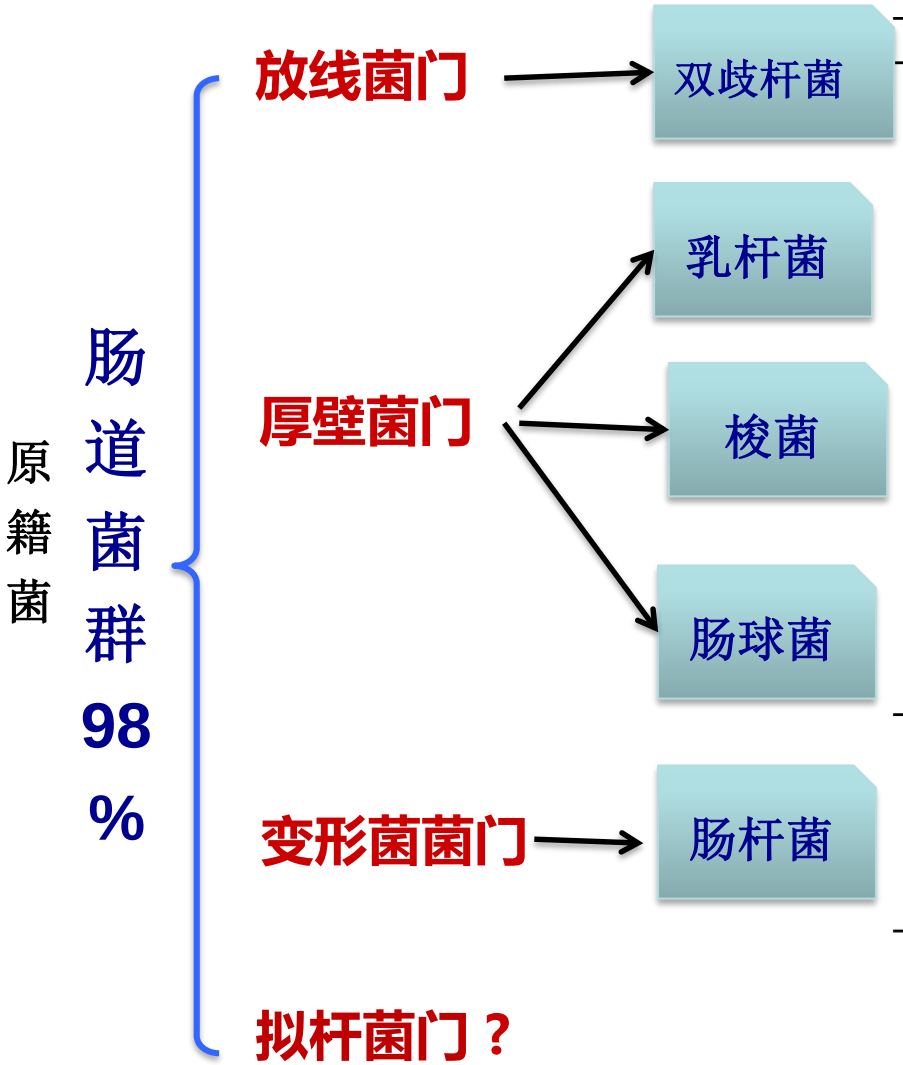
Published 20 October 2017, *Science 358, 359-363 (2017)*
DOI: 10.1126/science.aan4526

其他文献也显示：

微生态很可能在IBD的发生发展中发挥着重要的作用

- IBD 的发病部位多为结肠、直肠、回肠等与细菌接触最多的部位，使用广谱抗生素可改善肠道炎症 (Thompson-Chagoyan, 2005； Greenberg, 2004)
- 对IBD患者病变部位的粪流进行转移，有利改善肠道炎症，而重新恢复粪流则导致炎症复发 (Shanahan, 2004; Fichera,2005）
- 基因连锁分析显示编辑细菌识别受体的CARD15/NOD2基因与IBD的发展倾向有关 (Mathew and Lewis, 2004)
- 无细菌，无肠炎：在许多IBD的动物模型上得到验证 (Sellon , 1998)

益生菌的临床应用现状



来源	商品名	主要成分
国	金双歧	长双歧杆菌、保加利亚乳杆菌、嗜热链球菌
	丽珠肠乐	青春型双歧、长双歧杆菌、嗜酸乳杆菌、粪肠球菌
	培菲康	长双歧杆菌、嗜酸乳杆菌、粪肠球菌
	贝飞达	长双歧杆菌、嗜酸乳杆菌、粪肠球菌
	思连康	婴儿双歧杆菌、嗜酸乳杆菌、粪肠球菌、蜡样芽孢杆菌
	聚克	乳酸乳杆菌、嗜酸乳杆菌、乳酸链球菌、酪酸梭状芽孢杆菌
	常乐康	婴儿双歧杆菌、酪酸梭状芽孢杆菌
	宝乐安	酪酸梭状芽孢杆菌
	美常安	屎肠球菌、枯草芽孢杆菌
	爽舒宝	酪酸梭菌、地衣芽孢杆菌
产	整肠生	地衣芽孢杆菌
	乐腹康	蜡样芽孢杆菌
	米桑	酪酸梭菌
	阿泰宁	酪酸梭状芽孢杆菌
	常立宁	酪酸梭状芽孢杆菌
进	忆活	布拉氏酵母菌
	乐托尔	嗜酸乳杆菌
	米雅	酪酸梭菌
	适怡	乳酸菌、酪酸梭菌、糖化菌
口	妈咪爱	屎肠球菌、枯草杆菌

“粪菌”移植?

- 1958年Eiseman等首次报道用FMT治疗对抗生素无效的伪膜性肠炎
- 1989年Bennet 首次报道用FMT治疗自身的UC，并证实有效
- 2003 年报道了6 例重度复发性UC，在通过粪菌移植之后取得了临床、内镜及组织上的完全缓解
- 目前，国内有补充粪菌液/胶囊的应用，但成为药物近期内尚无可能。

Table 1. FMT treatment for IBD

Study	Disease	Years of diagnosis	Prior treatments	Pretreatment	Mode of administration	Documented remission
Bennet and Brinkman, 1989 (81)	UC	7	AS, S	Antibiotics and PEG	Retention enema	6 months
Borody et al., 1989 (78)	UC	1.5	AS	Not reported	Retention enema	3 months
"	CD	0	None	Not reported	Retention enema	4 months
Borody et al., 2003 (82)	UC	6	AS, S	V, M, R, PEG	Retention enema	13 years
"	UC	20	AS, S	V, M, R, PEG	Retention enema	12 years
"	UC	5	AS, S, A, C	V, M, R, PEG	Retention enema	4 years
"	UC	14	AS, S, A	V, M, R, PEG	Retention enema	2 years
"	UC	15	AS, S, A	V, M, R, PEG	Retention enema	1 year
"	UC	10	AS, S, A	V, M, R, PEG	Retention enema	1 year

A, azathioprine; AS, aminosalicylates; C, cyclosporine; CD, Crohn's disease; M, metronidazole; PEG, polyethylene glycol; R, rifampicin; S, steroids; UC, ulcerative colitis; V, vancomycin.

FMT用于治疗IBD的部分案例显现出较好的疗效

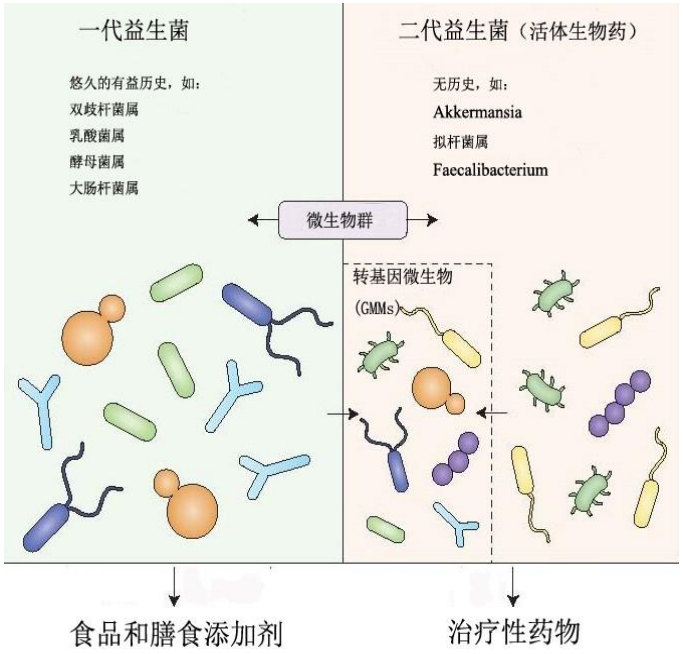
Christopher J. et al，2012

二代益生菌/活体生物药

2017年4月25日出版的Nature Microbiology：

O‘Toole PW发表了 “Next-generation probiotics: the spectrum from probiotics to live biotherapeutics”

提出了“ 新一代益生菌：从益生菌到活体生物治疗药物”



O'Toole PW, Marchesi JR, Hill C. Nat Microbiol. 2017 Apr 25;2:17057. doi: 10.1038/nmicrobiol.2017.57

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二代益生菌（Next-generation probiotics，NGPs)

随着二代测序技术突飞猛进，人体微生物面纱逐步揭开，二代益生菌（Next-generation probiotics，NGP）将崭露头角。

活体生物药（Live biotherapeutic，LBPs)

FDA定义为具有如下特征的一种生物活性产品：

①含有活的生物体，如细菌

②用于预防、治疗人类疾病或适应症

③非疫苗

2016年FDA和CBER颁布了生物活性药物治疗指南

南方醫院
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1857-1874
巴斯德发现乳酸菌

1878
利斯特从牛奶中分离出乳酸菌

1889
双歧杆菌被Tissier 描述

1907
梅奇尼科夫宣布保加利亚杆菌与健康密切相关

1900
嗜酸杆菌被Moro描述

1903
基于干酪乳杆菌分离株Shirota 发酵乳商品化

1953
Kollath 将促进健康活性物质命名为"probiotika"

1965
Lilley 和 Stillwell 将益生菌定义为有助于其他微生物生长的微生物群

1989
富勒,将益生菌定义为有益的微生物添加剂

2001
FAO/WHO 定义益生菌

2003
基因组时代：植物乳杆菌，第一个完成基因组测序

2005
Relman 使用高通量 16S 扩增子测序将肠道微生物编入目录

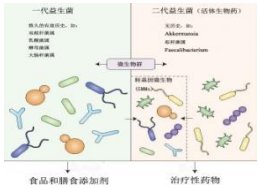
2016
FDA/CBER 颁布活性生物治疗药物指南

1850 1900 1950 2000

NGPs（Next-generation probiotics，二代益生菌）

=LBPs（live biotherapeutic，活体生物药）

一种生物活性产品，具有如下特征（FDA定义）：
(1) 含有活的生物体，如细菌；
(2) 用于预防、处理、治疗人类疾病或适应症；
(3) 非疫苗。

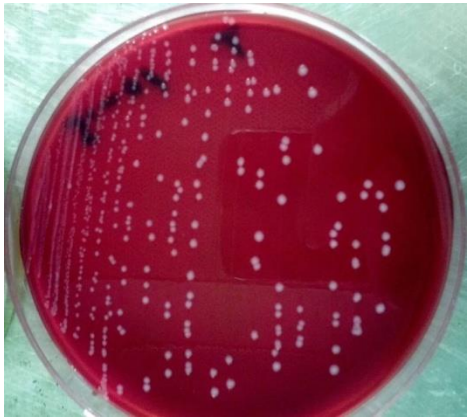


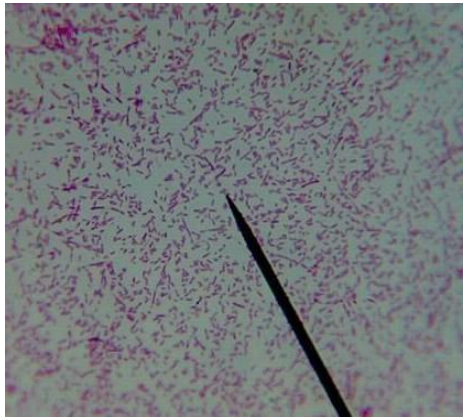
目前世界上最有希望的首个NGPs/LBPs--*脆弱拟杆菌*

关于脆弱拟杆菌

脆弱拟杆菌（bacteroides fragilis）

- 拟杆菌属，正常寄居于人体肠道、口腔、呼吸道等
- 革兰氏染色阴性、无芽孢、专性厌氧的小杆菌
- 培养呈圆，微凸，灰白，表面光滑，边缘整齐
- 根据有无携带bft1、2、3基因分为有毒株和无毒株





Cell, Vol. 122, 107–118, July 15, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.05.007

An Immunomodulatory Molecule of Symbiotic Bacteria Directs Maturation of the Host Immune System

Sarkis K. Mazmanian,[1,3,*] Cui Hua Liu,[1,2] Arthur O. Tzianabos,[1,3] and Dennis L. Kasper[1,3,*]

Summary

The mammalian gastrointestinal tract harbors a complex ecosystem consisting of countless bacteria in homeostasis with the host immune system. Shaped by evolution, this partnership has potential for symbiotic benefit. However, the identities of bacterial molecules mediating symbiosis remain undefined. Here we show that, during colonization of animals with the ubiquitous gut microorganism *Bacteroides fragilis*, a bacterial polysaccharide (PSA) directs the cellular and physical maturation of the developing immune system. Comparison with germ-free animals reveals that the immunomodulatory activities of PSA during *B. fragilis* colonization include correcting systemic T cell deficiencies and T_H1/T_H2 imbalances and directing lymphoid organogenesis. A PSA mutant of *B. fragilis* does not restore these immunologic functions. PSA presented by intestinal dendritic cells activates $CD4^+$ T cells and elicits appropriate cytokine production. These findings provide a molecular basis for host-bacterial symbiosis and reveal the archetypal molecule of commensal bacteria that mediates development of the host immune system.

2005年Mazmanian SK 等在无菌鼠中进行实验，发现无毒脆弱拟杆菌株中的菌体成分多聚糖（PSA）可以调节机体T细胞缺乏和TH1/TH2比例的平衡，从而调节机体免疫功能

B. Fragilis有可能成为一种益生菌

nature Vol 453|29 May 2008|doi:10.1038/nature07008

ARTICLES

A microbial symbiosis factor prevents intestinal inflammatory disease

Sarkis K. Mazmanian[1]*, June L. Round[1]* & Dennis L. Kasper[2,3]

Humans are colonized by multitudes of commensal organisms representing members of five of the six kingdoms of life; however, our gastrointestinal tract provides residence to both beneficial and potentially pathogenic microorganisms. Imbalances in the composition of the bacterial microbiota, known as dysbiosis, are postulated to be a major factor in human disorders such as inflammatory bowel disease. We report here that the prominent human symbiont *Bacteroides fragilis* protects animals from experimental colitis induced by *Helicobacter hepaticus*, a commensal bacterium with pathogenic potential. This beneficial activity requires a single microbial molecule (polysaccharide A, PSA). In animals harbouring *B. fragilis* not expressing PSA, *H. hepaticus* colonization leads to disease and pro-inflammatory cytokine production in colonic tissues. Purified PSA administered to animals is required to suppress pro-inflammatory interleukin-17 production by intestinal immune cells and also inhibits *in vitro* reactions in cell cultures. Furthermore, PSA protects from inflammatory disease through a functional requirement for interleukin-10-producing $CD4^+$ T cells. These results show that molecules of the bacterial microbiota can mediate the critical balance between health and disease. Harnessing the immunomodulatory capacity of symbiosis factors such as PSA might potentially provide therapeutics for human inflammatory disorders on the basis of entirely novel biological principles.

结果：①B. fragilis可以预防肝螺杆菌诱导的实验性肠炎

②这种作用通过菌体成分PSA发挥

③主要通过抑制炎症因子IL-17 和促进调节因子IL-10的产生发挥作用

结论：B. fragilis有望成为治疗肠炎的手段

Proc Natl Acad Sci U S A. 2010 Jul 6;107(27):12204-9

Inducible Foxp3$^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota

June L. Round and Sarkis K. Mazmanian[1]

Division of Biology, California Institute of Technology, Pasadena, CA 91125

Edited* by Richard A. Flavell, Yale University School of Medicine, Howard Hughes Medical Institute, New Haven, CT, and approved June 2, 2010 (received for review August 13, 2009)

结果：①B. fragilis可以诱导Treg细胞分化，进而产生调节性因子IL-10和TGF-β2，对机体产生免疫调节作用

②这种作用依赖于PSA的存在

③对TNBS诱导的肠炎具有治疗作用

结论：B. fragilis具有治疗IBD的可能

LETTER

Nature. 2013 Sep 19;501(7467):426-9

doi:10.1038/nature12447

Bacterial colonization factors control specificity and stability of the gut microbiota

S. Melanie Lee[1], Gregory P. Donaldson[1]*, Zbigniew Mikulski[2]*, Silva Boyajian[1], Klaus Ley[2] & Sarkis K. Mazmanian[1]

结果：①**B. fragilis**可以影响其他细菌的定植

②这种作用依赖于其**ccf**基因的存在

结论：**B. fragilis**有望成为一种益生菌

Cell

Volume 155, Issue 7, 19 December 2013, Pages 1451–1463

Article

Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders

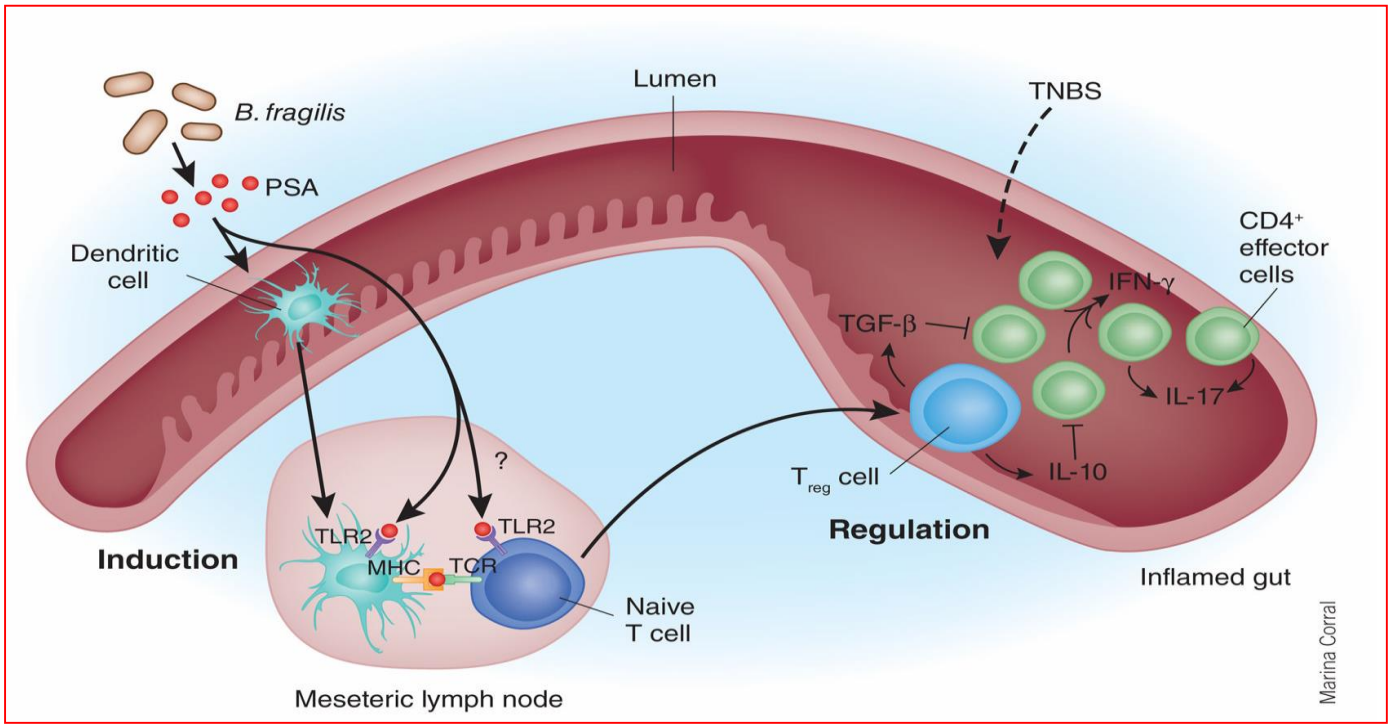
Elaine Y. Hsiao[1, 2], Sara W. McBride[1], Sophia Hsien[1], Gil Sharon[1], Embriette R. Hyde[3], Tyler McCue[3], Julian A. Codelli[2], Janet Chow[1], Sarah E. Reisman[2], Joseph F. Petrosino[3], Paul H. Patterson[1, 4], Sarkis K. Mazmanian[1, 4]

a metabolite that is increased by MIA and restored by *B. fragilis* causes certain behavioral abnormalities, suggesting that gut bacterial effects on the host metabolome impact behavior. Taken together, these findings support a gut-microbiome-brain connection in a mouse model of ASD and identify a potential probiotic therapy for GI and particular behavioral symptoms in human neurodevelopmental disorders.

明确指出：脆弱拟杆菌有治疗疾病的潜力

B. fragilis 具体作用机理

如图：**B. fragilis 通过菌体成分PSA被树突状细胞吞噬后，提呈给初始CD4+ T细胞，进而与CD4+ T细胞表面Toll样受体2结合，从而诱导固有层或者肠系膜淋巴结中的Treg细胞分化，分化后的Treg细胞迁移到炎症部位，通过分泌调节性因子IL-10和TGF- β2，抑制促炎因子IFN-γ和 IL-17而发挥治疗炎症作用。**



脆弱拟杆菌有望成为治疗IBD的药物

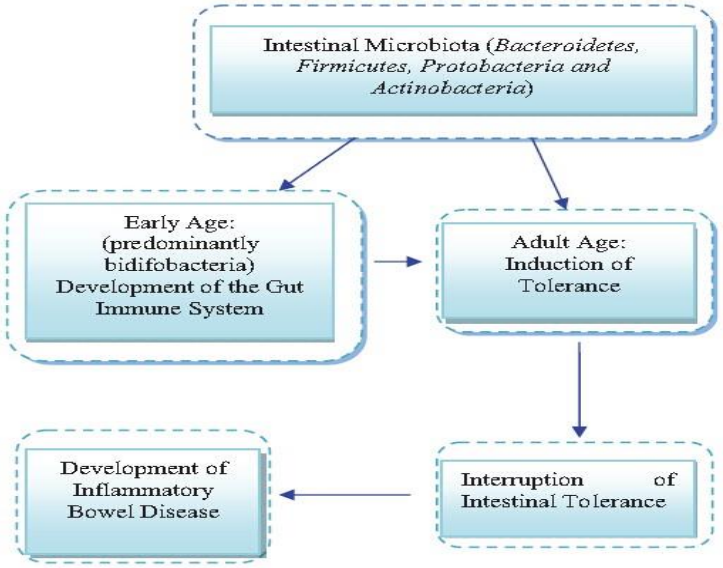
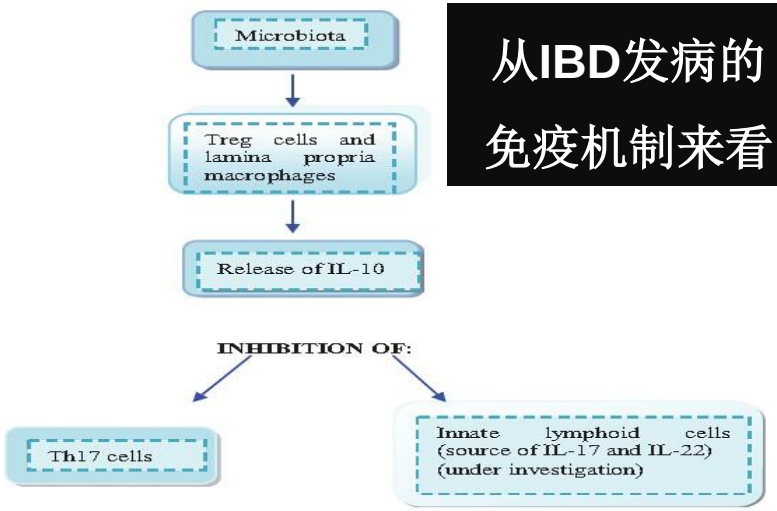


Fig. (1). Influence of the intestinal microbiota on gut immunity.



从IBD发病的免疫机制来看

Fig. (2). The intestinal microbiota induces the tolerogenic pathway in the bowel.

在人出生后早期，肠道菌群的定植促使了肠道免疫功能的成熟，成年后，肠道菌群平衡诱导了肠道对于细菌的免疫耐受，一旦这种耐受被打破，就有可能发展成为**IBD**

这种免疫耐受主要是由微生态与固有层免疫细胞作用，通过**Treg**细胞分泌**IL-10**进而调节炎性细胞**Th17**与**IL-17**、 **IL-22**的活性

虽然**IBD**发病机制是由于机体对菌体成分炎症反应过强、还是对机体免疫调节能力下降所致仍未清楚，但是脆弱拟杆菌具有影响细菌定植、抑制炎症反应、调节免疫耐受等功能，均有利于治疗炎症，故其很可能成为治疗**IBD**的一种新型药物

本团队关于脆弱拟杆菌的研究工作

南方醫院 NANFANG HOSPITAL

中华医学杂志 2014 年 8 月 12 日第 94 卷第 30 期 Natl Med J China, August 12, 2014, Vol. 94, No. 30

·临床研究·

健康婴儿体内的无毒脆弱拟杆菌的分离及鉴定

刘洋洋 张文娣 白杨 智发朝

【摘要】 **目的** 对分离到的 1 株无毒脆弱拟杆菌进行鉴定。**方法** 2012 年 7 月从健康足月新生儿的粪便中分离得到 1 株脆弱拟杆菌，观察其形态特征、培养特征，并进行生理生化鉴定、药敏试验及 16s rRNA 序列测定和分析。急慢性毒性试验检测毒性。**结果** 分离得到的菌株在细菌形态、培养特性、生理生化反应结果与脆弱拟杆菌相似。经 BLASTN 序列比对，所分离菌株与脆弱拟杆菌标准株 NCTC9343 同源性达 99%。药敏实验提示，新分离菌株对头孢拉定、阿莫西林、庆大霉素、磺胺甲噁唑、甲氧苄啶不敏感，急慢性毒性试验提示无毒性。**结论** 分离的细菌为 1 株无毒的脆弱拟杆菌。

【关键词】 拟杆菌，脆弱； 生物学鉴定法； 婴儿

Isolation and identification of a non-enterotoxigenic strain of *Bacteroides fragilis* from a healthy term infant

*Liu Yangyang**, *Zhang Wendi*#, *Bai Yang*, *Zhi Fachao*. **Guangzhou Zhiyi Biotechnology Limited Liability Company, Guangzhou 510515, China*; #*Department of Gastroenterology, Nanfang Hospital of Southern Medical University, Guangzhou 510515, China*

Zhang Wendi and Liu Yangyang are the first authors who contributed equally to the article

Corresponding author: Zhi Fachao, Email: zhifc41532@163.com

P1. 菌株鉴定及评价

全基因组测序

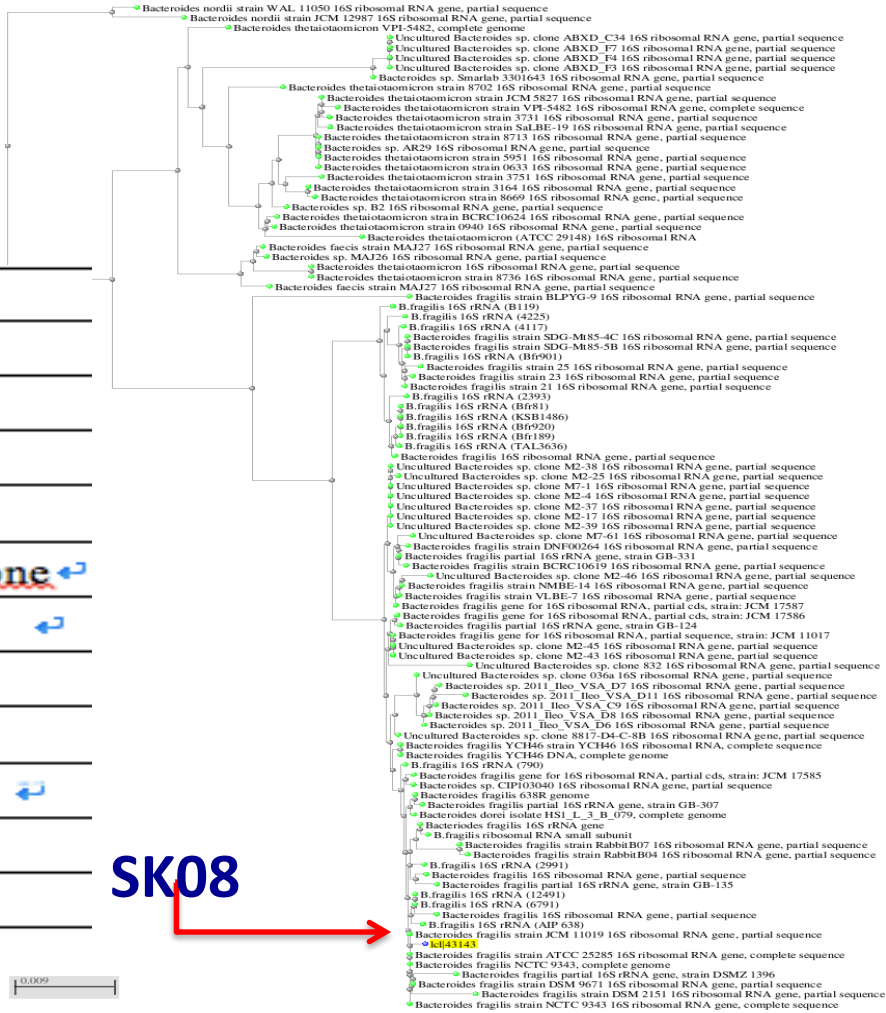
潜在毒力基因

Name
Alginate algI
Capsule cap8E
Capsule cap8G
Capsule cap8O
Capsule cps4I
Capsule cps4I
Capsule cpsJ
Capsule fcl
Capsule glf
ClpC clpC
Dispersin aatC
Flagella fleQ
HSI-I PA0073
Hsp60 htpB
LPS gmd
MgtBC mgtB

耐药基因

Drug
bacitracin
cephalosporin
trimethoprim
macrolide
chloramphenicol, fluoroquinolone
aminoglycoside, glycylcycline
fosmidomycin
tetracycline
vancomycin, teicoplanin
streptogramin_a
na_antimicrobials

系统发育树

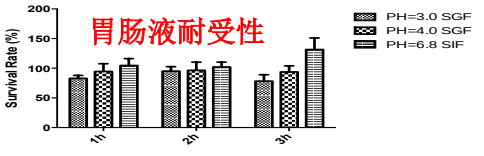


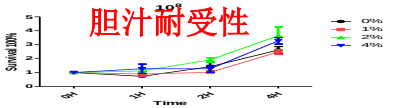
SK08安全性评价

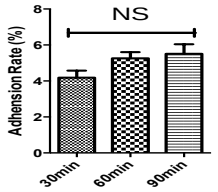
序号	名称
1	SK08 项目原始种子形态学检查
2	SK08 项目原始种子生理生化实验
3	SK08 项目原始种子杂菌检查
4	SK08 项目原始种子抗微生物药敏感实验
5	SK08 项目原始种子分子生物学鉴定
6	SK08 项目原始种子人工胃肠液耐受评价
7	SK08 项目原始种子耐受胆粉实验
8	SK08 项目原始种子菌体 LoVo 细胞毒性实验
9	SK08 项目原始种子细胞黏附实验
10	SK08 项目原始种子代谢产物小鼠毒性实验
11	SK08 项目原始种子小鼠毒性实验
12	SK08 项目原始种子耐药性实验
13	SK08 项目原始种子稳定性考察实验
14	SK08 项目原始种子（HT-29）细胞毒性
15	SK08 项目原始种子菌体裂解液细胞毒性
16	SK08 菌体裂解液对原代巨噬细胞吞噬致病菌的影响
17	SK08 菌体裂解液对原代巨噬细胞吞噬荧光微球的影响
18	SK08 菌体裂解液对原代巨噬细胞表达细胞因子的影响

菌种鉴定结果及评价

- ZY-312为一株脆弱拟杆菌，和ATCC25285同源理化性质及产物相似
- 主要代谢产物：乙酸、琥珀酸、丙酸、苯乙酸乳酸、异丁酸
- 不含有明显毒力基因
- 耐药基因存在于染色体，没有水平转移的风险
- 生长迅速、耐氧能力强、具有较好的黏附性能
- 耐受pH 3.0人工胃液，耐受肠液、胆汁
- 具有动物安全性和细胞安全性







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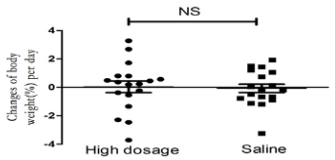
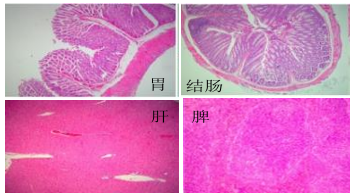
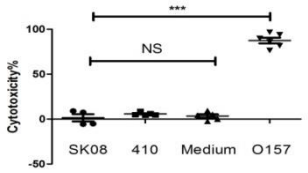
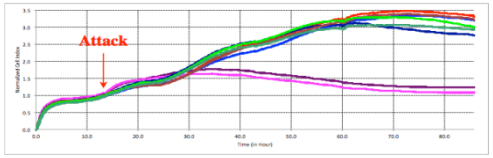


5×10^{11}CFU/天
连续5天







frontiers in Microbiology

ORIGINAL RESEARCH
published: 17 March 2017
doi: 10.3389/fmicb.2017.00435

南方醫院 NANFANG HOSPITAL

Safety Evaluation of a Novel Strain of *Bacteroides fragilis*

Ye Wang[1,2†], Huimin Deng[2,3†], Zhengchao Li[2,3†], Yafang Tan[3], Yanping Han[3], Xiaoyi Wang[3], Zongmin Du[3], Yangyang Liu[4], Ruifu Yang[3], Yang Bai[2], Yujing Bi[3]* and Fachao Zhi[2]**

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Commensal non-toxigenic *Bacteroides fragilis* confers powerful health benefits to the host, and has recently been identified as a promising probiotic candidate. We previously isolated *B. fragilis* strain ZY-312 and identified it as a novel strain based on 16S rRNA sequencing and morphological analyses. We also determined that ZY-312 displayed desirable probiotic properties, including tolerance to simulated digestive fluid, adherence, and *in vitro* safety. In this study, we aim to investigate whether ZY-312 meets the safety criteria required for probiotic bacteria through comprehensive and systematic evaluation. Consequently, the fatty acid profile, metabolite production, and biochemical activity of strain ZY-312 were found to closely resemble descriptions of *B. fragilis* in Bergey's manual. Taxonomic identification of strain ZY-312 based on whole genome sequencing indicated that ZY-312 and ATCC 25285 showed 99.99% similarity. The 33 putative virulence-associated factors identified in ZY-312 mainly encoded structural proteins and proteins with physiological activity, while the lack of *bft* indicated that ZY-312 was non-toxigenic. *In vivo* safety was proven in both normal and immune-deficient mice. The 11 identified antibiotic resistance genes were located on the chromosome rather than on a plasmid, ruling out the risk of plasmid-mediated transfer of antibiotic resistance. *In vitro*, ZY-312 showed resistance to cefepime, kanamycin, and streptomycin. Finally, and notably, ZY-312 exhibited high genetic stability after 100 passages *in vitro*. This study supplements the foundation work on the safety evaluation of ZY-312, and contributes to the development of the first probiotic representative from the dominant Bacteroidetes phylum.

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本株脆弱拟杆菌属无毒株，安全性评价符合安全标准，具有益生菌潜质。

P2. 菌株的药效学研究

南方醫院 NANFANG HOSPITAL

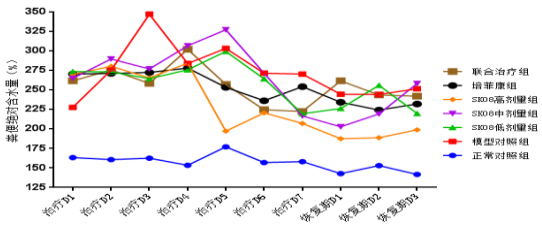
1. SK08治疗单纯性AAD动物的药效试验

表 2 各组软便动物数

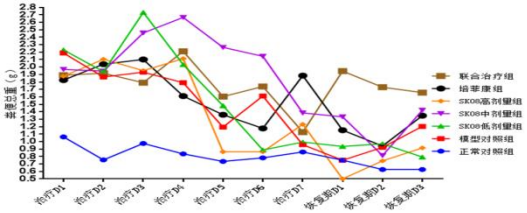
组别	治疗期							恢复期		
	D1	D2	D3	D4	D5	D6	D7	D1	D2	D3
正常对照组	2	0	1	1	0	0	0	0	0	0
模型对照组	16	16	15	14	8	8	6	3	3	3
SK08 低剂量组	16	13	14	12	8	3	2	4	4	0
SK08 中剂量组	16	12	11	11	7	3	3	1	2	2
SK08 高剂量组	16	12	12	11	0	1	1	1	0	0
培菲康组	16	13	13	13	4	2	4	4	3	2
联合治疗组	16	13	9	13	3	2	3	6	4	0

注：治疗期 D1 至 D4，n=16；治疗期 D5 至恢复期 D3，n=8；表中数值为软便（稀便）动物数

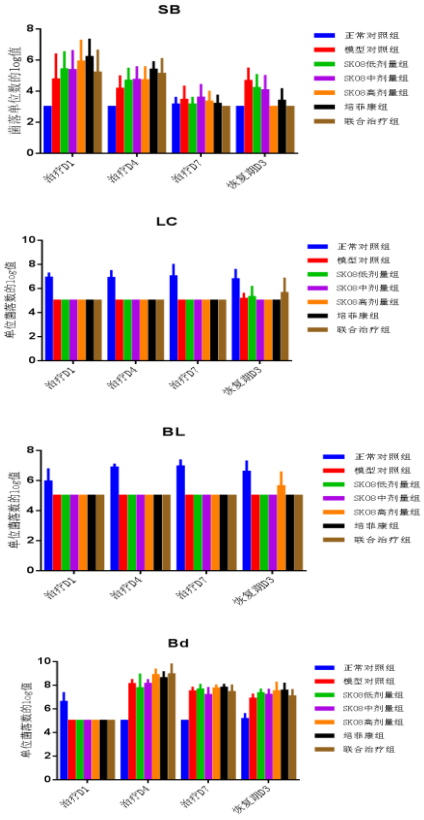
腹泻率：治疗期第5天开始，SK08高剂量组动物软便数降为0-1只，相比其余各组都显著降低



粪便绝对含水量：治疗后，SK08高剂量组的粪便含水量明显下降，下降趋势最明显



2h粪便总重:治疗后，SK08高剂量组的2h粪便总重明显下降，治疗结束后下降至正常对照组水平



治疗后，各组的LC、BL（乳酸、双歧杆菌）均未恢复，Bd显著增加，SK08高剂量组的酵母菌SB显著下降

frontiers in Immunology

ORIGINAL RESEARCH
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Bacteroides fragilis Protects Against Antibiotic-Associated Diarrhea in Rats by Modulating Intestinal Defenses

Wandi Zhang[1*], Bo Zhu[1*], Jiahui Xu[1], Yangyang Liu[2], Enqi Qiu[1], Zhijun Li[1], Zhongchao Li[1], Yan He[3], Hongwei Zhou[3], Yang Bai[1] and Fachao Zhi[1*]

Results: Rats exposed to adequate antibiotics developed diarrhea symptoms, indicating successful establishment of the model. These rats also showed microbiota alterations, with overgrowth of some pathogenic bacteria, and exhibited gastrointestinal barrier defects, including low aquaporin expression, aberrant tight junction proteins, and decreased mucus-filled goblet cells compared to control rats. Oral treatment with *B. fragilis* ameliorated AAD-related diarrhea symptoms by reversing the microbiota changes, restoring barrier function, and promoting enterocyte regeneration.

Conclusion: We identified a potential probiotic therapeutic strategy for AAD and established vital roles of *B. fragilis* in reshaping the colonic bacteria and microbiota-modulated epithelial differentiation

2. SK08治疗副溶血弧菌感染的动物实验

南方醫院 NANFANG HOSPITAL

实验结果/结论：

① B.f培养上清能够抑制副溶血弧菌的生长

② B.f能够减少副溶血弧菌对于细胞的损伤

③ B.f能够缩短副溶血弧菌发光株在小鼠体内的停留时间

认为：本株脆弱拟杆菌有可能在副溶血弧菌引起的肠道感染之防治中发挥重要作用

frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
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南方醫院 NANFANG HOSPITAL

Bioluminescence Imaging to Track *Bacteroides fragilis* Inhibition of *Vibrio parahaemolyticus* Infection in Mice

Zhongchao Li[1,2†], Huimin Dong[1,2†], Yazhou Zhou[2], Yafang Tan[2], Xiaoyi Wang[2], Yanping Han[2], Yangyang Liu[3], Ye Wang[3], Ruifu Yang[2], Yujing Bi[2*] and Fachao Zhi[1*]*

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Bacteroides fragilis is an anaerobic, Gram-negative, commensal bacterium of the human gut. It plays an important role in promoting the maturation of the immune system, as well as suppressing abnormal inflammation. Many recent studies have focused on the relationship between *B. fragilis* and human immunity, and indicate that *B. fragilis* has many useful probiotic effects. As inhibition of intestinal pathogens is an important characteristic of probiotic strains, this study examined whether *B. fragilis* could inhibit pathogenic bacteria. Results showed that *Vibrio parahaemolyticus* was inhibited by *B. fragilis in vitro*, and that *B. fragilis* could protect both RAW 264.7 and LoVo cells from damage caused by *V. parahaemolyticus*. Using *in vivo* imaging, we constructed a light-emitting *V. parahaemolyticus* strain and showed that *B. fragilis* might shorten the colonization time and reduce the number of *lux*-expressing bacteria in a mouse model. These results provide useful information for developing *B. fragilis* into a probiotic product, and also indicate that this commensal bacterium might aid in the clinical treatment of gastroenteritis caused by *V. parahaemolyticus*.

Keywords: *Bacteroides fragilis*, *Vibrio parahaemolyticus*, real-time cell analysis, bioluminescence, *in vivo* imaging

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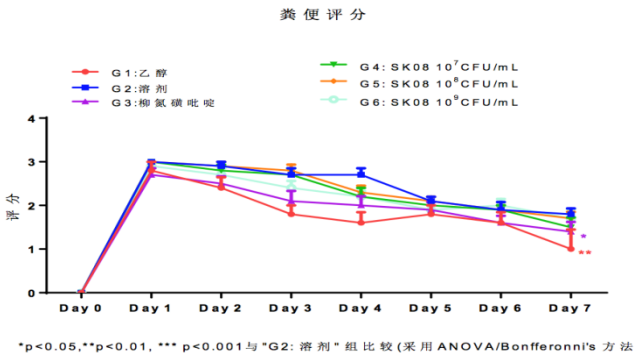
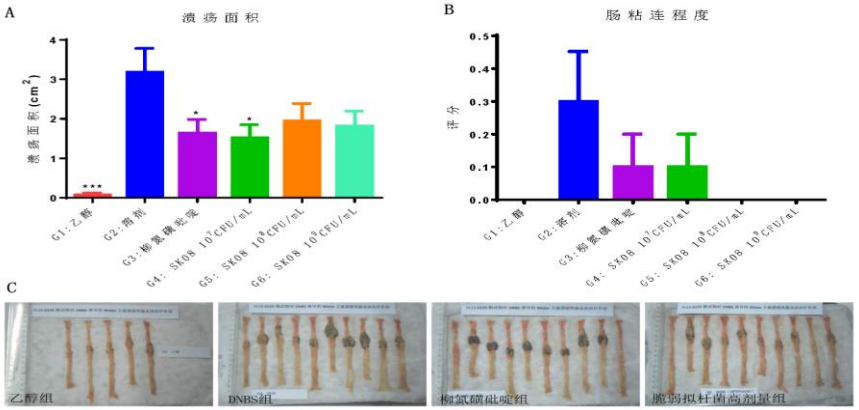
INTRODUCTION

本株脆弱拟杆菌有可能在副溶血弧菌引起的肠道感染之防治中发挥重要作用

3. SK08治疗DSS诱导的肠炎动物药效试验





SK08显著降低腹泻评分

SK08显著降低溃疡面积、肠粘连程度，且效果优于SASP

4. SK08预防阪崎肠杆菌诱发的肠炎

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RESEARCH ARTICLE
Host-Microbe Biology

Bacteroides fragilis Strain ZY-312 Defense against *Cronobacter sakazakii*-Induced Necrotizing Enterocolitis *In Vitro* and in a Neonatal Rat Model

Hongying Fan,[a] Zhenhui Chen,[a] Ruqin Lin,[a] Yangyang Liu,[c] Xianbo Wu,[a] Santhosh Puthiyakunnon,[a] Ye Wang,[c] Bo Zhu,[b] Qiwei Zhang,[a] Yang Bai,[b] Fachao Zhi[b]

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SK08可预防阪崎肠杆菌诱发的肠炎

其机制是调节肠上皮屏障功能、减少细胞凋亡及焦亡

P3. 菌株的药理学研究

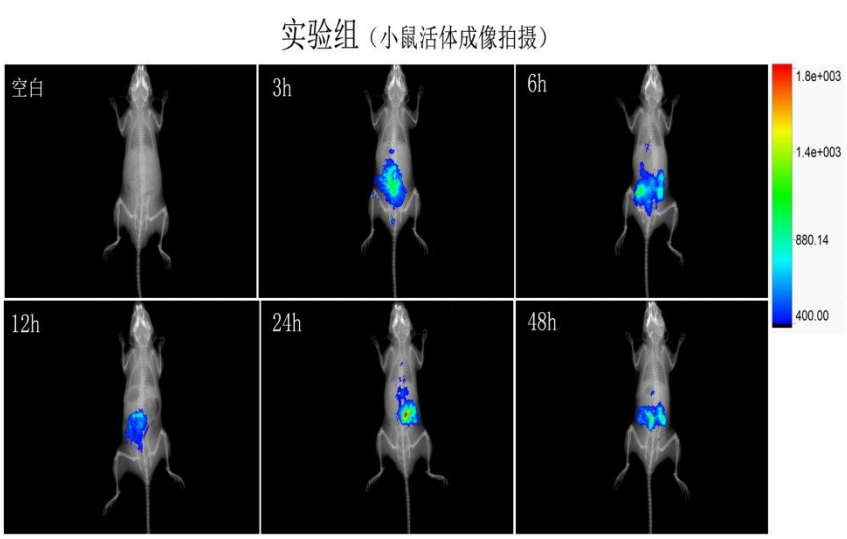
- **SK08促进IEC6增殖，对抗有害菌内毒素**
- **SK08可能通过下调 p38介导的通路而抑制细胞凋亡及炎症反应**
- **SK08可以影响其他菌群的定植**
- **免疫调节作用：***维持Th1/Th2平衡，下调CDC42影响Th1细胞反应（维持促炎反应和抑炎反应平衡）*
- **SK08与巨噬细胞相互作用***----增强巨噬细胞吞噬致病菌*

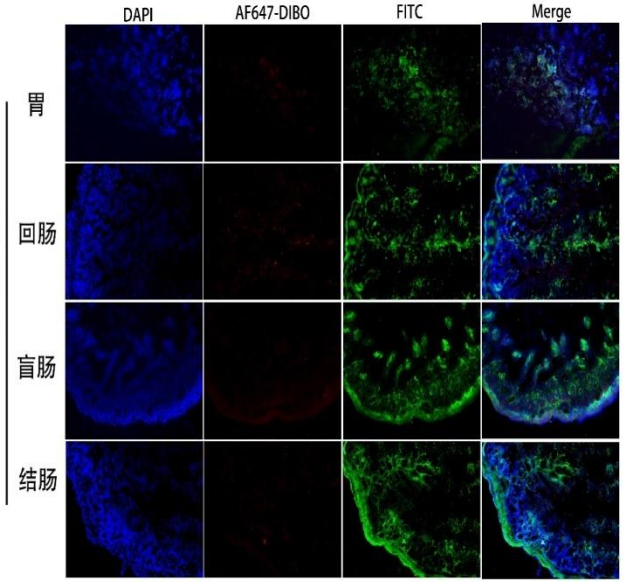
Huimin Deng/Fachao Zhi.. Scientific Reports | 6:29401 | DOI: 10.1038/srep29401,1-11 （IF= 5.228）

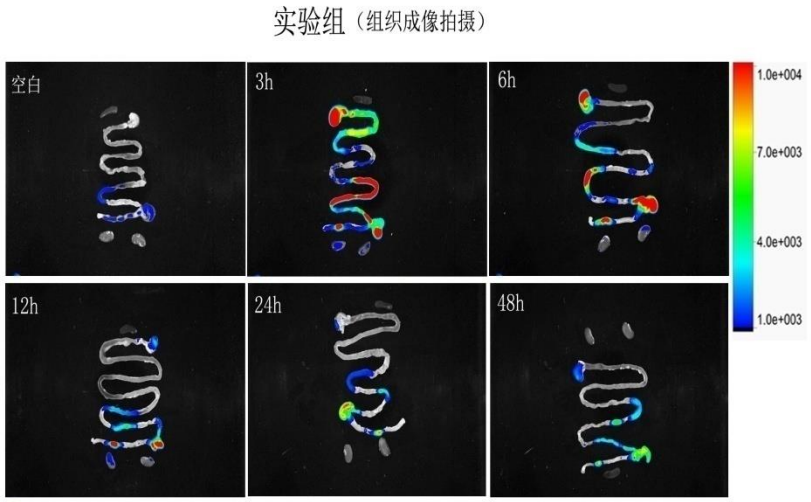
Ye Wang/Fachao Zhi. Frontiers in Microbiology 2017;8: 435. doi: 10.3389/fmicb.2017.00435（IF=4.164）

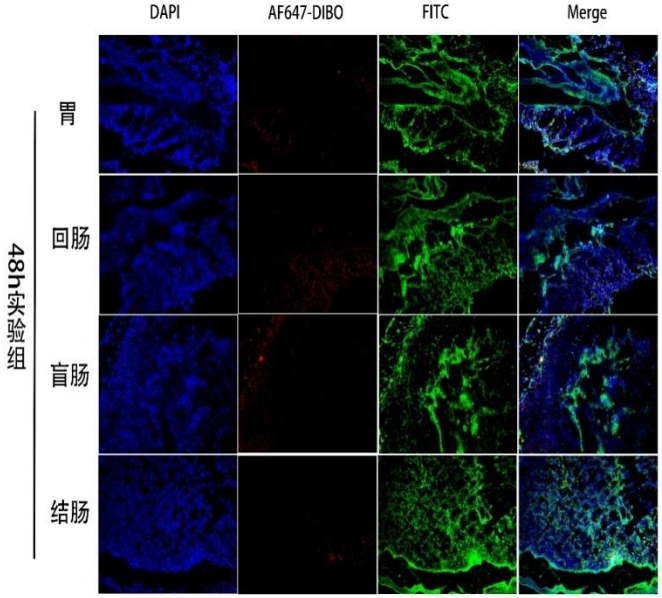
Zhengchao Li/Fachao Zhi. Frontiers in Cellular and Infection Microbiology 2017;7(Article 170):1-9 doi: 10.3389/fcimb.2017.00170 （Ⅱ区，IF=5.218）

SK08 体内定植分析



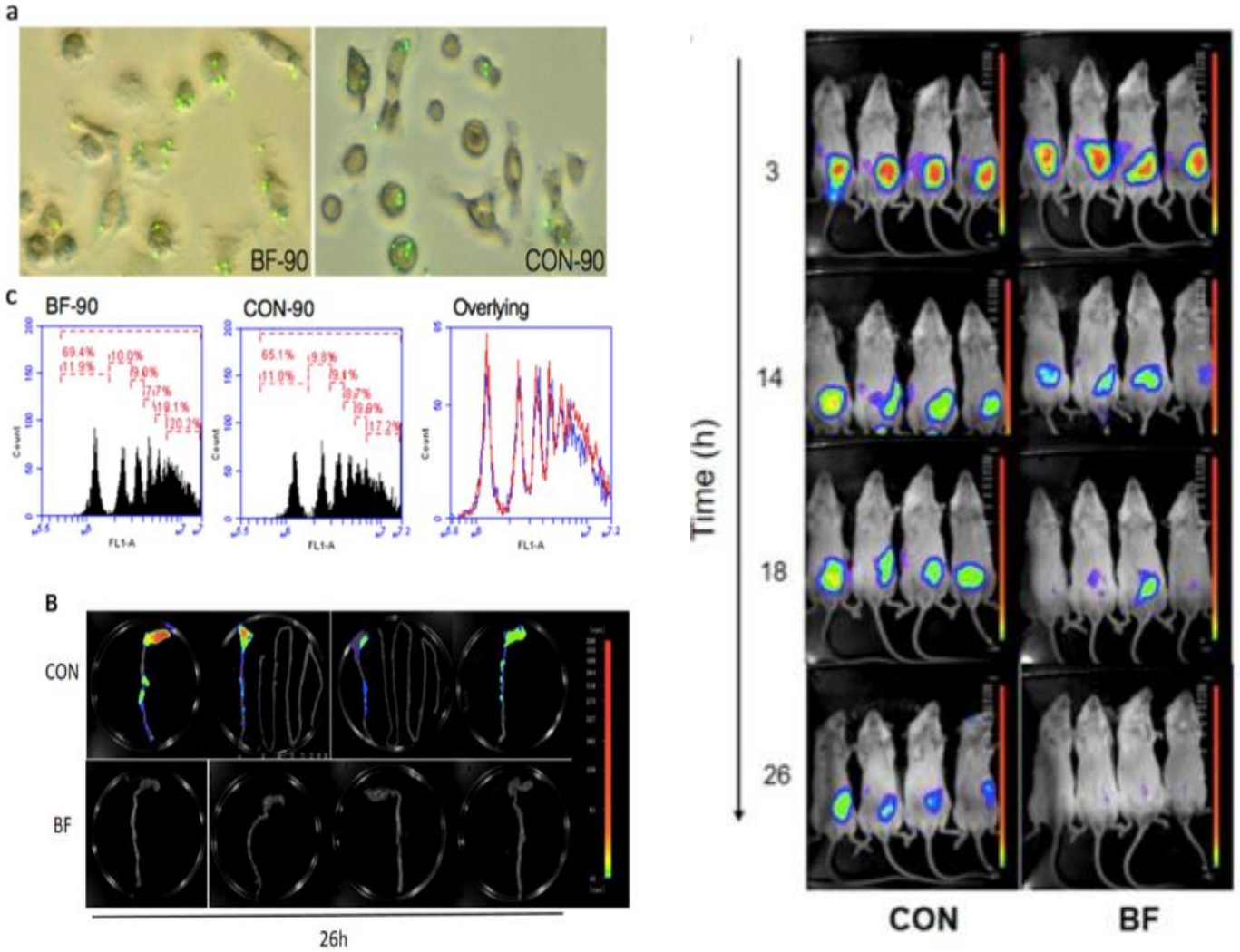






SK08菌灌胃给予小鼠，24h后主要分布在肠道，48h在盲肠和结肠定植。

主要定植部位在小鼠盲肠和结肠。



Sci rep. 2016, 6: 29401
Front Microbiol. 2017,8:435
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SCIENTIFIC REPORTS

OPEN

A novel strain of *Bacteroides fragilis* enhances phagocytosis and polarises M1 macrophages

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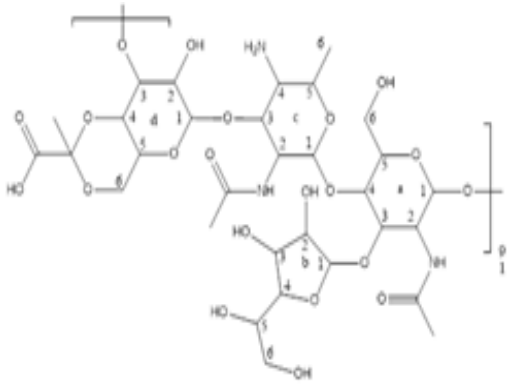
Huimin Deng[1,2,*], Zhengchao Li[1,2,*], Yafang Tan[2], Zhaobiao Guo[2], Yangyang Liu[3], Ye Wang[3], Yuan Yuan[2], Ruifu Yang[2], Yujing Bi[2], Yang Bai[1] & Fachao Zhi[1]

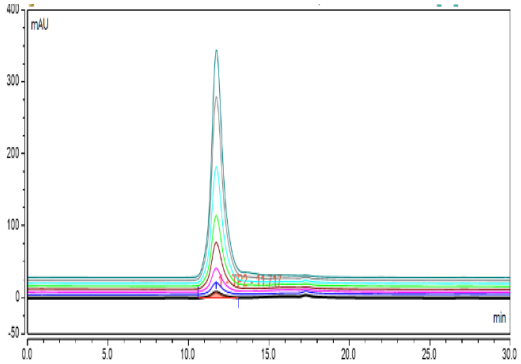
Commensal *Bacteroides fragilis* possesses immune-regulatory characteristics. Consequently, it has been proposed as a potential novel probiotic because of its therapeutic effects on immune imbalance, mental disorders and inflammatory diseases. Macrophages play a central role in the immune response

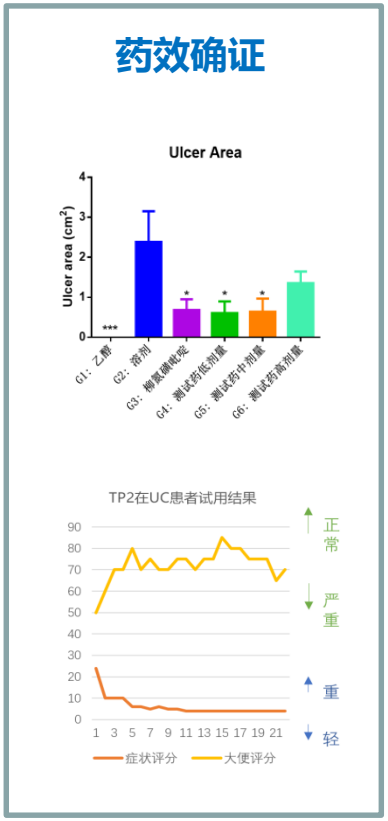
enhances the phagocytic functions of macrophages, polarising them to an M1 phenotype. Our findings provide insight into the close relationship between *B. fragilis* and the innate immune system.

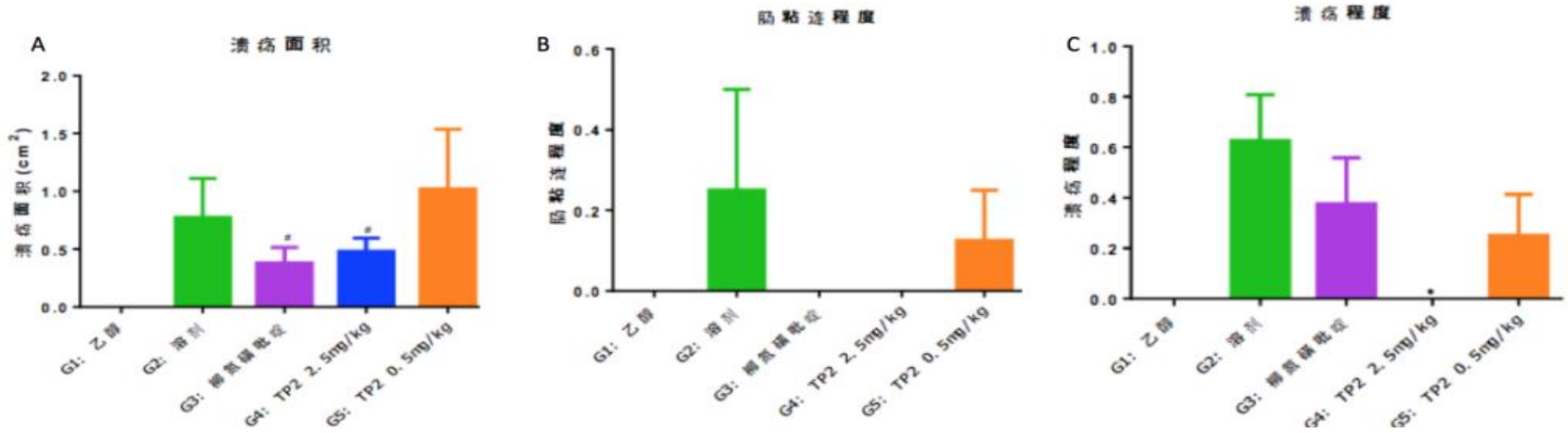
- 体外增强骨髓诱导巨噬细胞（BMDM）吞噬能力，促进吞噬更多荧光微球及致病菌（肠出血性大肠杆菌O157: H7，EHEC）
- 诱导M1型巨噬细胞，经菌体及裂解液刺激后BMDM表达NO、TNF-α、IL-1β和IL-12明显上调。

P4. 国际上首次大规模提取并纯化了本株B. f的荚膜多糖（PSA，代号TP2），确证了其分子结构，优化了提取工艺，完成了DSS诱导大鼠肠炎的药效学研究



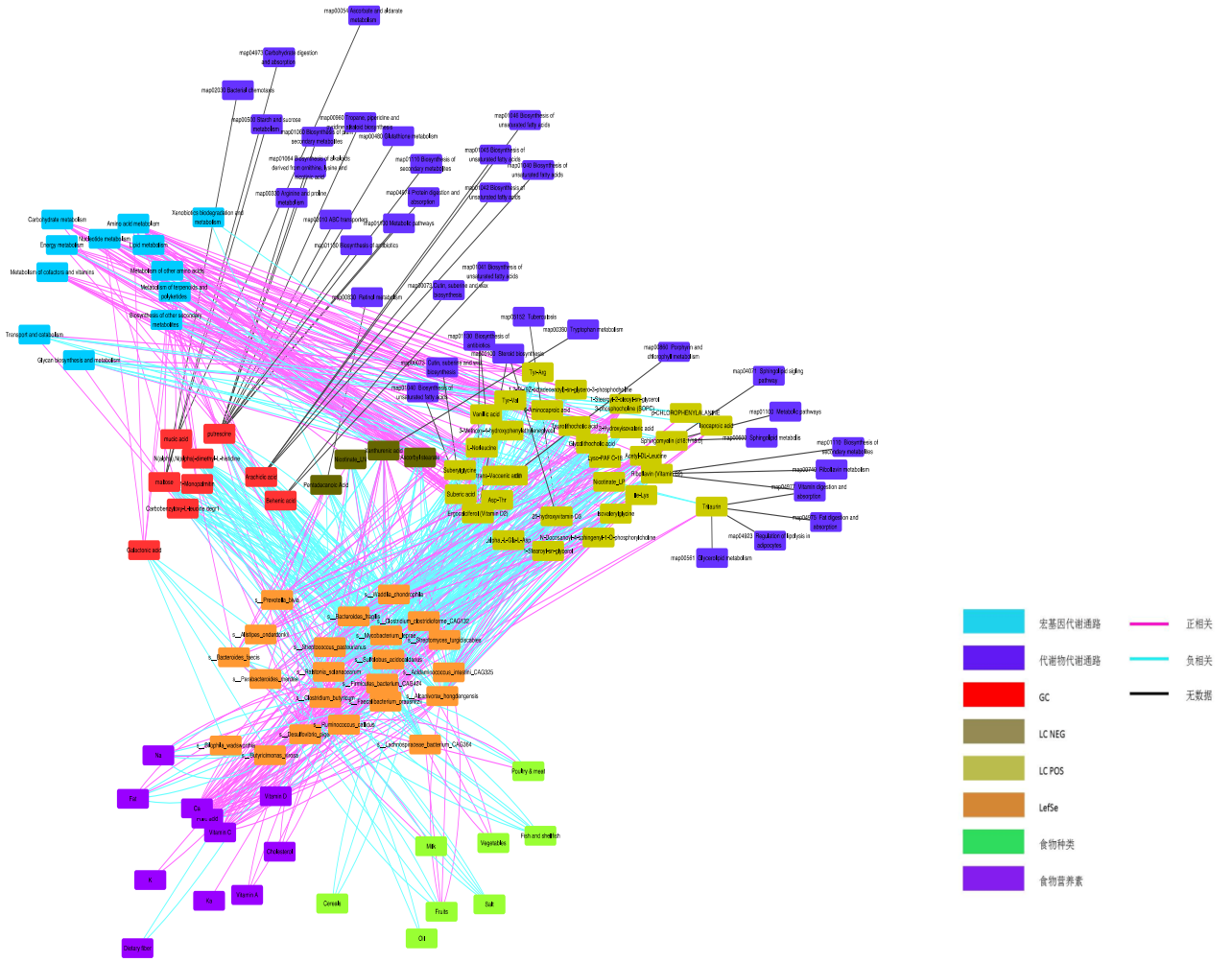






明显降低肠粘连程度和肠壁增厚程度，且能明显减少肠道溃疡的面积，效果优于SASP

P5. 系统绘制了IBD患者饮食、肠道菌群、代谢产物、代谢通路多组学关联图



（Unpublished data)

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小　结

- 肠道微生态对人体的健康很重要
- 肠道微生态失衡会引起肠道及某些肠外疾病
- 脆弱拟杆菌有望成为肠道疾病防治的二代益生菌

THANK YOU!