聚焦益生元，解读与肠道健康的“因果关系”

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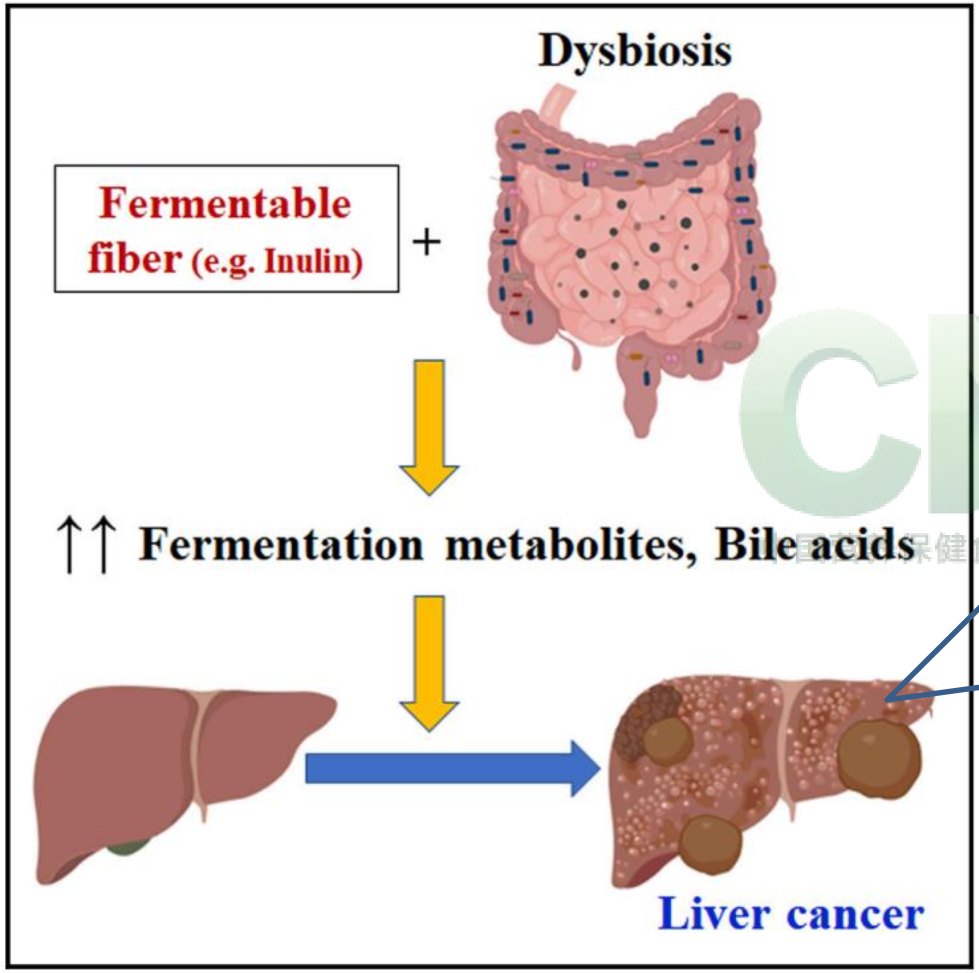
Zhejiang Academy of Agricultural Sciences

一篇引起悍然大波的文章：

菊粉在菌群失衡的小鼠模型中引起肝癌



划重点：

1. 发生在菌群失衡 (Dysbiosis)状态下；

2. 饲喂了过量的可发酵膳食纤维或者益生元；

3. 可发酵膳食纤维造成菌群进一步失衡，导致细菌与宿主代谢失衡。

Singh et al., 2018, Cell *175*, 679–694

科学问题：

1. 什么是正常人群的正常肠道菌群？
2. 中国正常人群肠道菌群是否失衡？
3. 如何通过膳食补充剂维持正常人群的肠道菌群平衡？
3. 什么样的疾病状态下肠道菌群处于失衡状态？
4. 如何帮助失衡的肠道菌群达到平衡？

什么是正常的、平衡的人体肠道菌群?

frontiers in Genetics

只能按照西方饮食方式定义:

- 菌群的a-多样性高
- 双岐杆菌数量丰富
- 丁酸产生菌数量丰富，丁酸产量高
- 粘液素降解菌(Akkermansia)数量丰富

Advances in Gut Microbiome research, opening new strategies to cope with a Western lifestyle

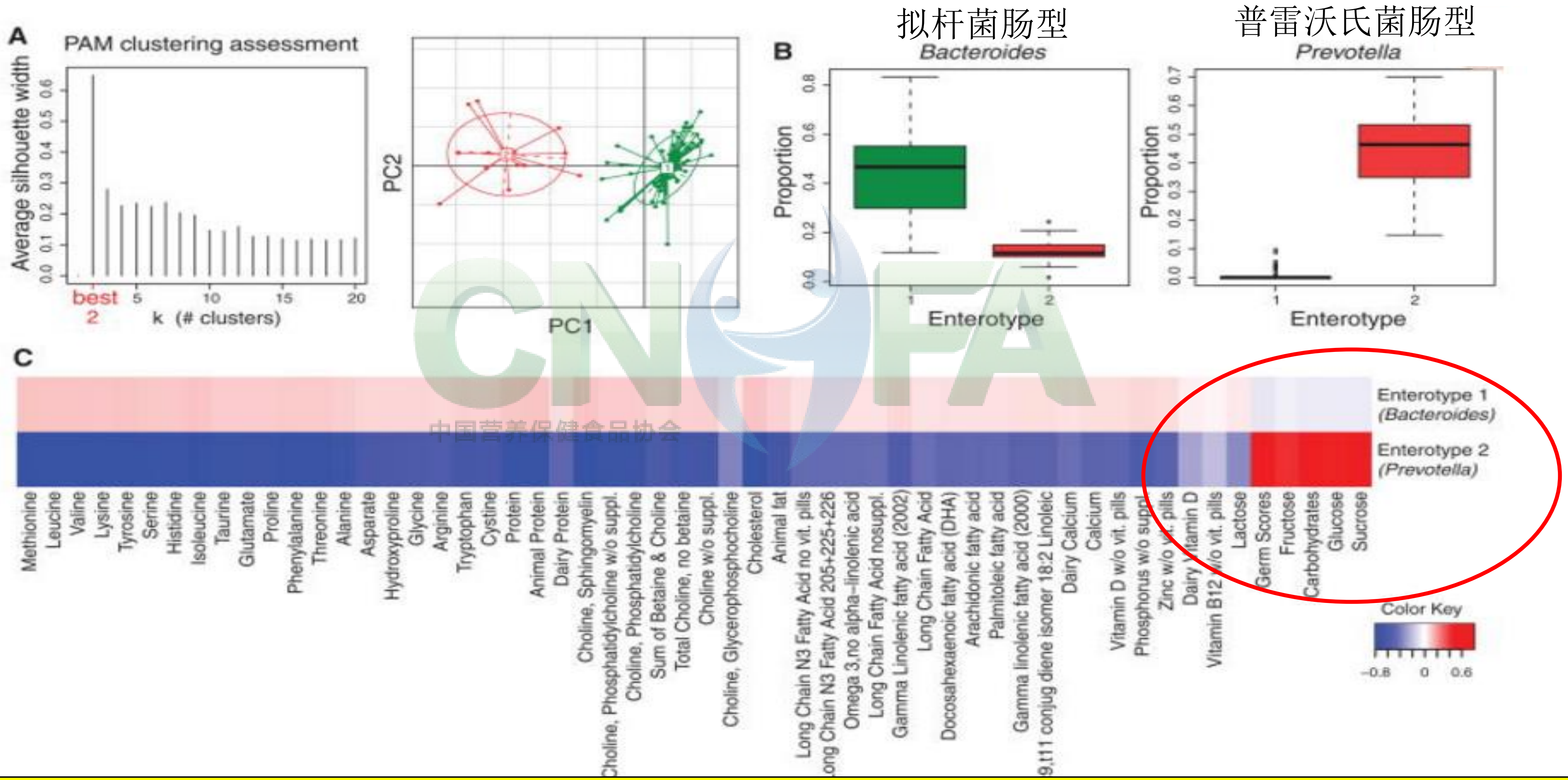
Gina Paola Rodriguez-Castano[1,2], Alejandro Caro-Quintero[3], Alejandro Reyes[4], Fernando Lizcano[1*]

[1]Biosciences, UNIVERSIDAD DE LA SABANA, Colombia, [2]Doctorado en Biociencias, Universidad de La Sabana, Colombia, [3]Corporación de investigación agropecuaria CORPOICA, Colombia, [4]Department of Biological Sciences, Universidad de Los Andes, Colombia

Bifidobacteria contributes to colon health through the production of organic acids, like acetate and lactate, that are then used by butyrate-producing bacteria. Thus, a high abundance of butyrate-producers, mucin-degraders, and bifidobacteria could be an indicator of good health.

Another common feature in some studies is greater gut diversity in healthy states. In lean twins, a greater bacterial diversity has been observed compared to their obese twins[33], in patients with morbid obesity

普雷沃氏菌和拟杆菌肠型都是人体肠道微生态正常类型



Science文章证明葡萄糖类型的碳水化合物是决定肠型的主要营养元素（Science. 334 (6052): 105–108）

国家允许在食品中使用的寡糖、 多糖类产品

- 乳果糖
- 阿拉伯半乳聚糖(落叶松来源)（Arabinogalactan）
- 多聚果糖 (Polyfructose)
- 燕麦β-葡聚糖 (Oat β-Glucan)
- 低聚木糖 (XOS)
- L-阿拉伯糖 (L-Arabinose)
- 低聚半乳糖（GOS）
- 棉籽低聚糖 (Raffino-OS)
- 酵母β-葡聚糖（Yeast β-Glucan）
- 低聚甘露糖 (MOS)
- 壳寡糖（Chitosan OS）
- 水苏糖（Stachyose）
- 抗性糊精（Resistant Dextrin）
- 果胶

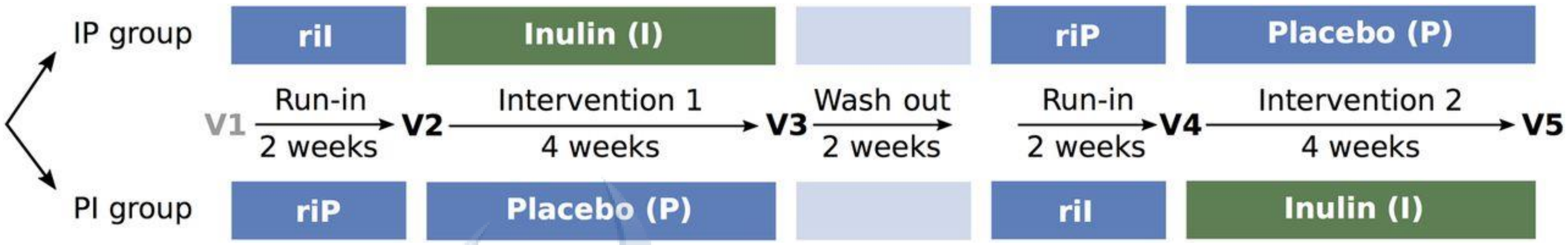
这些多糖对人体肠道菌群的调控作用是否相同?

化学结构不清晰的植物多糖、寡糖

枸杞多糖、灰树花多糖、
银耳多糖、灵芝多糖等等

在正常便秘人群中菊粉促进双歧和丁酸产生菌的生长

- 随机双盲试验
- 健康便秘人群
- 日摄入量 12g/人
- 试验人数 n=44



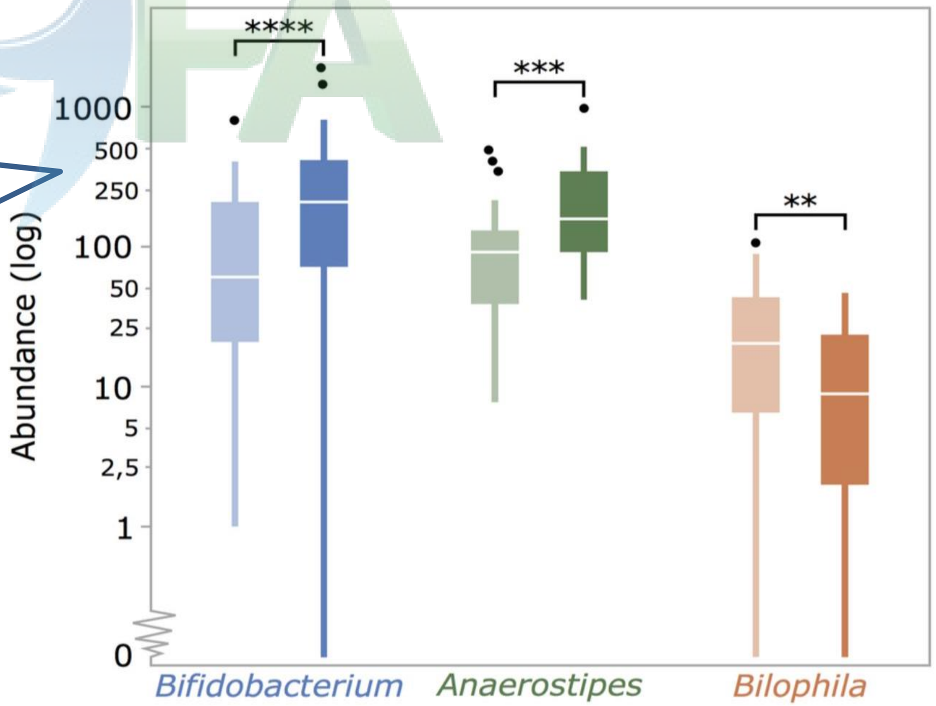
划重点：

菊粉提高健康便秘人群粪便中：

- *Bifidobacteria*和*Anaerostipes*的含量；
- 降低*Bilophila*含量。

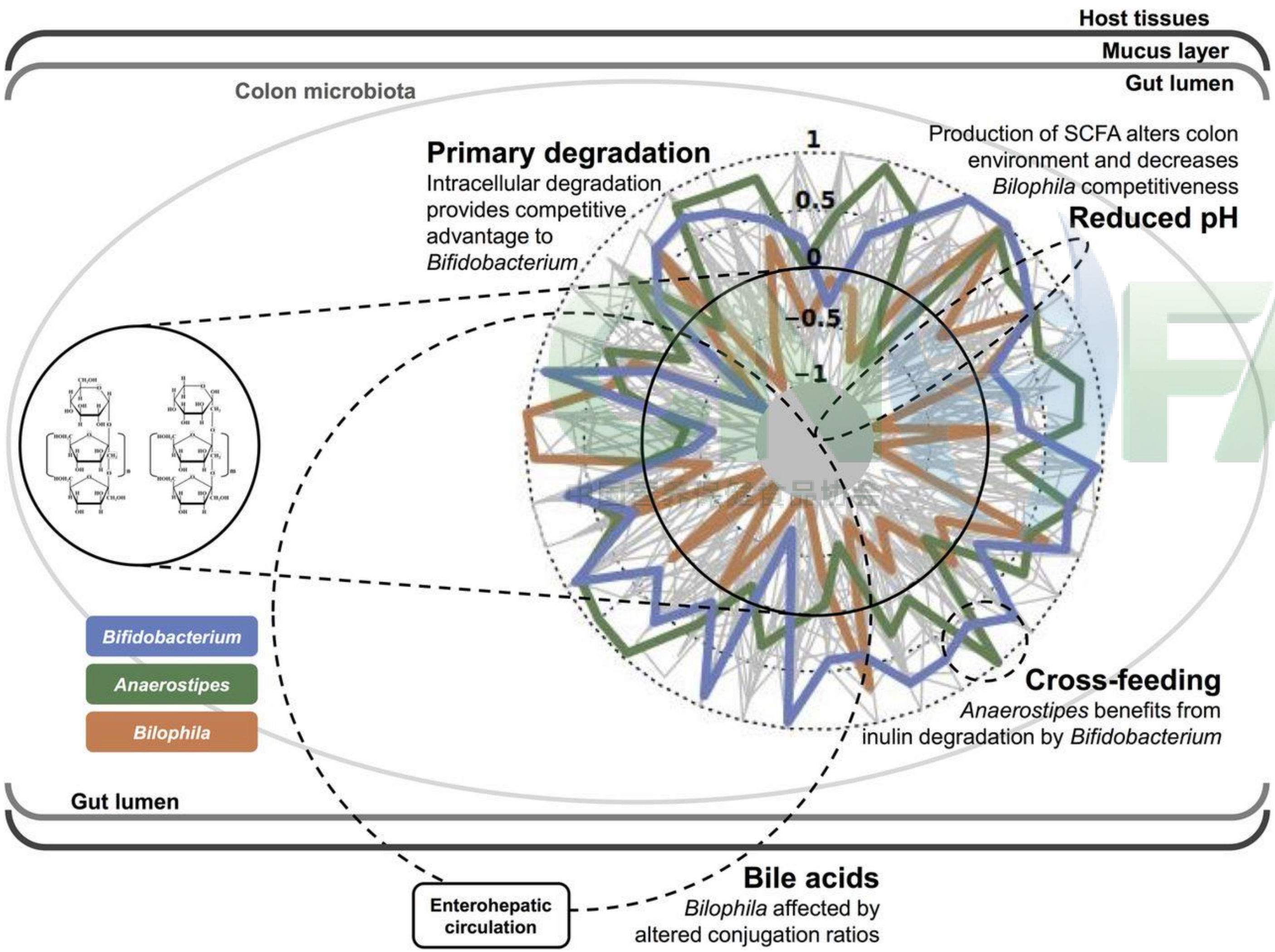
其中：*Anaerostipes*为丁酸产生菌

Bilophila 为胆汁酸降解菌



Doris Vandeputte et al. Gut doi:10.1136/gutjnl-2016-313271

菊粉在便秘人群中可以降低胆汁酸降解细菌的数量



人体每天摄入12克菊粉

刺激双歧杆菌的生长

降低肠腔内的pH值

降低肠腔内胆汁酸含量

降低胆汁酸分解细菌*Bilophila*含量

有明显缓解便秘的效果

体外连续发酵模型研究发现了控制肠型的主要营养因素

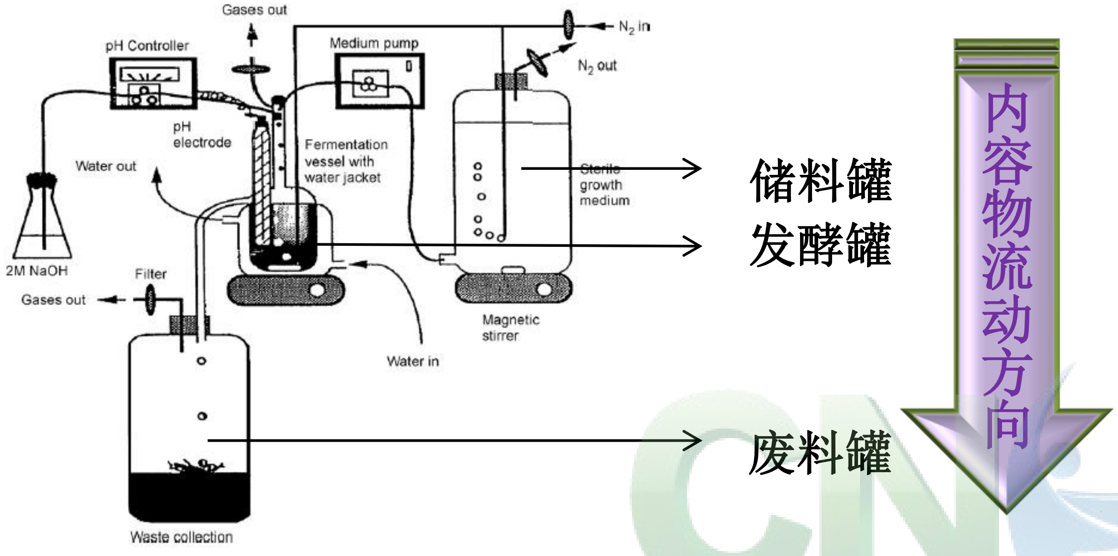
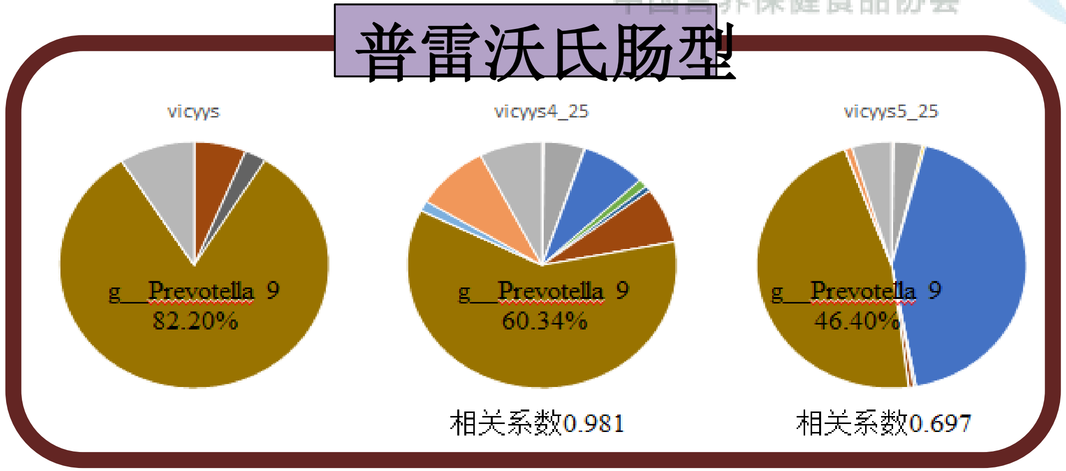
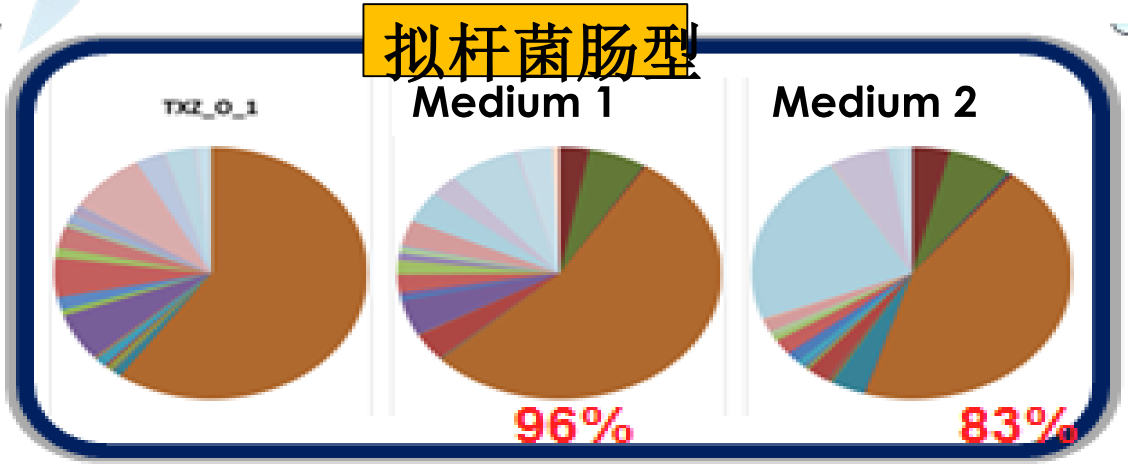


Fig. 3. Single-stage continuous culture system (reproduced with permission from Macfarlane et al., 1989).

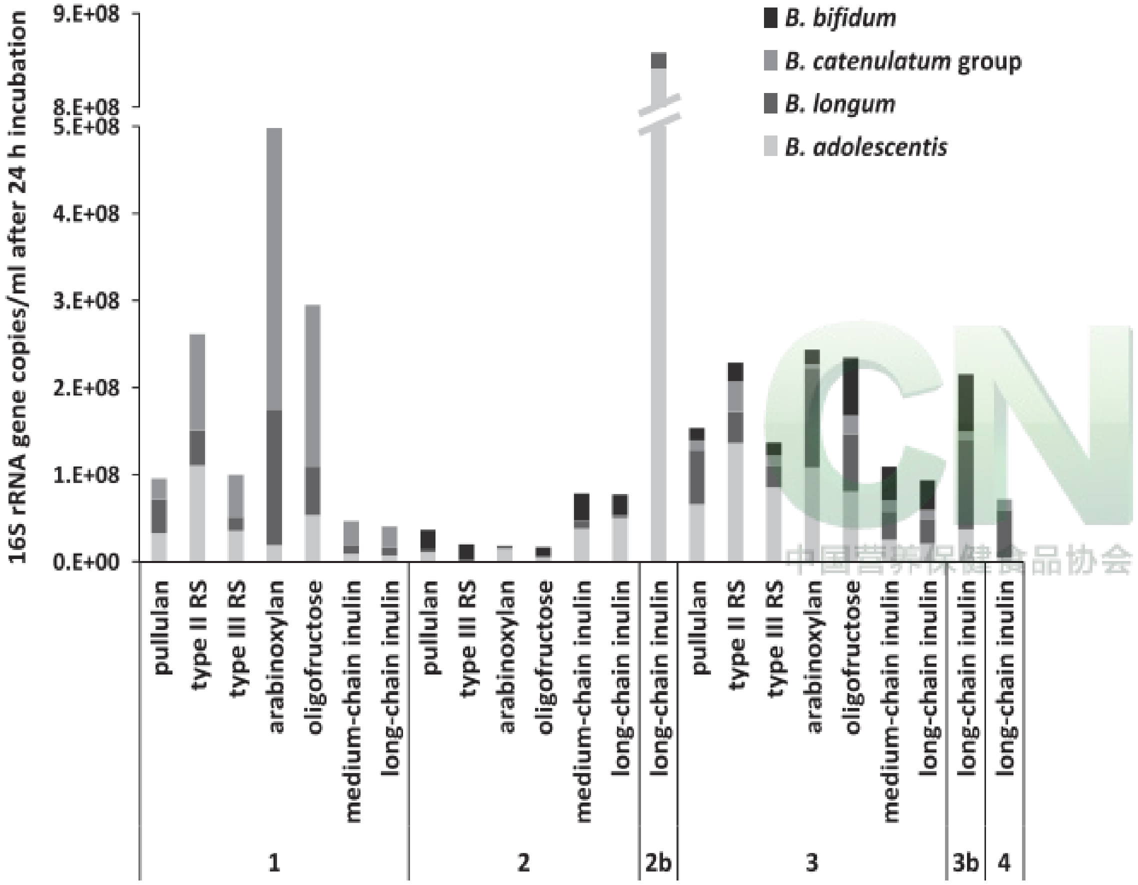




粪便 发酵罐1 发酵罐2

粪便 发酵罐1 发酵罐2

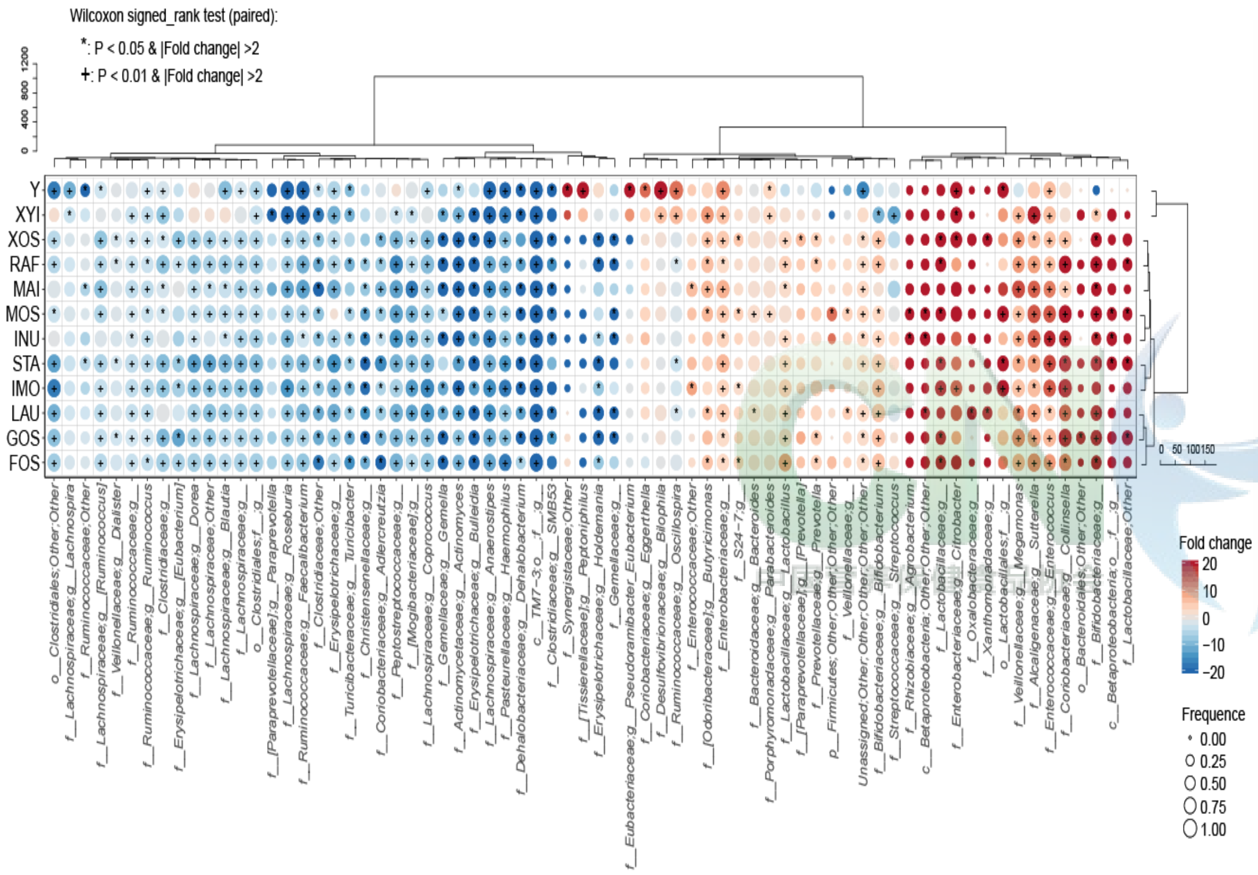
体外模型研究阿拉伯木聚糖等对菌群结构影响

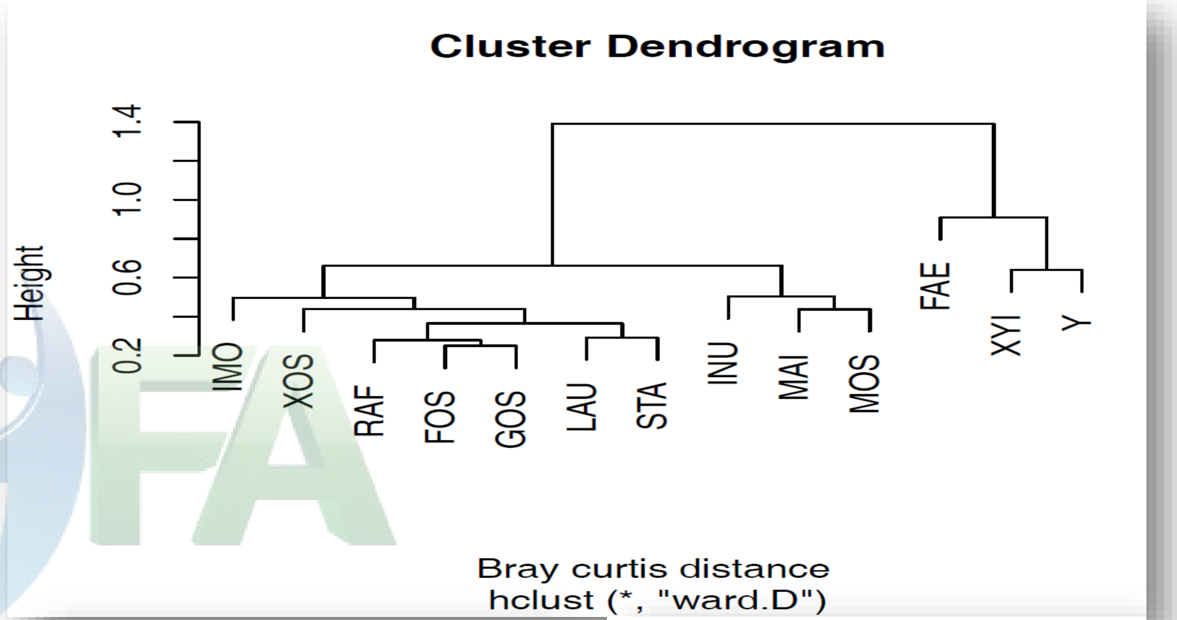


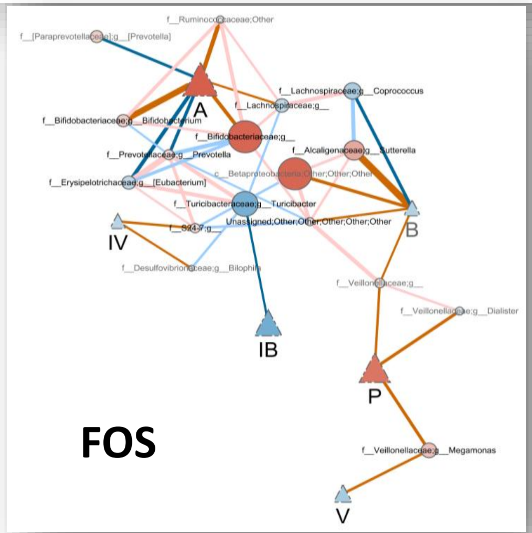
OTU no.	Closest relative bacterial species (BLAST)	Identity	Non-digestible carbohydrate	
			pH 5.5	pH 6.5
Otu0002	*Faecalibacterium prausnitzii*	99%	Apple pectin[a,b]	Apple pectin[a,b] Carob galactomannan[a,b]
Otu0003	*Clostridium spiroforme*	93%	Laminarin[a,b]	
Otu0005	*Bacteroides uniformis*	100%		Pyrodextrin[a,b] Laminarin[a,b] Guar galactomannan[a,b]
Otu0006	*Blautia faecis*	99%	Rhamnose[a,b]	Rhamnose[a,b]
Otu0010	*Fusicatenibacter saccharivorans*	99%	Laminarin[a] Carob galactomannan[a,b]	
Otu0013	*Subdoligranulum variabile*	99%	Arabinoxylan[a,b]	
Otu0017	*Oscillibacter ruminantium*	96%		Rhamnogalacturonan[a]
Otu0018	*Dorea longicatena*	99%	Pullulan Guar galactomannan Medium-chain inulin[a,b]	
Otu0024	*Lactobacillus rogosae*	96%	Rhamnogalacturonan[a] Guar galactomannan	

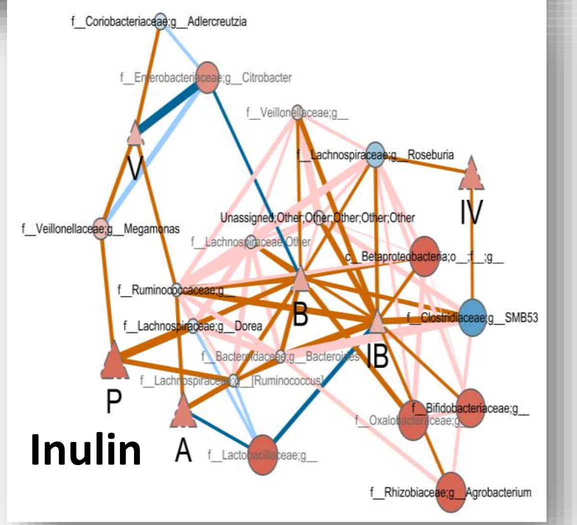
The ISME Journal (2017), 1–13

体外发酵模型确定益生元的化学结构和调控菌群之间的关系





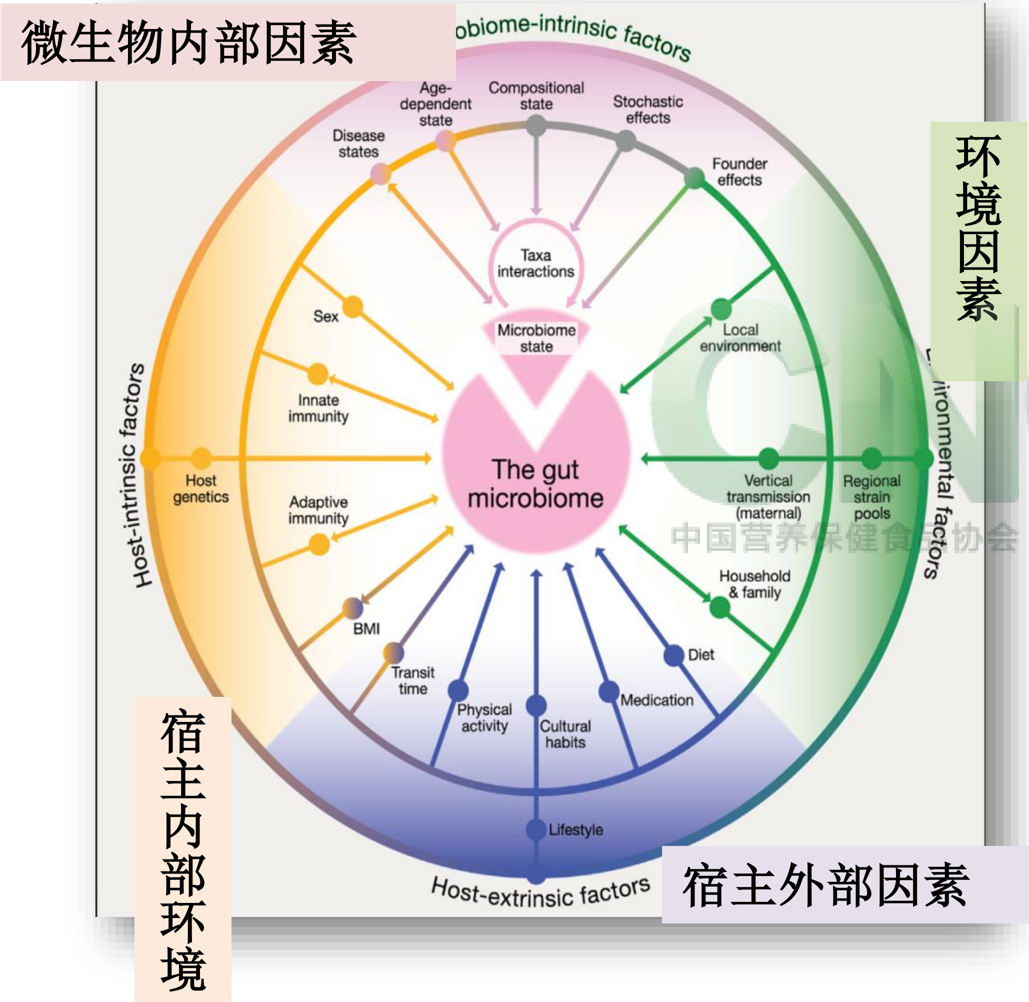




相同化学结构、不同分子量的益生元对健康人体肠道微生物的调控作用展现出显著差异

陈军奎，朱立颖，刘伟、皮雄娥、王欣等，未发表结果

环境因素在肠道菌群形成中起到决定性作用



最近基于696个个体的研究发现宿主遗传背景对肠道菌群不同结构的贡献率只有2%。

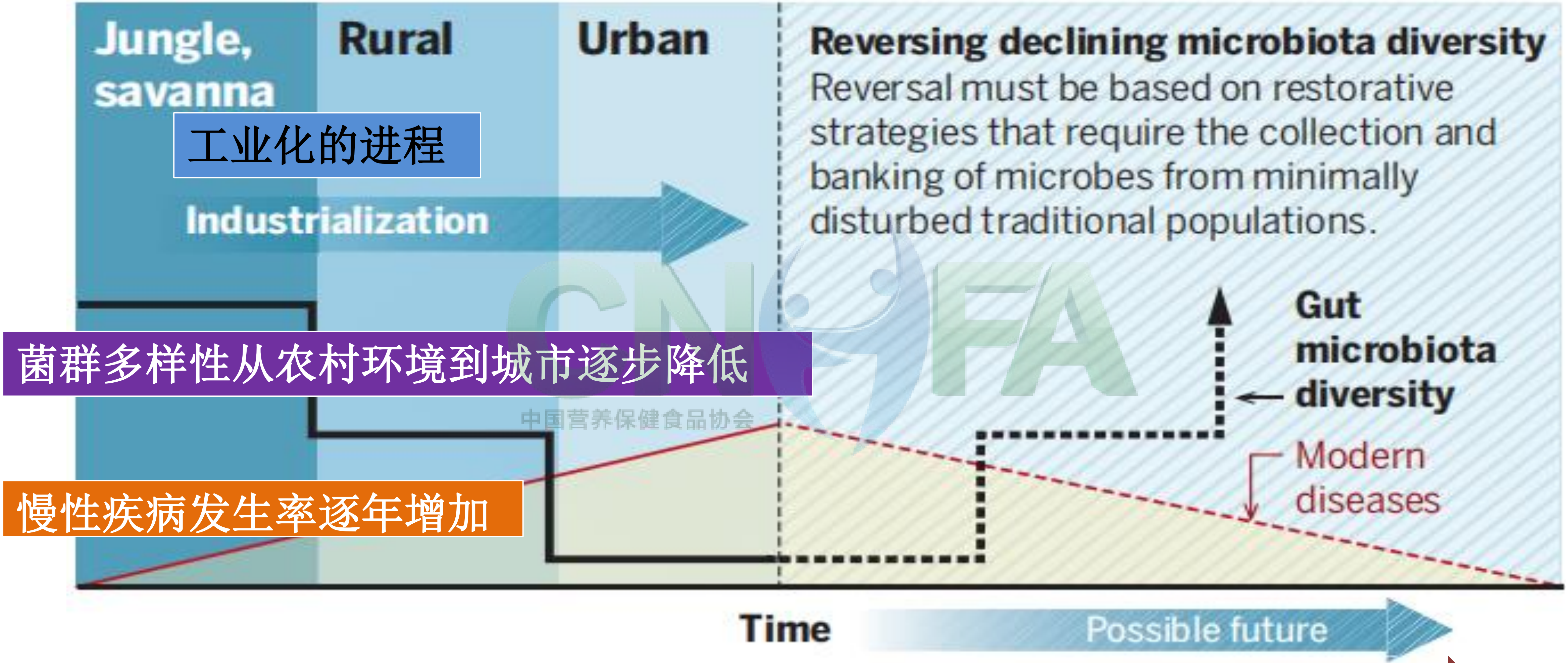
影响肠道菌群多样性的因素：

- 抗生素
- 药物
- 剖腹产
- 母乳/配方奶喂养
- 现在食品加工
- 日常清洁剂

……等等

Rothschild D, et al. Nature , 2018

全球工业化进程伴随着人类肠道菌群多样性的丧失



增加菌群多样性是我们今后的努力方向

Science **362** (6410), 33-34.

菌群失衡的特征 --a多样性、丁酸产生菌、双歧杆菌含量下降、变形杆菌等兼性厌氧菌数量升高

Bacterial families that dominate the adult human gut in health and durin iversity dysbiosis

正常　　失衡

Healthy	Low-diversity dysbiosis
Firmicutes	Firmicutes
Clostridia	Bacil
Clostridiales	Lactobacillales
Lachnospiraceae (Clostridia XIVa)	Lactobacillaceae
(Clostridia IV)	Enterococcaceae
Bacteroidetes	Proteobacteria
Bacteroidia	Gammaproteobacteria
Bacteroidales	Enterobacteriales
Bacteroid eae	Enterobacteraceae
Prevotella	

厌氧芽孢梭菌目

拟杆菌菌目

肠球菌，链球菌

肠杆菌科

目前发现和菌群多样性下降相关的疾病：

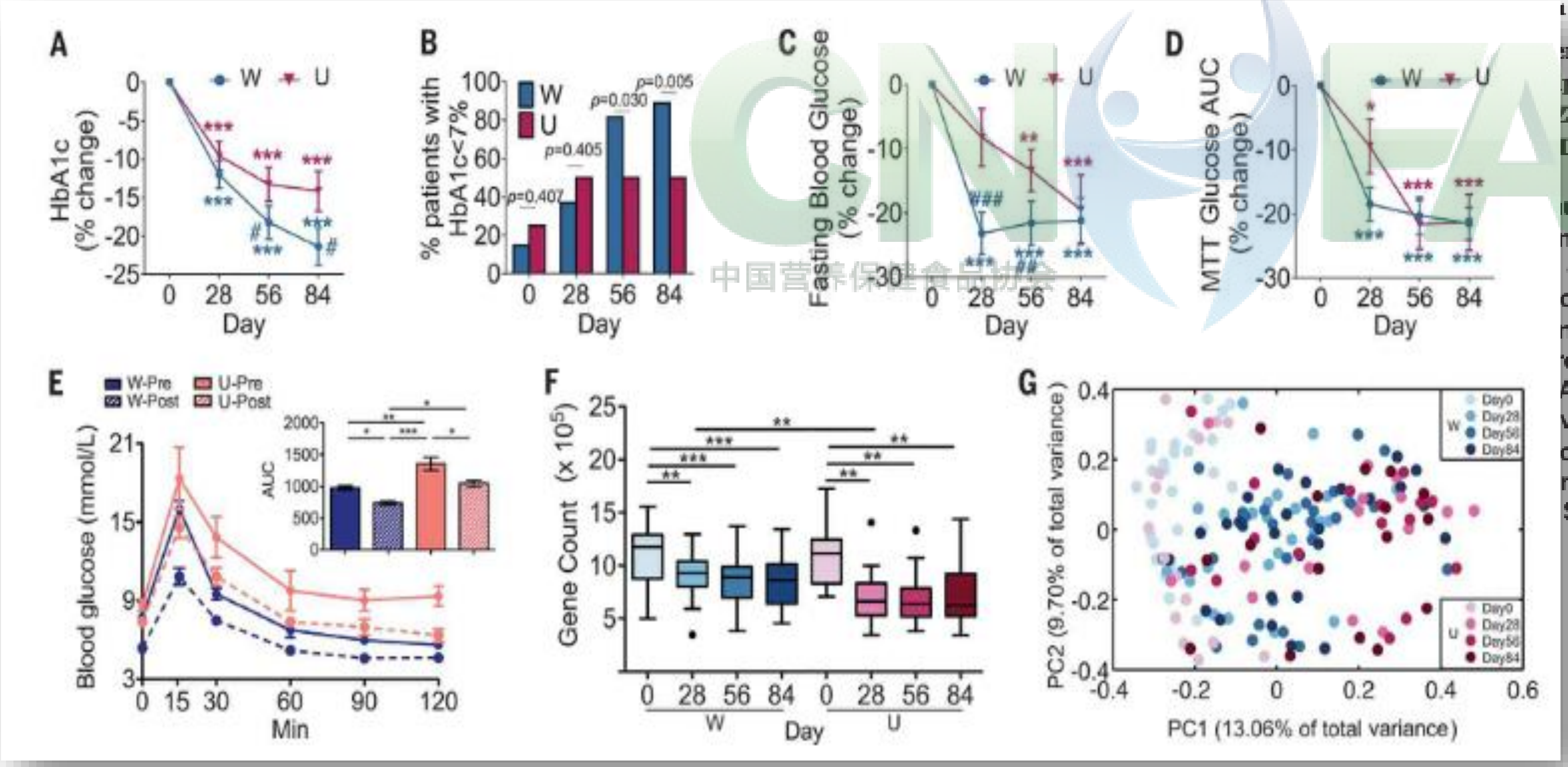
- **IBD**
- 急性腹泻
- **CDI**
- 结肠癌
- 孤独症
- 肝脏疾病等

II糖尿病患者肠道菌群结构失衡丁酸产生菌降低

MICROBIOTA

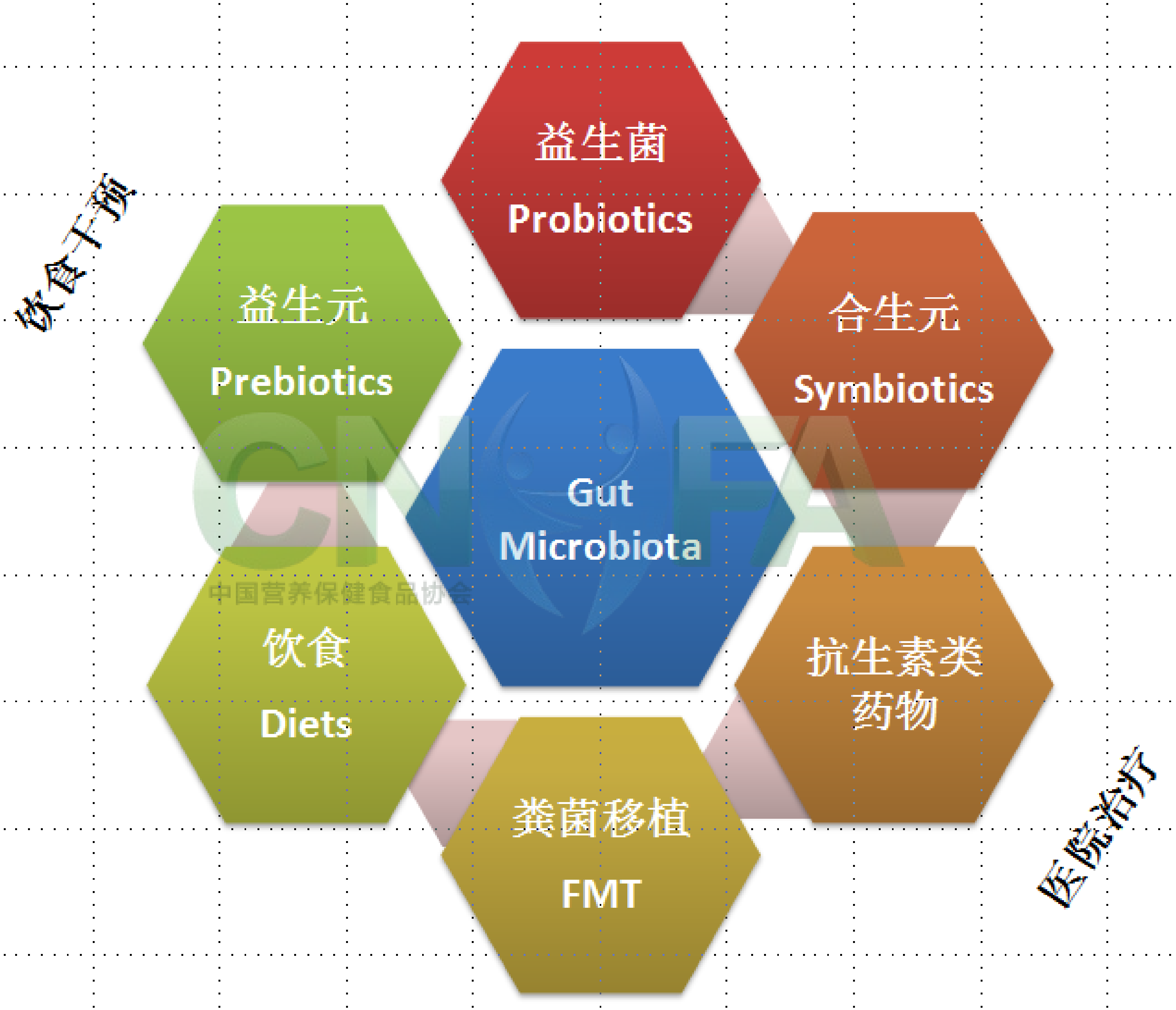
Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes

Liping Zhao,[1,2*†] Feng Zhang,[1*] Xiaoying Ding,[3*] Guojun Wu,[1*] Yan Y. Lam,[2*]
[1] Xinhe Xue,[1] Chunhua Lu,[4] Jilin Ma,[4] Lihua Yu,[4]
n,[4] Ying Xu,[5] Songmei Xu,[5] Hongli Shen,[5] Xiuli Zhu,[5]
ping Dong,[3] Rui Liu,[1] Yunxia Ling,[3] Yue Zeng,[7]
Zhang,[1] Jing Wang,[1] Linghua Wang,[1] Yanqiu Wu,[1]
Ienghui Zhang,[1] Yongde Peng,[3†] Chenhong Zhang[1†]



umans via short-chain fatty acid (SCFA) production from
nd deficiency in SCFA production is associated with type 2
conducted a randomized clinical study of specifically
ogether with fecal shotgun metagenomics, to show that a
ng strains was promoted by dietary fibers and that most
e either diminished or unchanged in patients with T2DM.
A producers were present in greater diversity and abundance,
vement in hemoglobin A1c levels, partly via increased
ction. Promotion of these positive responders diminished
rimental compounds such as indole and hydrogen sulfide.
SCFA producers may present a novel ecological approach

恢复肠道菌群平衡的可能方法

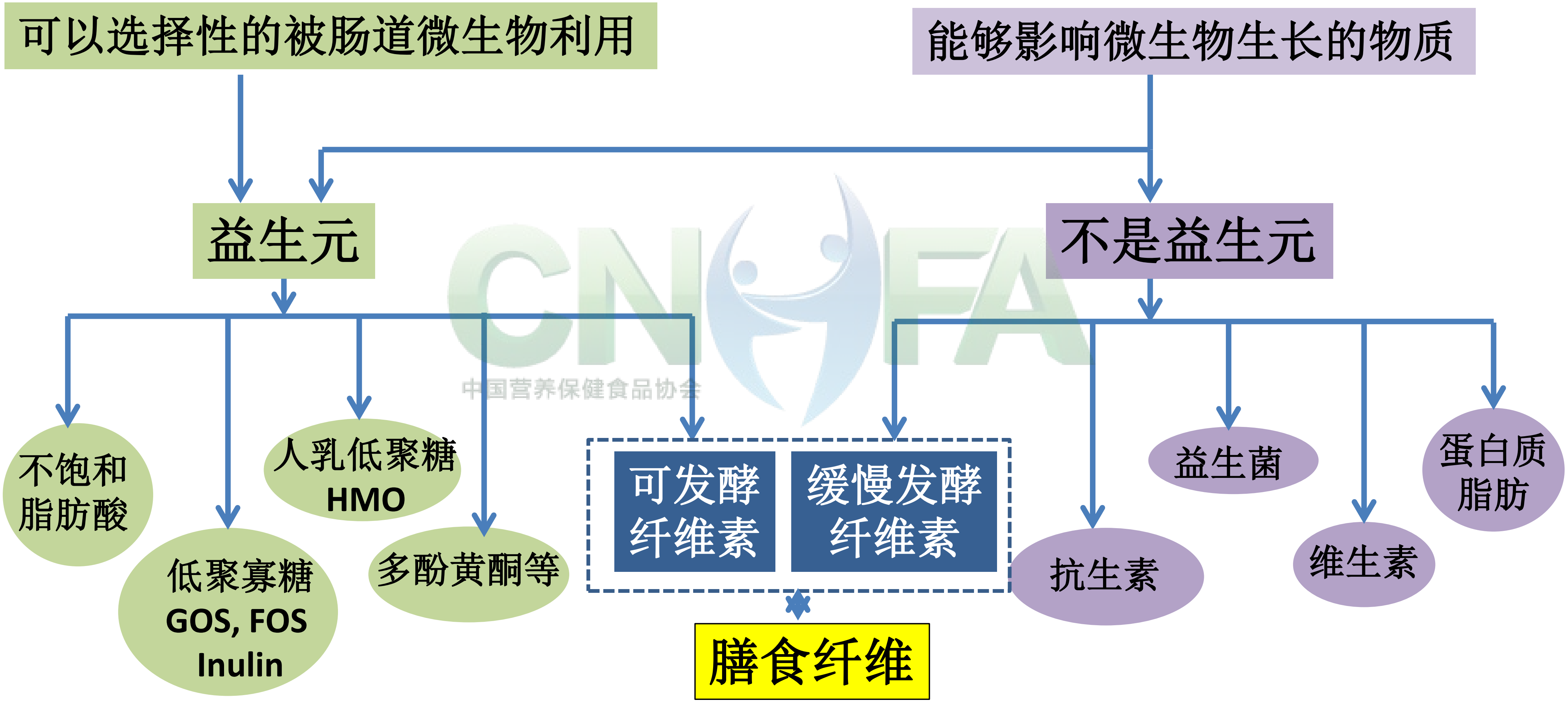


什么是益生元?

International Scientific Association for Probiotics and Prebiotics

- ✓ 能够被宿主体内的菌群选择性利用并转化为有益于宿主健康的物质；
- ✓ 益生元不仅仅可以通过口服作用于肠道，也可以作用于其它部位，如阴道或皮肤；
- ✓ 功效包括：
  - 有利于肠道健康
  - 有利于心血管代谢
  - 有利于精神健康
  - 有利于骨骼健康
- ✓ 目前承认的益生元大部分为寡糖和多糖，其它物质如多酚，不饱和脂肪酸等也被列为益生元；
- ✓ 要有充分证据证明这些物质是通过调节共生菌群对宿主产生有益的影响。

益生元的定义



Nature Rev. Gastroenterol. & Hepatol. 2017.75

对失衡的肠道菌群如自闭症儿童，能否通过调整饮食组份缓解临床症状

益生元能调整CD病人的肠道菌群吗？

正常对照 n=15

新鲜粪便样品

自闭症儿童，n=15

新鲜粪便样品

GOS

FOS

IMO

菊粉

XOS

木糖醇

对照

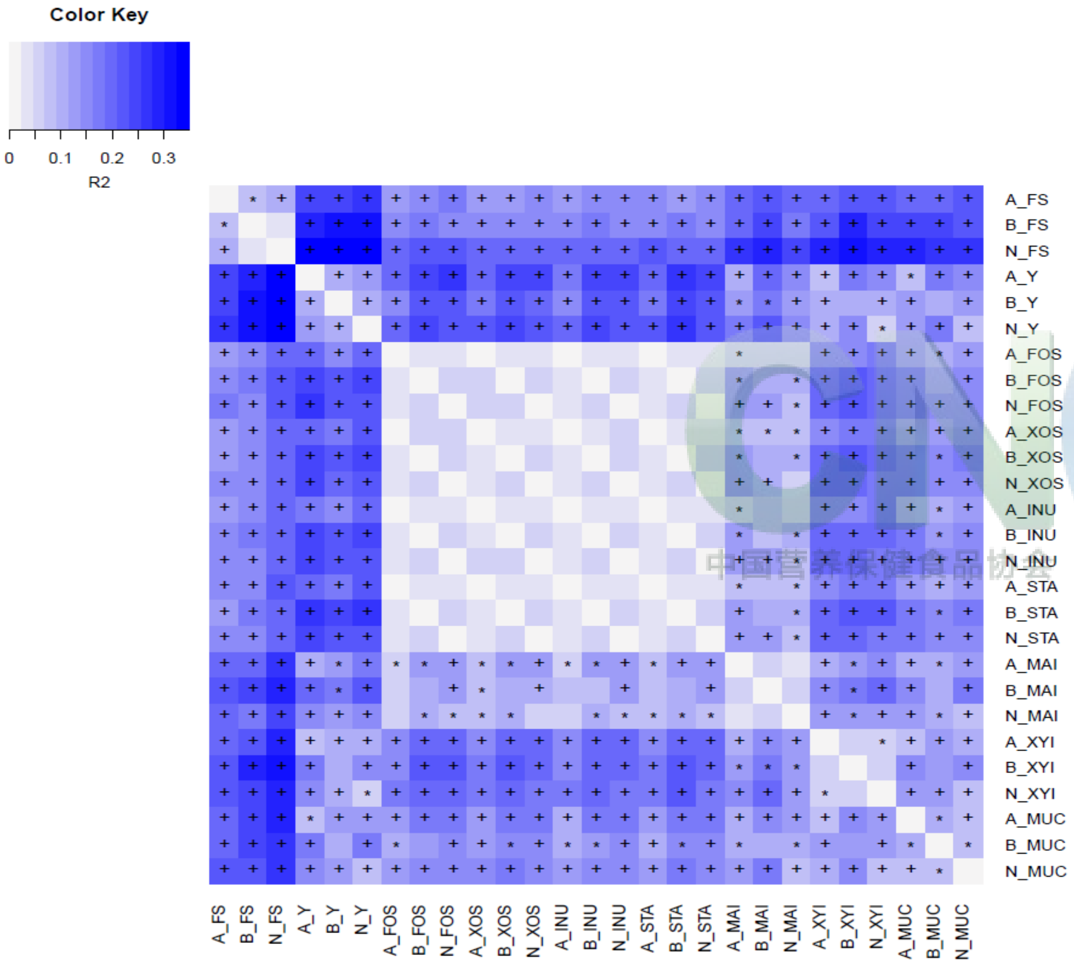
气体产量

短链脂肪酸产量

16S分析菌群结构

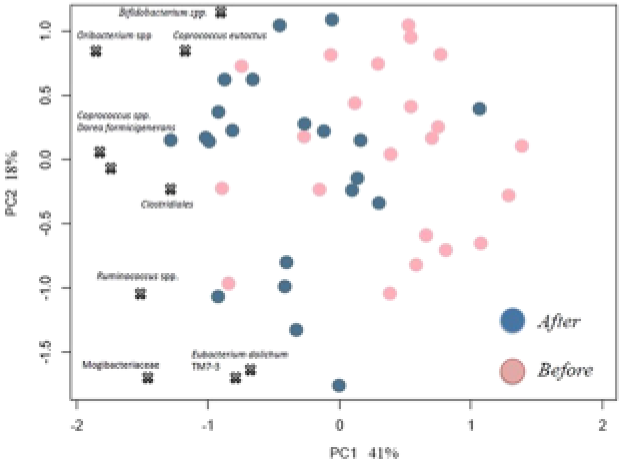
有益菌数量

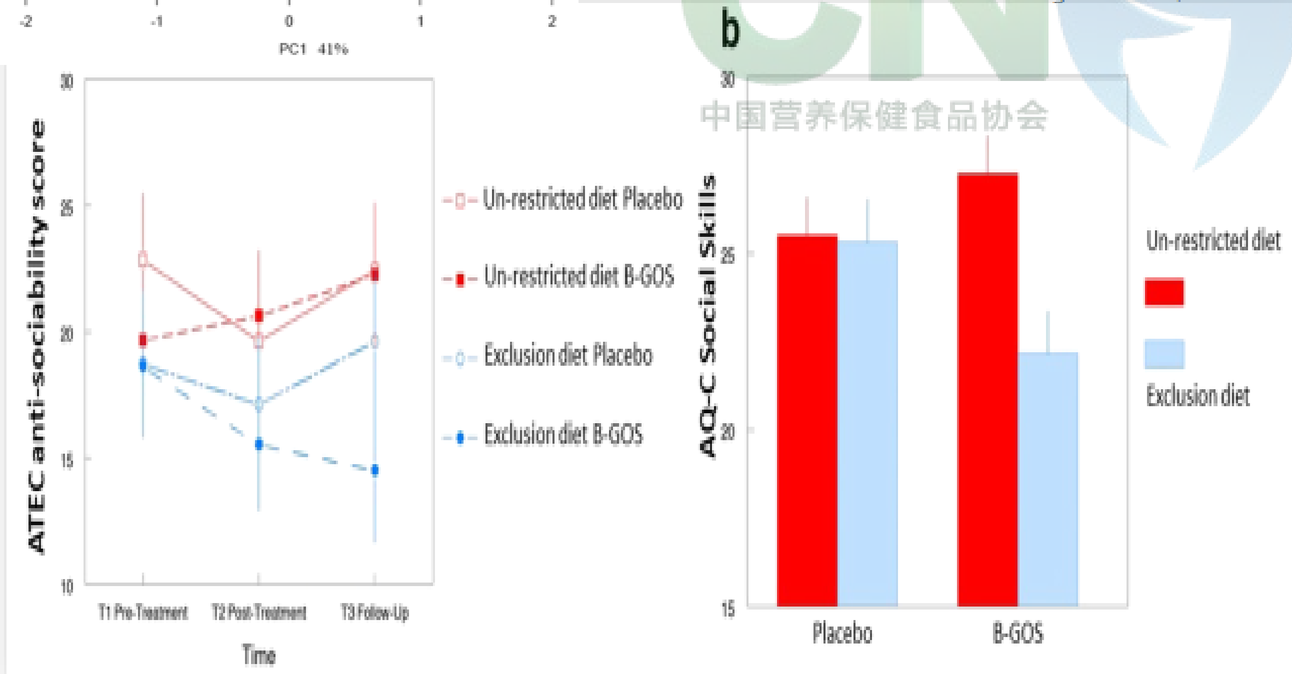
ASD儿童粪便体外发酵益生元后菌群结构更接近正常儿童



王雷、李正鹏、王欣、杨云生等，未发表结果

益生元干预能够改善自闭症儿童临床症状





Grimaldi *et al. Microbiome* (2018) 6:133
https://doi.org/10.1186/s40168-018-0523-3
Microbiome

RESEARCH Open Access

CrossMark

A prebiotic intervention study in children with autism spectrum disorders (ASDs)

Roberta Grimaldi[1,2*], Glenn R. Gibson[1], Jelena Vulevic[2], Natasa Giallourou[3], Josué L. Castro-Mejía[4], Lars H. Hansen[5], E. Leigh Gibson[6], Dennis S. Nielsen[4] and Adele Costabile[6]

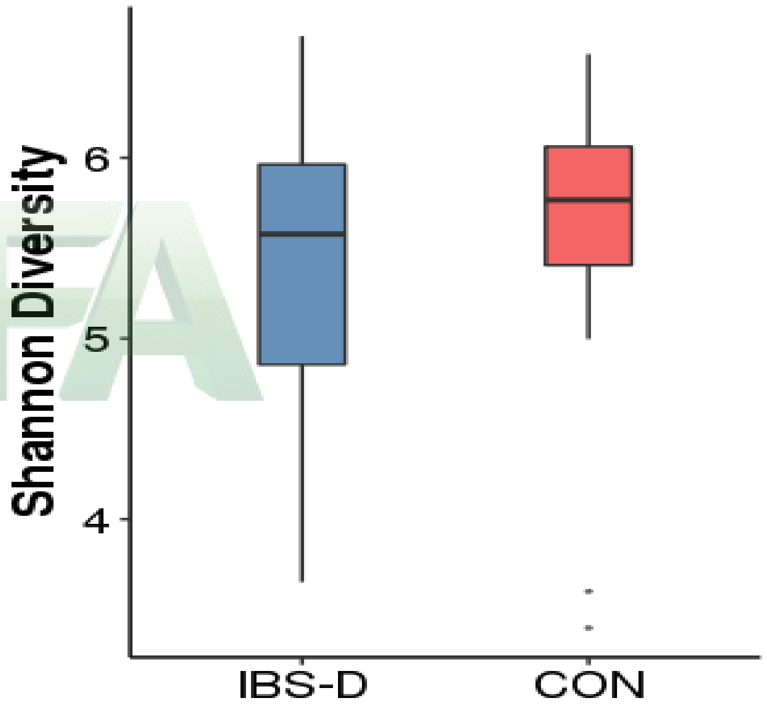
...tary approaches, such as gluten and casein free diets, or the use of probiotics and prebiotics ...tistic spectrum disorders in order to reduce gastrointestinal (GI) disturbances. GI symptoms ...this population due to prevalence and correlation with the severity of behavioural traits. ...strong evidence about the effect of dietary interventions on these problems, particularly ...sessed the impact of exclusion diets and a 6-week Bimuno® galactooligosaccharide (B-GOS®) ...autistic children.

...d that children on exclusion diets reported significantly lower scores of abdominal pain and ...s lower abundance of *Bifidobacterium* spp. and Veillonellaceae family, but higher presence ...*tzii* and *Bacteroides* spp. In addition, significant correlations were found between bacterial ...no acids in this group, compared to children following an unrestricted diet. Following B-

IBS-D肠道菌群失衡状态的特征（实验室结果）

健康人（对照组）与IBS-D患者的粪便中短链脂肪酸含量分析			
SCFA（mmol/L）	正常人群 (No=38)	IBS-D (No=47)	P value
总SCFA	6.06	11.44	P<0.001
乙酸	3.49	7.39	P<0.001
丙酸	1.32	2.03	P<0.001
丁酸	0.78	1.00	p=0.152

两组数据采用了非参数Mann-Whitney U检验



IBS-D病人产生更多的乙酸和丙酸

张雅雯、朱立颖、戴宁等，未发表结果

正常人的益生元变成了 IBS-D病人的FODMAPs

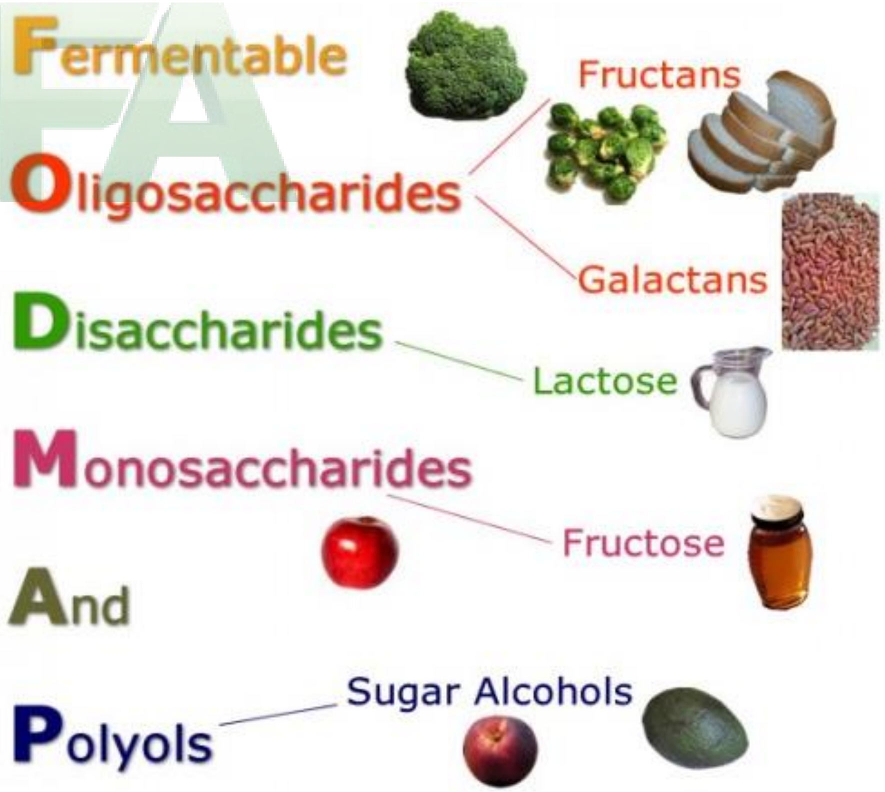
•益生元低聚糖

- 阿拉伯半乳聚糖（Arabinogalactan）
- 低聚果糖 （FOS）
- 燕麦β-葡聚糖（Oat β-Glucan）
- 低聚木糖（XOS）
- L-阿拉伯糖（L-Arabinose）
- 低聚半乳糖（GOS）
- 棉籽低聚糖（Raffino-OS）
- 酵母β-葡聚糖（Yeast β-Glucan）
- 低聚甘露糖（MOS）
- 壳寡糖（Chitosan OS）
- 水苏糖（Stachyose）
- 抗性糊精（Resistant Dextrin）

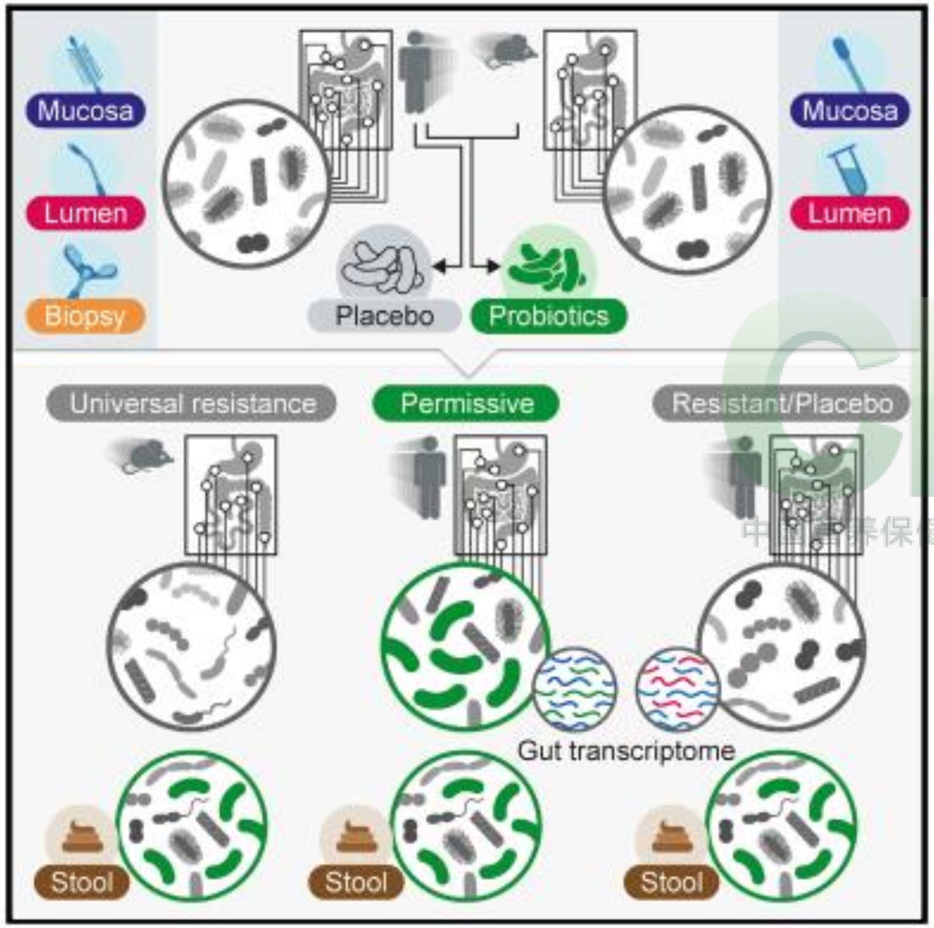
饮食中尽量减少摄入

• FODMAPs

肠道难以吸收的短链碳水化合物，包括低聚糖、双糖、单糖、糖醇等，例如乳糖、果糖、果聚糖、低聚寡糖、木糖醇、山梨醇等



越来越多的观点强调肠道菌群的个性化干预



Zmora et al., 2018, Cell 174, 1388–1405

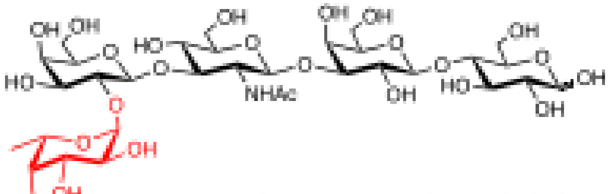
- **Cell， Cell Host and Microbe, Genome Research**等杂志联合发声提出下列观点：

1. 益生菌只能在部分人肠道内定植；
2. 只用乳杆菌和双歧杆菌益生菌制剂阻碍抗生素引起的肠道菌群失衡的重建；
3. 粪便排出物不能代表肠道内黏膜定植菌群。而益生菌的作用需要在肠道内的有效定植；

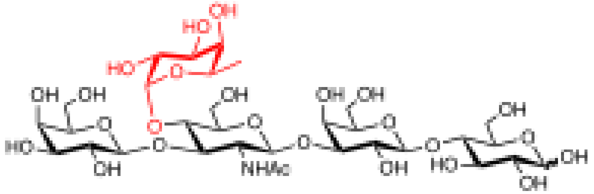
由于是环境影响了个体肠道菌群结构，干预方法可能要根据不同疾病状态和个体生理状态来制订

人乳低聚糖具有直接抑制病原菌生长的效果

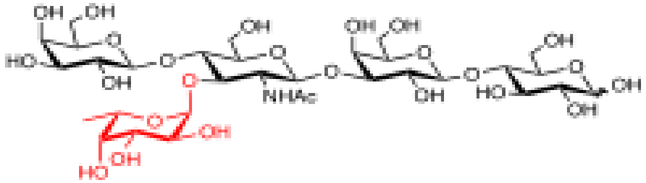
Lacto-*N*-tetraose (LNT), (4)



Lacto-*N*-fucopentaose I (LNFP I), (12)



Lacto-*N*-fucopenatose II (LNFP II), (13)



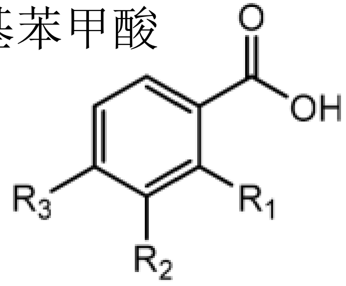
Lacto-*N*-fucopenatose III (LNFP III), (14)

	S. agalactiae strain GB590		*S. agalactiae* strain GB2	
human milk oligosaccharide	antimicrobial activity	antibiofilm activity	antimicrobial activity	antibiofilm activity
lactose	No	No	No	No
lacto-*N*-tetraose (LNT)	Strong	No	No	Weak
lacto-*N*-neotetraose (LNnT)	Strong	No*	No	No
2′-fucosyllactose (2′-FL)	No	No	Weak	No
3-fucosyllactose (3-FL)	No	No	No	No
difucosyllactose (DFL)	Strong	No*	Weak	No
lacto-*N*-fucopentaose I (LNFP I)	Strong	Weak	Weak	No
lacto-*N*-fucopentaose II (LNFP II)	Strong	No	Weak	No
lacto-*N*-fucopentaose III (LNFP III)	Strong	No	Weak	No
lacto-*N*-triose II (LNT II)	Strong	No*	Weak	No
para-lacto-*N*-neohexaose (*para*-LNnH)	No	No	Weak	No
lacto-*N*-hexaose (LNnH)	Strong	No*	Strong	No

ACS Infect. Dis., 2018

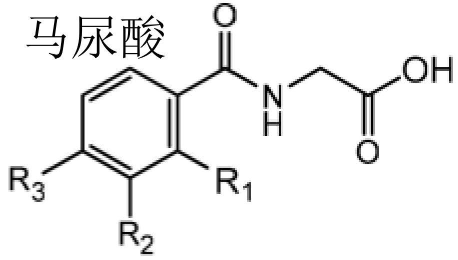
植物多酚对人体肠道微生态调控同样起到重要作用

羟基苯甲酸

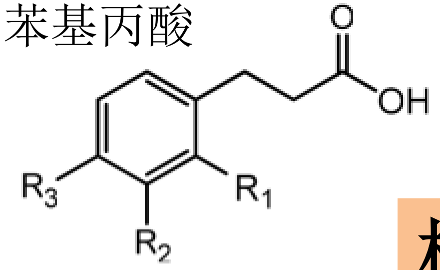


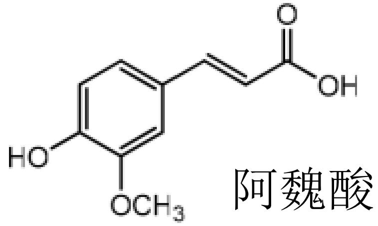
苯基乙酸

马尿酸



苯基丙酸





阿魏酸

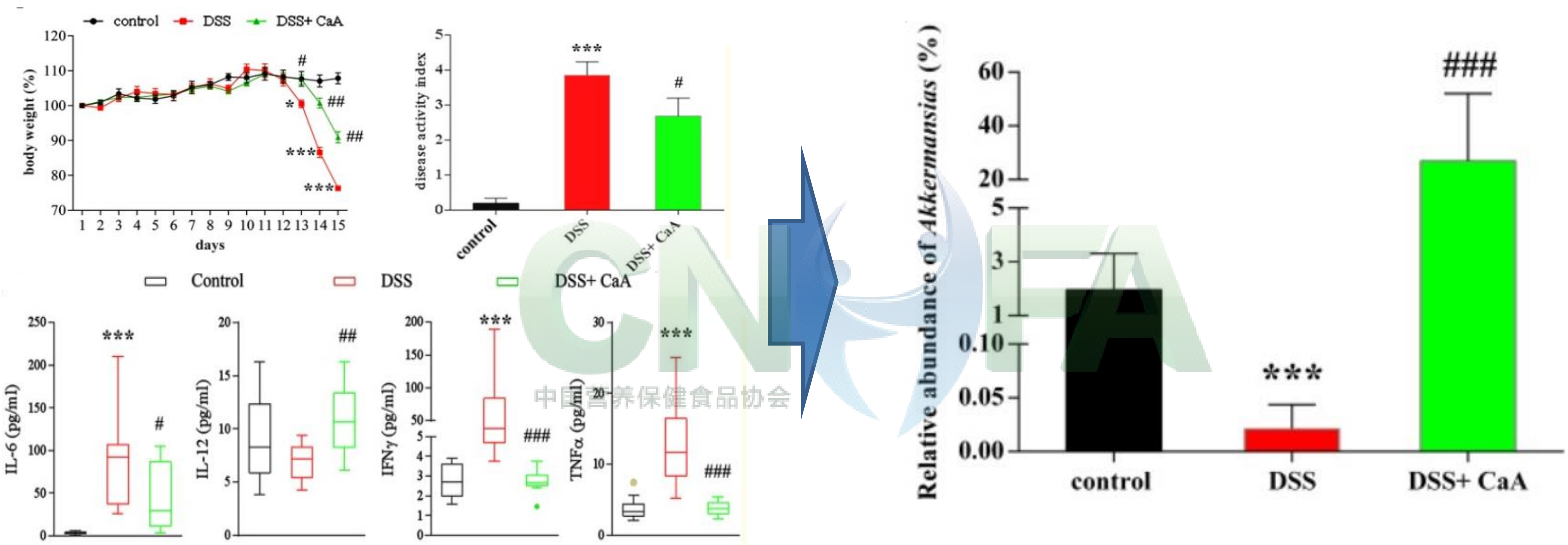
In vitro antibacterial activity of polyphenols

Polyphenol	Minimum inhibitory concentration (μg/ml)[a]			
	Escherichia coli	*Staphylococcus aureus*	*Salmonella typhimurium*	*Lactobacillus rhamnosus*
Caffeic acid	500	125	500	≤250
Catechin	1000	125	1000	≤250
Chlorogenic acid	1000	125	1000	≤250
Epicatechin	1000	125	1000	500
o-coumaric acid	250	125	250	250
p-coumaric acid	500	125	500	500
Phloridzin	1000	125	1000	1000
Rutin	>1000	>1000	>1000	>1000
Naringenin	125	62.5	125	125
Daidzein	1000	125	1000	1000
Genistein	1000	125	1000	1000
Quercetin	125	62.5	125	250
			8	32

植物多酚类物质有抑制病原菌生长的功能

International Journal of Food Microbiology 124 (2008) 295–298

肠道微生物代谢绿原酸变成咖啡酸可以缓解DSS诱导的结肠炎症



- 流行病学调查发现饮用咖啡或者绿茶对溃疡性结肠炎的发生起到保护作用；
- 小鼠试验发现肠道微生物代谢绿原酸变成咖啡酸，增加肠道中有益菌Akkermansia含量

益生元的筛选流程

Prebiotic selection criteria

- Resistance to digestion in the upper sections of the alimentary tract.
- Fermentation by intestinal microbiota.
- Beneficial effect on host's health.
- Selective stimulation of growth of probiotics.
- Stability in various food/feed processing conditions.

氨基酸?

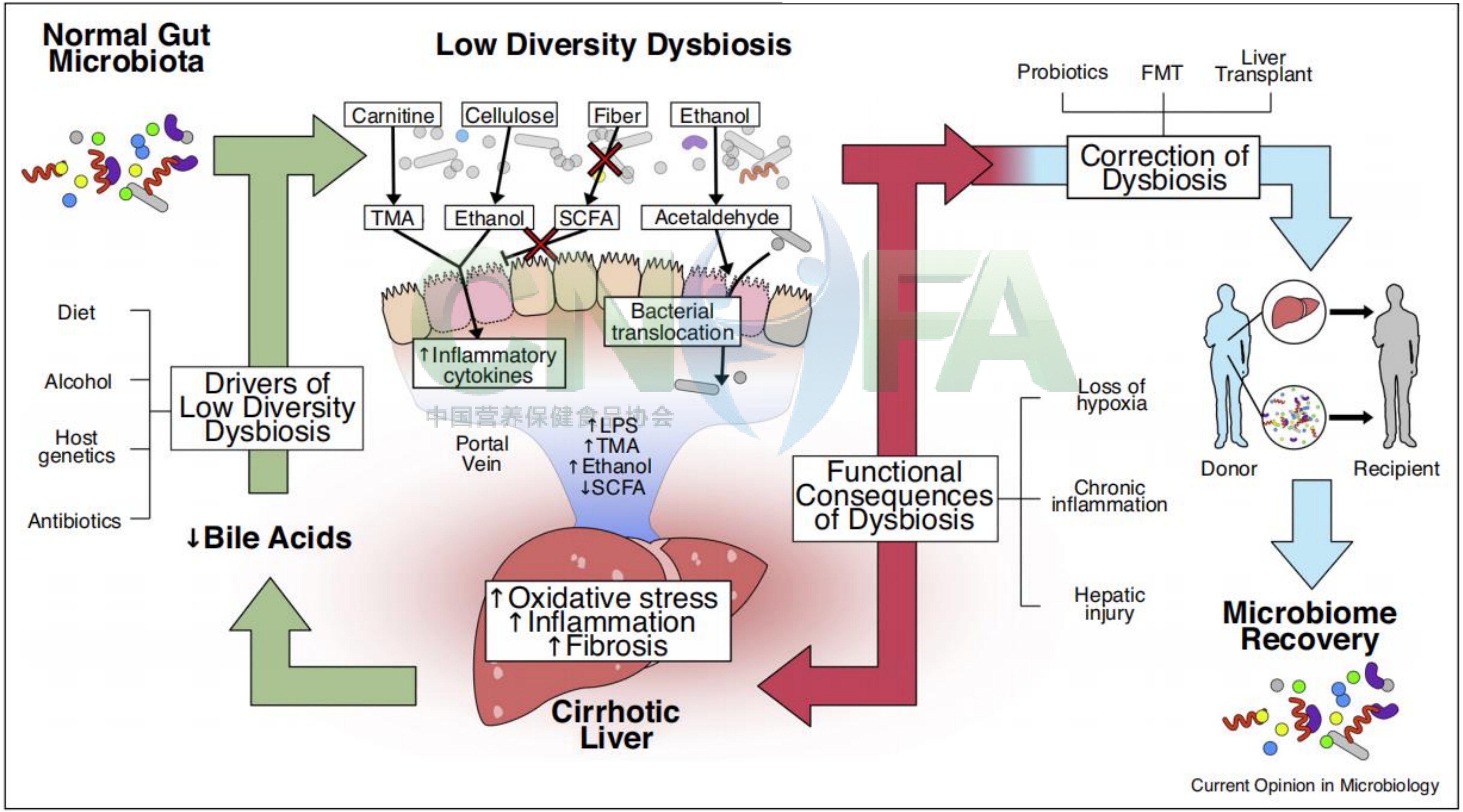
是否要考虑肠道微生物代谢产物的特征？
以及代谢产物对宿主健康的影响？

Effects of Probiotics, Prebiotics, and Synbiotics on Human Health

Nutrients. 2017 Sep; 9(9): 1021.

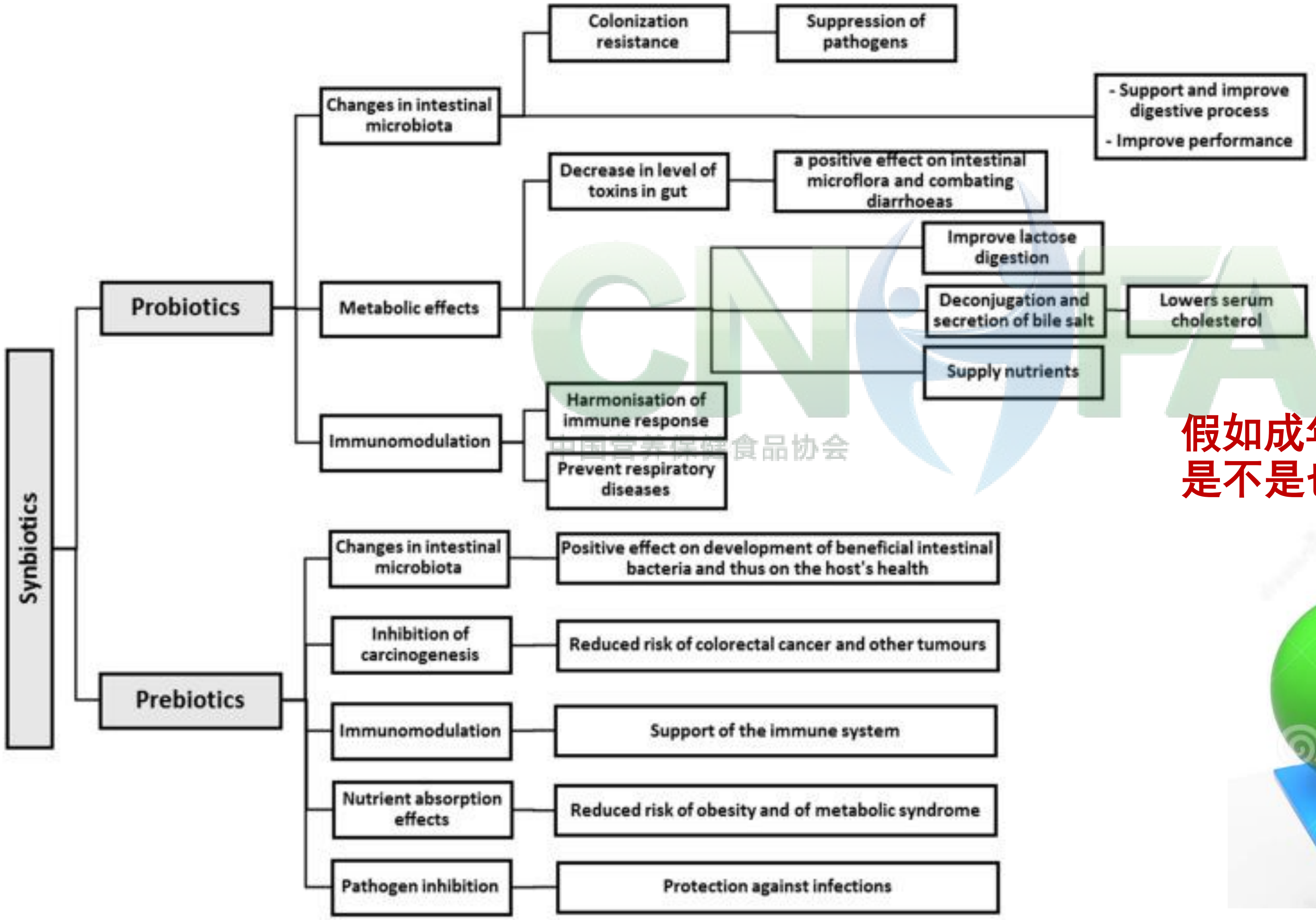
失衡的肠道菌群是不是“万恶”之源

Current Opinion in Microbiology 2018, **44**:34–40

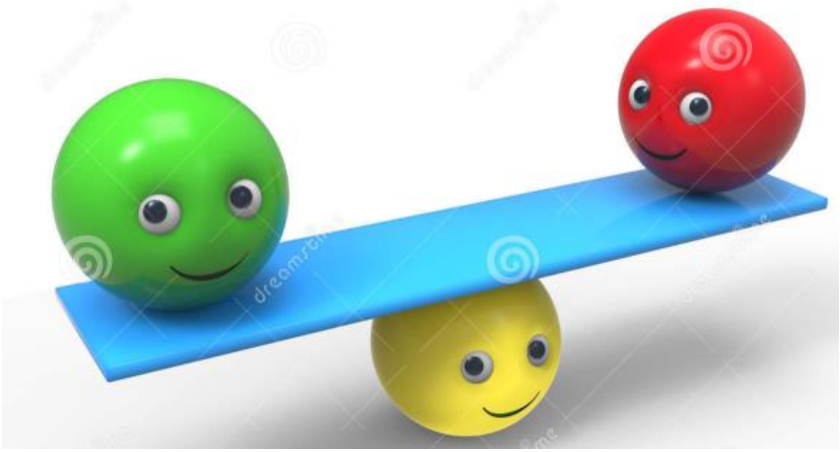


维持正常人群肠道菌群平衡、纠正失衡人群的不平衡

合生元：促进肠道内有益菌的生长



假如成年人体肠道中全部都是双歧杆菌，
是不是也是一种失衡？



展望

- 需要建立创新而高效的检测方法，研究益生元、膳食纤维、多酚等营养素对人体肠道微生态的影响；
- 通过更多的基础研究，寻找有效逆转微生态失衡的有效干预产品，达到调节肠道微生态提升健康的目的。

Thank you for your attention!