



# 读书报告

READING REPORTS

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# TOPIC



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## Fish and Shellfish Immunology

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Full length article

Effects of dietary *Lactobacillus rhamnosus* JCM1136 and *Lactococcus lactis* subsp. *lactis* JCM5805 on the growth, intestinal microbiota, morphology, immune response and disease resistance of juvenile Nile tilapia, *Oreochromis niloticus*



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# Introduction

# 1 . Introduction

益生菌（Probiotics）是一类对宿主有益的活性微生物，是定植于人体肠道、生殖系统内，能产生确切健康功效从而改善宿主微生态平衡、发挥对肠道有益作用的活性有益微生物的总称。人体、动物体内有益的细菌或真菌主要有：酪酸梭菌、乳酸菌、双歧杆菌、嗜酸乳杆菌、放线菌、酵母菌等。

益生元(Prebiotics)是这样一种物质：它是一种膳食补充剂，通过选择性的刺激一种或少数种菌落中的细菌的生长与活性而对寄主产生有益的影响从而改善寄主健康的不可被消化的食品成分（Gibson and Roberfroid, 1995）。成功的益生元应是在通过上消化道时，大部分不被消化而能被肠道菌群所发酵的。最重要的是它只是刺激有益菌群的生长，而不是有潜在致病性或腐败活性的有害细菌。



# 1 . Introduction

鼠李糖乳杆菌(*L.rhamnosus*), 从属于乳杆菌属, 是人体正常菌群之一, 肠道黏着率高, 定植能力强, 并具有高效降胆固醇, 促进细胞分裂, 可起到调节肠道菌群、预防和治疗腹泻、提高机体免疫力功能。水产中已被广泛应用控制嗜水气单胞菌和黄杆菌引起的虹鳟中的感染和爱德华氏菌和链球菌引起的罗非鱼的感染。

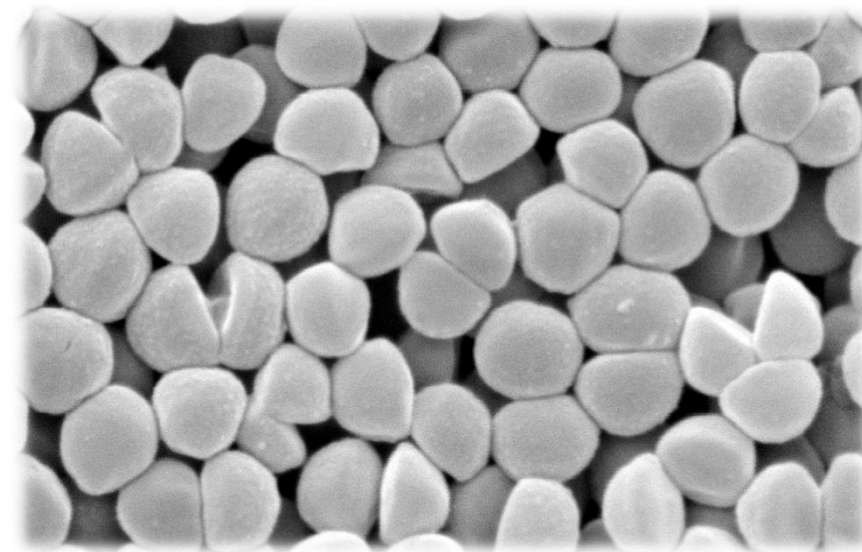


The screenshot displays a journal website interface. On the left, there are two article listings from Elsevier. The first article is titled "Viability and morpho encapsulated *Lacti tilapia gastrointesti performance, intes Streptococcus aga" and lists authors Nopadon Pirarat, Komkiew Takayuki Katagiri, and Masashi Mait. The second article is titled "Protective effec *Lactobacillus ri tarda infection i" and lists author Nopadon Pirarat. On the right, there is a search bar with the text "search" and a magnifying glass icon, and a link for "Advanced Search". Below the search bar, there is a navigation menu with links for "Home", "Articles", "For Authors", "About the Journal", and "Subscribe". The main content area features a large article titled "Characterization of the Properties of Human- and Dairy-Derived Probiotics for Prevention of Infectious Diseases in Fish" by Sami Nikoskelainen, Seppo Salminen, Göran Bylund, and Arthur C. Ouwehand. The DOI for this article is 10.1128/AEM.67.6.2430-2435.2001.**

# 1 . Introduction

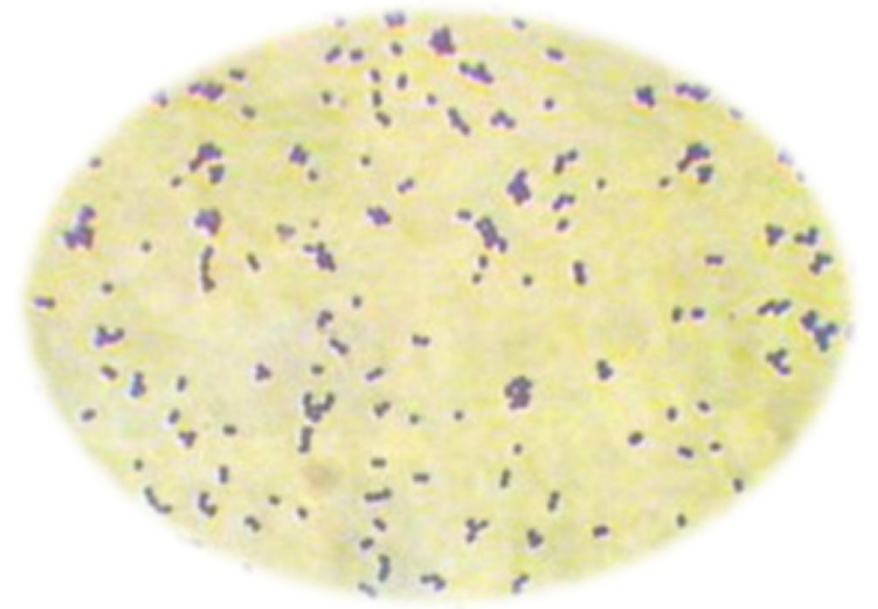
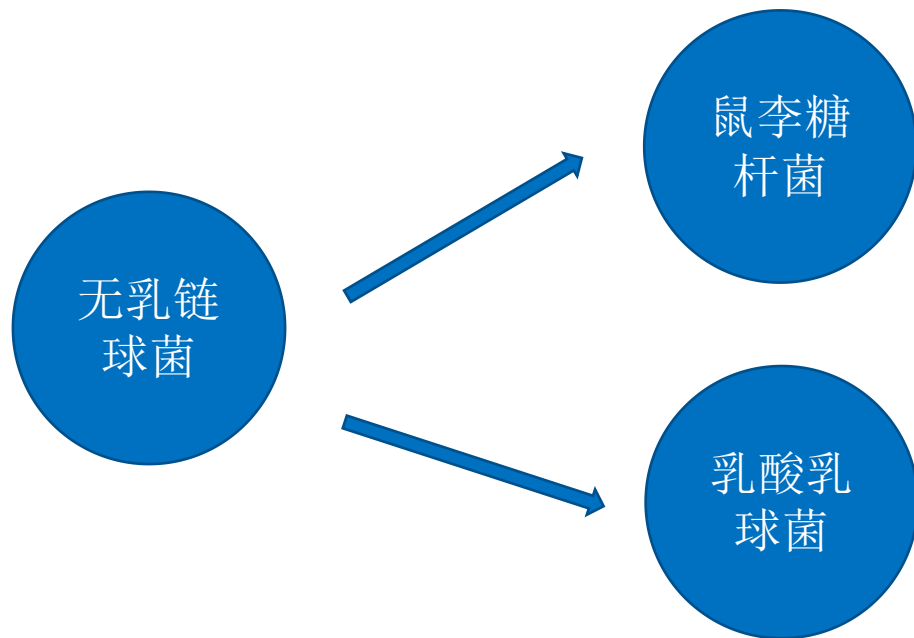
**乳酸乳球菌**(*Lactococcus lactis*, *L. lactis*)是一种原核微生物，归属于硬壁菌门，杆菌纲，乳杆菌目，链球菌科，乳球菌属，同时是乳酸菌属中的一种重要模式菌。

乳酸乳球菌 **JCM5805** 被发现是一种可以直接激活小鼠和人类中独特的 pDCs（浆细胞，树状细胞）。口服 JCM5805 也已显示通过可以 pDC 增强 NK 细胞的细胞毒活性。pDC 可以表达 TLR-7 和 TLR-9 两种不同的类型 Toll样受体，以 MyD88 依赖性方式诱导I型 IFN 的产生。



# 1 . Introduction

无乳链球菌(*S.agalactiae*)是乳腺炎的**病原体**，常存在于乳牛的皮肤、乳头及乳房内，通过挤乳人员的手或挤乳机械以及蝇类的机械携带而传播。这种链球菌引起乳房炎后**不产生明显免疫力**，目前尚无可靠的多价菌苗。

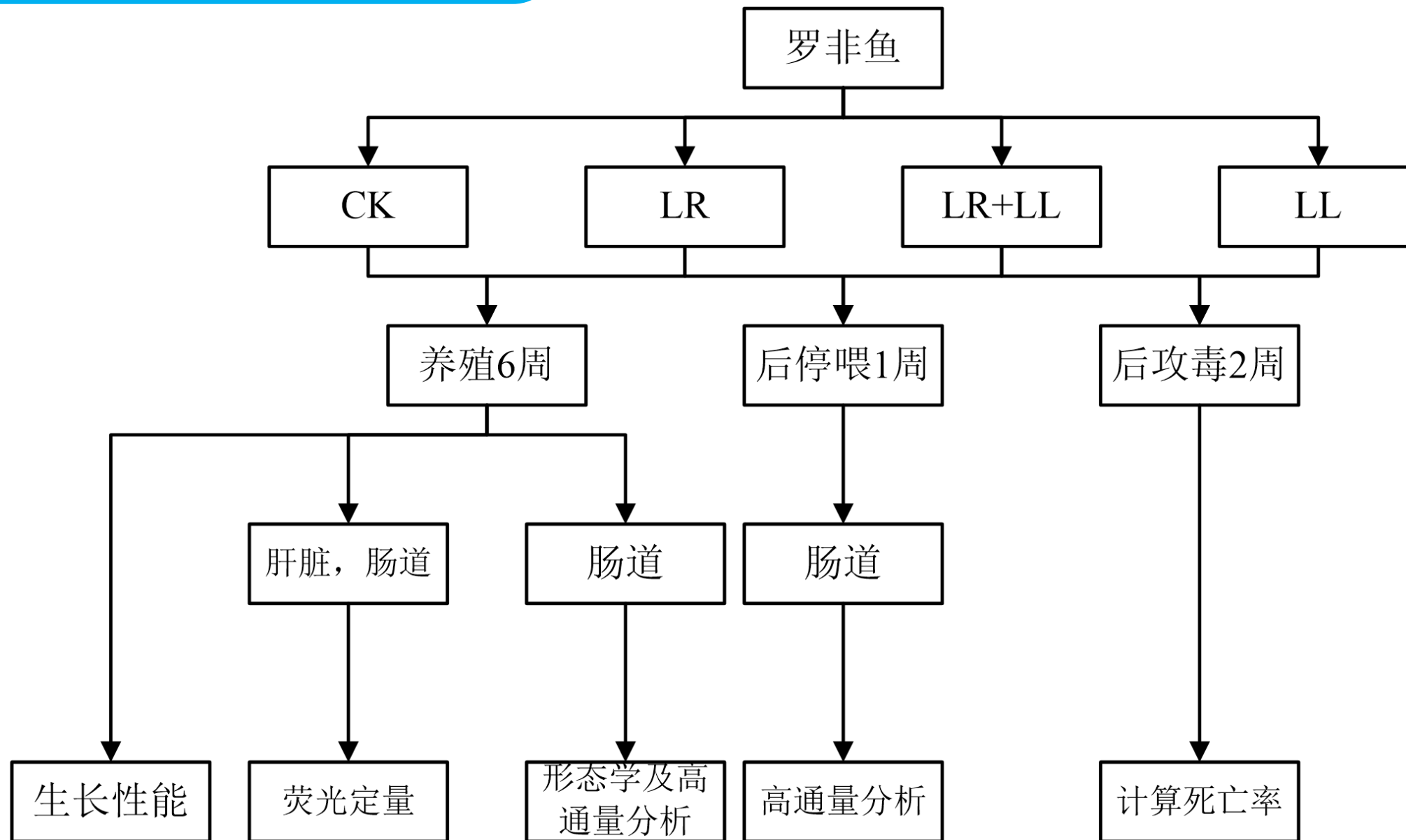




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# Materials and methods

## 2. Materials and methods



## 2. Materials and methods

乳酸菌（LAB）菌株均购自日本微生物保藏中心（JCM），在30°C下培养48小时，收集，5000g离心5分钟。无菌蒸馏水洗涤两次，冷冻干燥并悬浮于PBS中。细菌数量通过比浊法测定。

基础日粮的配方如图1，制备三种补充饮食（图2）。加入适当的LAB将饲料原料混合制粒。每天都会生产新批次的饲料储存在4°C的密封塑料袋中，以维持LR和LL的活力。

**Table 2**

The treatments of the experiment for comparison of *Lactobacillus rhamnosus* JCM1136 or/and *Lactococcus lactis* Subsp. *lactis* JCM5805 as dietary supplements in tilapia.

Treatments	Diet
CK	Basal
LR	Basal contained $1 \times 10^8$ CFU JCM 1136/g feed
LR+LL	Basal contained $0.5 \times 10^8$ CFU JCM 1136 and $0.5 \times 10^8$ CFU JCM 5805/g feed
LL	Basal contained $1 \times 10^8$ CFU JCM 5805/g feed

**Table 1**

Formulation and calculated chemical compositions of the basal diet.

Ingredients	Percentage (%)
Fish meal	48
Soybean meal	22
Wheat flour	25
Adhesives	0.2
Soybean oil	2.0
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	2.0
VCphosphate ester	0.1
Choline chloride (50%)	0.3
Vitamin mix <sup>a</sup>	0.2
Mineral mix <sup>b</sup>	0.2
Crude protein	42.0
Crude lipid	7.3
Ash	9.5
Crude fibre	3.1
N free extract	27.9

<sup>a</sup> Vitamin premix (g/kg): thiamine, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B-12, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; retinyl acetate, 0.677; cholecalciferol, 0.116; DL- $\alpha$ -tocopherol-acetate, 12.632.

<sup>b</sup> Mineral premix (g/kg): CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.074; CuSO<sub>4</sub>·5H<sub>2</sub>O, 2.5; FeSO<sub>4</sub>·7H<sub>2</sub>O, 73.2; NaCl, 40.0; MgSO<sub>4</sub>·7H<sub>2</sub>O, 284.0; MnSO<sub>4</sub>·H<sub>2</sub>O, 6.50; KI, 0.68; Na<sub>2</sub>SeO<sub>3</sub>, 0.10; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 131.93; cellulose, 501.09

## 2 . Materials and methods

养殖对象：尼罗罗非鱼幼鱼（ $0.20 \pm 0.05\text{g}$ ）

养殖时间：根据以往养殖情况养殖5周，但由于益生菌组与对照组之间生长性能没有显著性差异，所以养殖时间延长一周。在第7周，所有鱼都喂食基础日粮。

养殖条件：在 $28-29^{\circ}\text{C}$ 水中暂养一周。然后将体格健壮的鱼被随机分配到十二个玻璃缸（每个缸六十条鱼，每个处理三个重复）中。为了尽量减少外部环境的影响，水和饲料预先用紫外线灭菌。



## 2 . Materials and methods

取样:

6周后，禁食24小时，用过量MS-222麻醉处死。

轻轻剪取鱼的一段中肠（约1厘米），在PBS（pH7.2）中洗涤3次，每次1分钟以除去消化物，用于肠粘膜形态学观察。

中肠的其余部分以相同方式洗涤并用于RT-PCR分析。

另取实验鱼的无菌条件下打开腹腔，取出肠道并在无菌磷酸盐缓冲液（PBS，pH 7.4）中轻轻洗涤3次，每次1分钟以除去内容物和非粘附细菌，用于细菌群落分析。

称量鱼重以计算体重增加率（WG），饲料转化率（FCR）。

## 2 . Materials and methods

### 高通量测序分析

交由GENEWIZ,Inc.（金唯智，中国苏州）进行。

### 益生菌停喂处理

在第7周（益生菌喂养停止后第7天）后，每个重复取三条鱼，通过高通量测序以评估益生菌在中肠内存在的持久性。

### 免疫相关的基因表达

选择的免疫相关基因是TNF- $\alpha$ ，IFN- $\gamma$ ，lyz-c，hsp70和IL-1 $\beta$ ，以 $\beta$ -actin为内参。

## 2. Materials and methods

### 肠道组织学观察

每个重复取3条鱼的肠道进行扫描电子显微镜（SEM）和透射电子显微镜（TEM）观察。

将样品（约2mm）在1% S-羧甲基-L-半胱氨酸中洗涤30秒（仅SEM）以除去杂质，然后使用2.5%戊二醛缓冲液（0.1M pH 7.2）进行固定。

SEM图像（×30,000）用于测量微绒毛密度。TEM图像（×6000）用于测量微绒毛长度。

### 攻毒实验

6周后，每个重复选取25条鱼进行腹腔注射20μl无乳链球菌（LD<sub>50</sub> = 1 × 10<sup>5</sup> CFU/ml），无乳链球菌由患病的尼罗罗非鱼的鳃中分离出。将该菌株在37°C厌氧条件下于脑心浸液培养基中培养48小时。然后以2000g离心5分钟。用磷酸盐缓冲液洗涤沉淀两次，细菌数量通过比浊法测定。注射后2周内进行监测其临床症状，死后病变情况和每日死亡率。

03

## Results and discussion



### 3 . Results and discussion

**Table 3**  
Growth performance of Tilapia fed diets supplemented with *Lactobacillus rhamnosus* JCM1136 (LR) or/and *Lactococcus lactis* Subsp. *lactis* JCM5805 (LL) for 6 weeks.\*

	Experimental diets			
	CK	LR	LR+LL	LL
IBW (g)	0.20 ± 0.02	0.20 ± 0.02	0.19 ± 0.02	0.20 ± 0.02
WG (%)	2681.90 ± 166.47a	2772.65 ± 229.21a	2919.07 ± 256.48a	3532.20 ± 403.43b
FCR	1.39 ± 0.05b	1.33 ± 0.07b	1.29 ± 0.03b	1.04 ± 0.02a
SR (%)	94.07 ± 6.12	91.11 ± 2.22	89.63 ± 2.31	93.33 ± 2.94

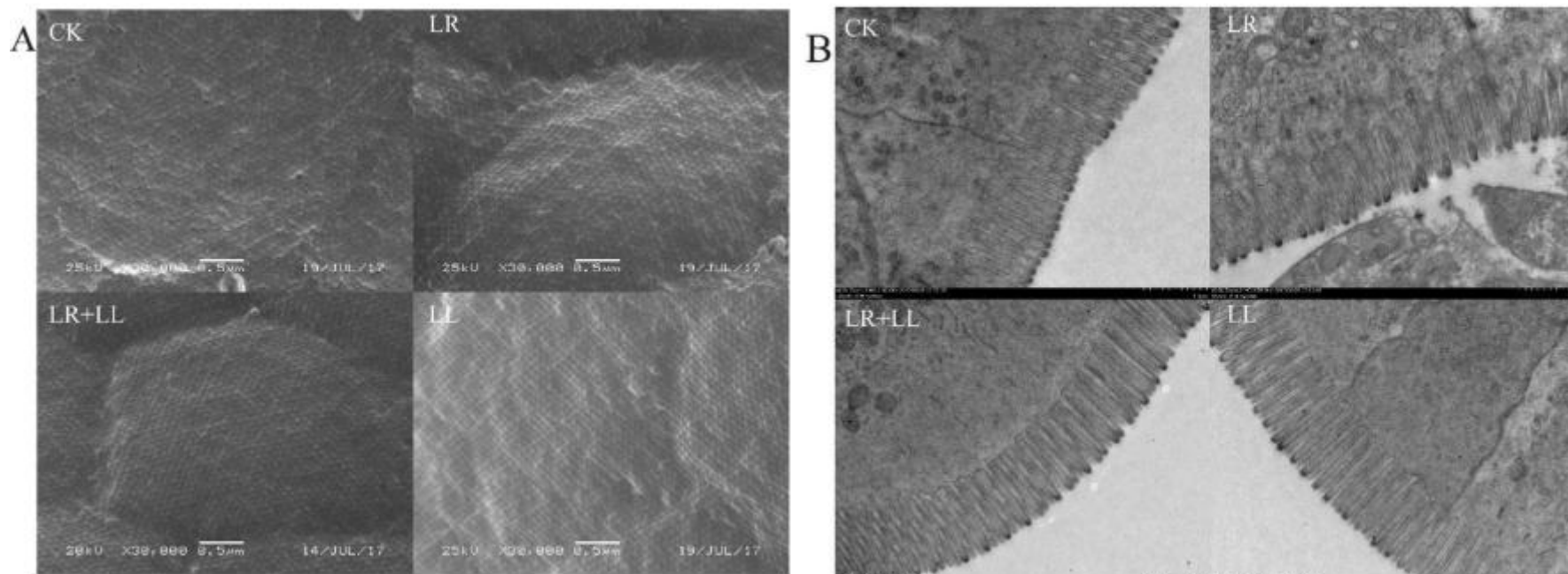
\*In the same line, means with different letters are significantly different ( $P < 0.05$ ), means with the same letters are not significantly different ( $P > 0.05$ ). Data represent means ± SD (n = 3 fish).

CK, basal diet; LR, basal diet with  $1 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136; LR+LL, basal diet with both  $0.5 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136 and  $0.5 \times 10^8$  cfu/g of *L. lactis* JCM 5805; LL, basal diet with  $1 \times 10^8$  cfu/g of *L. lactis* JCM 5805.

罗非鱼的生长性能的表现与特定益生菌菌株的抗菌活性差异以及个体差异有关。

综合其他文献结果，机体生长性能是益生菌，肠道微生物组，饮食，宿主，研究条件和处理实践等因素之间相互作用的结果。

### 3 . Results and discussion



结果表明：益生菌可以通过改善微绒毛密度和微绒毛长度增加肠道吸收表面积。此外，较高的微绒毛密度可以减少肠细胞暴露，增加紧密连接水平，对阻止潜在病原体提供更有效的屏障。

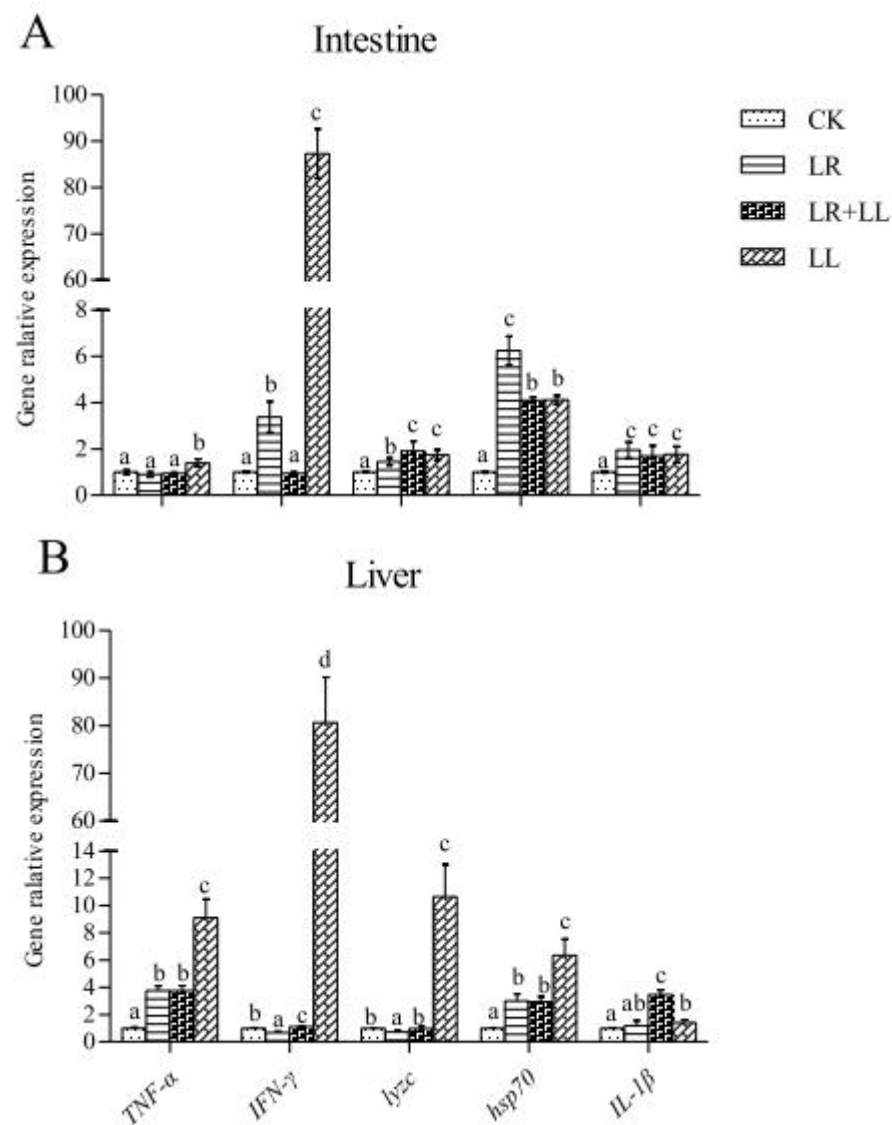
**Table 4**  
Intestinal microvilli density and length of Nile tilapia fed diets supplemented with *Lactobacillus rhamnosus* JCM1136 (LR) or/and *Lactococcus lactis* Subsp. *lactis* JCM5805 (LL) for six weeks.\*

	CK	LR	LR+LL	LL
Density (count/ $\mu\text{m}^2$ )	85.66 $\pm$ 7.45a	120.85 $\pm$ 8.23b	133.47 $\pm$ 9.00bc	134.79 $\pm$ 7.01c
Length ( $\mu\text{m}$ )	0.91 $\pm$ 0.06a	1.11 $\pm$ 0.05c	0.96 $\pm$ 0.04b	0.96 $\pm$ 0.03b

\*In the same line, means with different letters are significantly different ( $P < 0.05$ ), means with the same letters are not significantly different ( $P > 0.05$ ). Data represent means  $\pm$  SD (n = 3 fish).

CK, basal diet; LR, basal diet with  $1 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136; LR+LL, basal diet with both  $0.5 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136 and  $0.5 \times 10^8$  cfu/g of *L. lactis* JCM 5805; LL, basal diet with  $1 \times 10^8$  cfu/g of *L. lactis* JCM 5805.

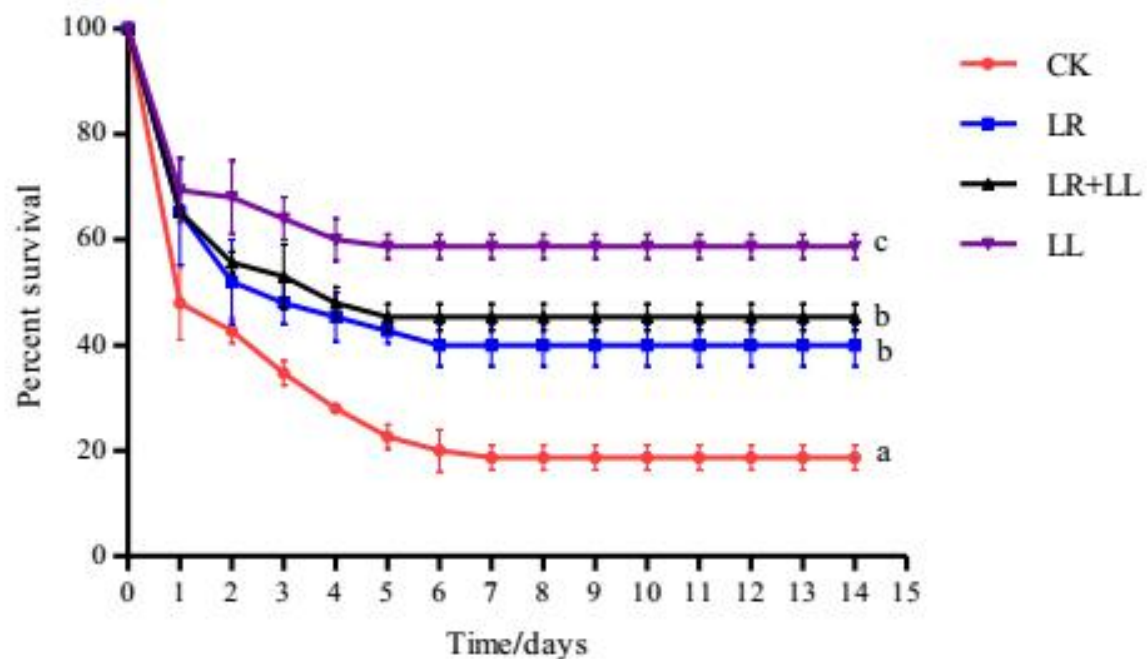
### 3 . Results and discussion



Hsp70可以促进蛋白质结构的保护作用，增强免疫系统并停止细胞凋亡机制。研究发现了乳酸乳球菌能诱导更高的全身hsp70。

IFN- $\gamma$ 在先天性和适应性细胞介导的免疫反应中起主要作用，它主要去除细胞内的病原体，可以通过它来提高TNF- $\alpha$ 和IL-1 $\beta$ 。IL-1 $\beta$ 和TNF- $\alpha$ 的表达变化，可能与细菌对肠壁的粘附特性有关（JCM5805是一种肠粘性细菌）。C型溶菌酶参与对细菌病原体的反应，研究表明，这种转录被强烈的细菌攻击或LPS注射而快速激活，当以组合形式进行益生菌补充时，不同的益生菌应该会以协同作用来影响机体健康。

### 3 . Results and discussion



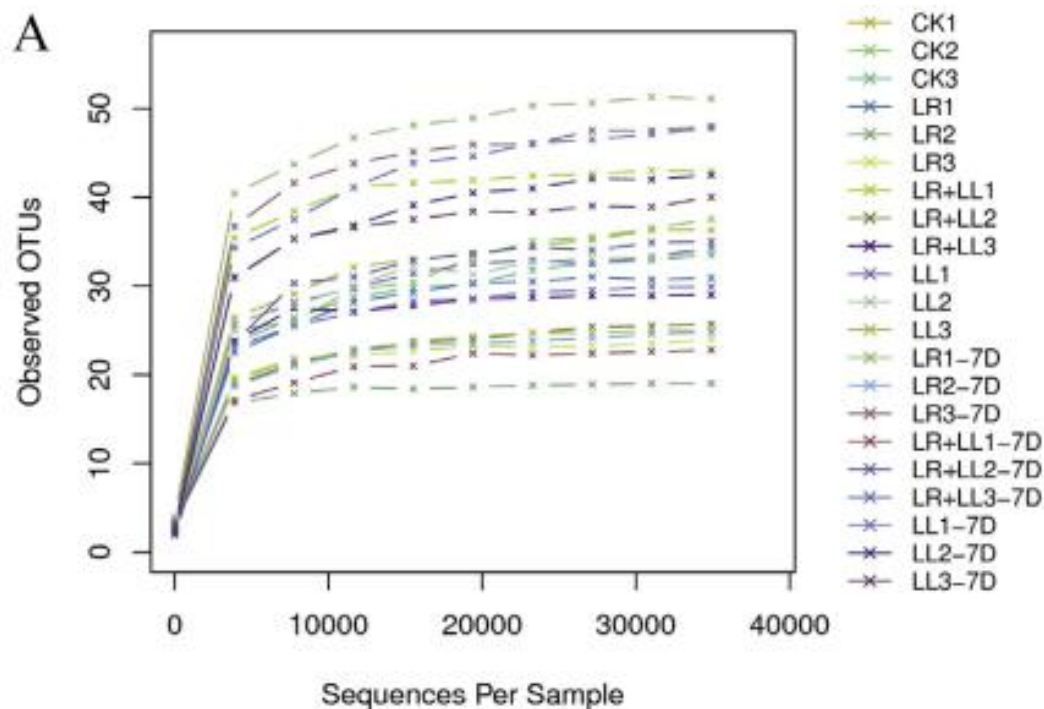
**Fig. 3.** Cumulative survival rates of tilapia challenged by injection of *S. agalactiae* after administering either basal diet or basal diet with probiotics for six weeks (means  $\pm$  SD). Different letters denote significant differences ( $P < 0.05$ ). CK, basal diet; LR, basal diet with  $1 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136; LR+LL, basal diet with both  $0.5 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136 and  $0.5 \times 10^8$  cfu/g of *L. lactis* JCM 5805; LL, basal diet with  $1 \times 10^8$  cfu/g of *L. lactis* JCM 5805.

攻毒后罗非鱼的存活率表明，每组均在第一天达到最高每日死亡率。与对照组相比，罗非鱼饲喂益生菌的存活率显著提高。在LL组中观察到最高的存活率，而LR组和LR + LL组之间存活率的差异没有统计学意义 ( $P > 0.05$ )，但与LL组相比，显著降低 ( $P < 0.05$ )。

实验结果表明：益生菌的协同作用并不一定都是正向的。

### 3 . Results and discussion

共1,631,877序列，平均长度为443 bp。共识别出222个OUT。



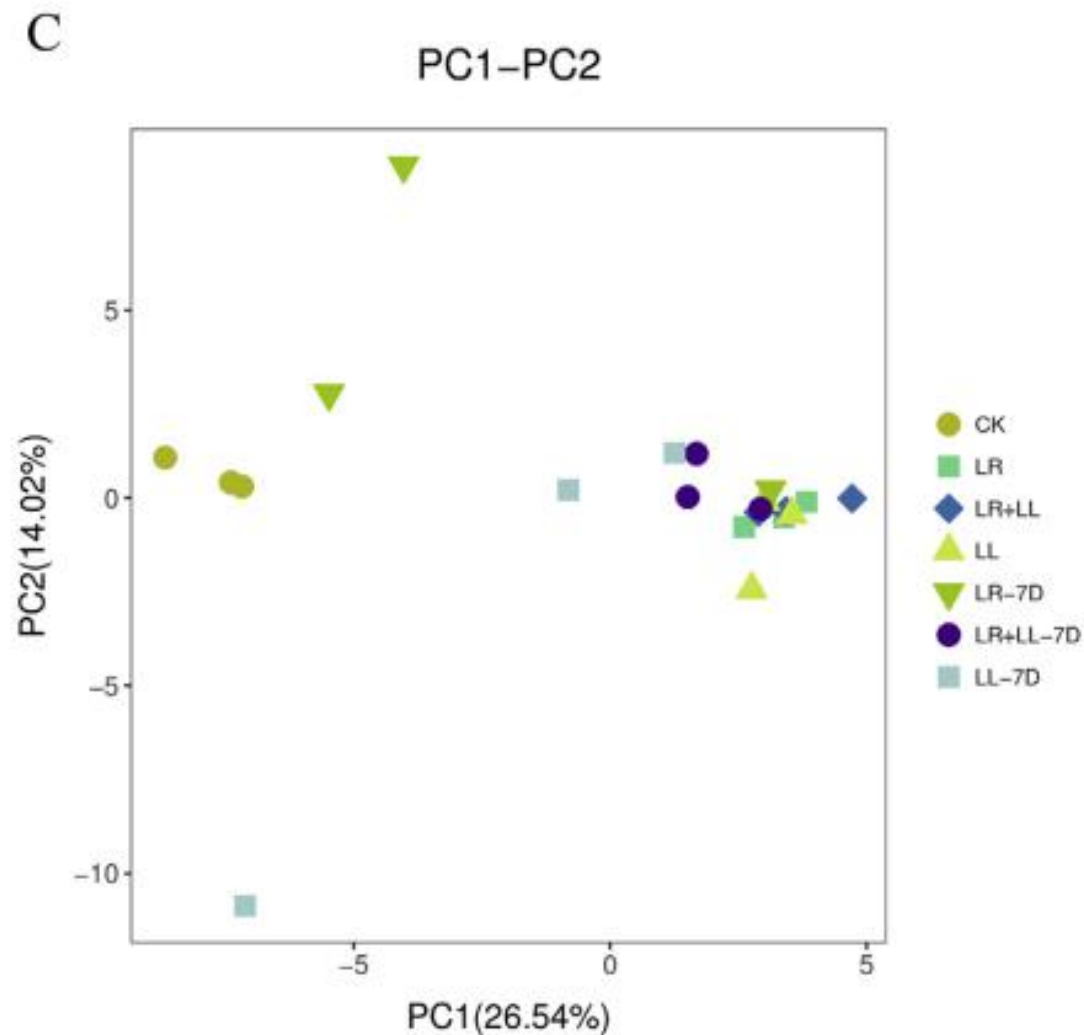
**Table 5**  
Diversity indices used in this study.\*

sample	ace	chao1	shannon	simpson	goods_coverage
CK	43.29 ± 4.56b	40.11 ± 2.67b	1.15 ± 0.23	0.31 ± 0.08	0.95 ± 0.01
LR	25.37 ± 6.03a	25 ± 6.00a	1.40 ± 0.02	0.39 ± 0.01	0.95 ± 0.01
LR+LL	33.88 ± 8.20ab	33.17 ± 8.55ab	1.50 ± 0.32	0.40 ± 0.07	0.96 ± 0.04
LL	33.23 ± 7.66ab	33 ± 7.55ab	1.55 ± 0.09	0.42 ± 0.01	0.96 ± 0.03
LR-7D	42.85 ± 15.42ab	42.73 ± 15.73ab	1.64 ± 0.62	0.46 ± 0.18	0.97 ± 0.01
LR+LL-7D	29.58 ± 5.88ab	29.33 ± 6.03ab	1.25 ± 0.24	0.33 ± 0.09	0.96 ± 0.03
LL-7D	45.92 ± 3.90ab	48.83 ± 4.19ab	1.46 ± 0.23	0.42 ± 0.15	0.96 ± 0.03

\*In the same line, means with different letters are significantly different ( $P < 0.05$ ), means with the same letters are not significantly different ( $P > 0.05$ ). Data represent means ± SD (n = 3 fish).

CK, basal diet; LR, basal diet with  $1 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136; LR+LL, basal diet with both  $0.5 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136 and  $0.5 \times 10^8$  cfu/g of *L. lactis* JCM 5805; LL, basal diet with  $1 \times 10^8$  cfu/g of *L. lactis* JCM 5805; LR-7D, the LR group cessation in probiotic consumption for 1 week; LR+LL-7D, the LR+LL group cessation in probiotic consumption for 1 week; LL-7D, LL group cessation in probiotic consumption for 1 week.

### 3 . Results and discussion



结果显示：停止喂食益生菌后LR组和LL组（即LR-7D和LL-7D）散乱无序。表明其组间成分差异较大。

实验结果表明：益生菌在肠道中的定植与益生菌种类，喂食的剂量和在肠道内的保留时间有关。而益生菌的保留时间也可能取决于动物的发育状态。

本实验中，单种益生菌的停止改变了罗非鱼肠道微生物群落，从而引起益生菌在肠粘膜区快速释放，而后生态失调，导致宿主具有更高的易感性。

### 3 . Results and discussion

总体来说，在益生菌组，变形杆菌（Proteobacteria）是最丰富的门，而CK组则由梭杆菌（Fusobacterium）占主导地位。

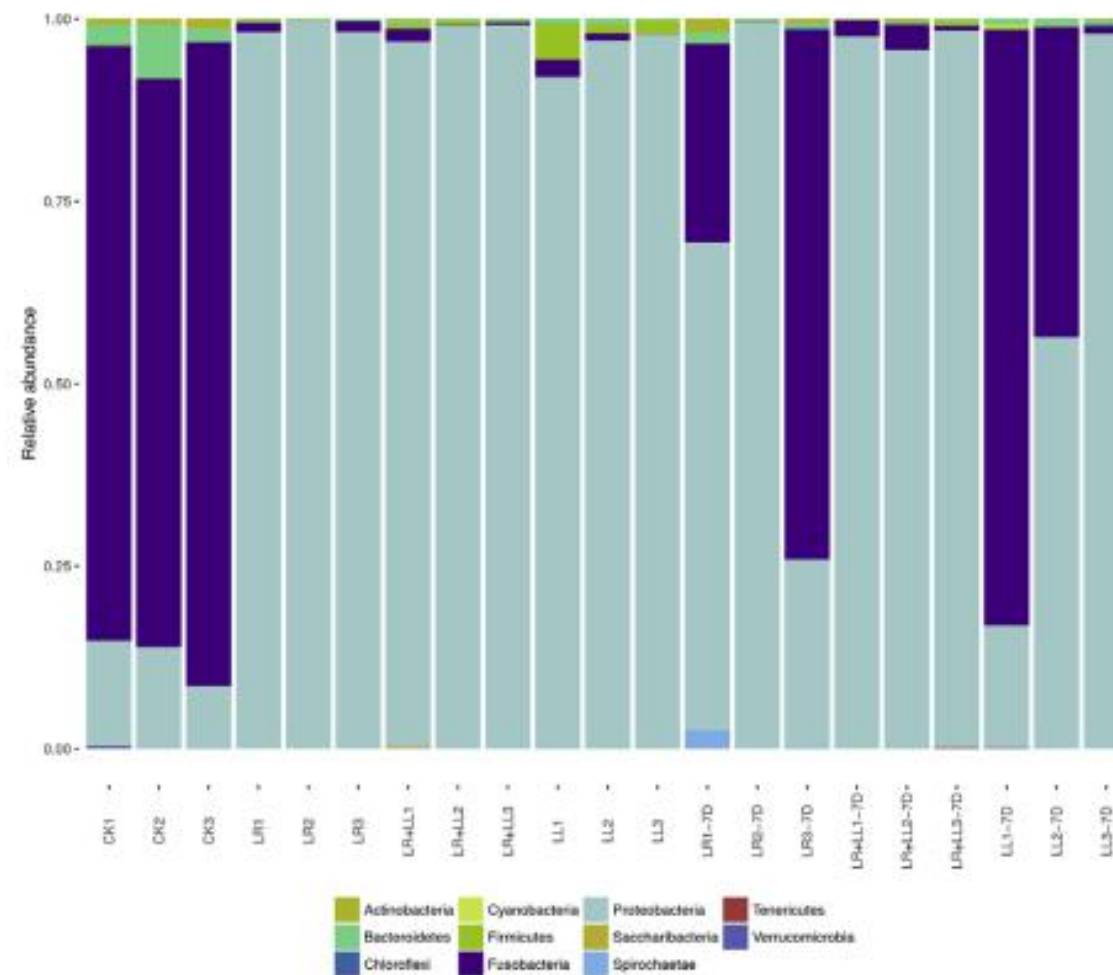


Fig. 5. Taxonomic distribution of gut samples at the phylum level.

CK, basal diet; LR, basal diet with  $1 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136; LR+LL, basal diet with both  $0.5 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136 and  $0.5 \times 10^8$  cfu/g of *L. lactis* JCM 5805; LL, basal diet with  $1 \times 10^8$  cfu/g of *L. lactis* JCM 5805; LR-7D, the LR group ceasing probiotic consumption for 1 week; LR+LL-7D, the LR+LL group ceasing probiotic consumption for 1 week; LL-7D, LL group ceasing probiotic consumption for 1 week.

### 3 . Results and discussion

**Table 6**  
Genus subdivision abundance in the four groups.

Abundance rank	Genus/Acidobacteria subdivision by source			
	CK	LR	LR + LL	LL
1	<i>Cetobacterium</i>	<i>Rhizobium</i>	<i>Rhizobium</i>	<i>Rhizobium</i>
2	<i>Unclassified</i>	<i>Unclassified</i>	<i>Unclassified</i>	<i>Unclassified</i>
3	<i>Plesiomonas</i>	<i>Escherichia-Shigella</i>	<i>Escherichia-Shigella</i>	<i>Escherichia-Shigella</i>
4	<i>Macellibacteroides</i>	<i>Achromobacter</i>	<i>Achromobacter</i>	<i>Achromobacter</i>
5	<i>Alpinimonas</i>	<i>Phyllobacterium</i>	<i>Phyllobacterium</i>	<b>Lactococcus</b>
6	<i>Bosea</i>	<i>Delftia</i>	<i>Delftia</i>	<i>Phyllobacterium</i>
7	<i>Alsobacter</i>	<i>Cetobacterium</i>	<i>Cetobacterium</i>	<i>Cetobacterium</i>
8	<i>Escherichia-Shigella</i>	<i>Burkholderia-Paraburkholderia</i>	<i>Lactococcus</i>	<i>Delftia</i>
9	<i>Vogesella</i>	<i>Chryseobacterium</i>	<i>Acinetobacter</i>	<i>Chryseobacterium</i>
10	<i>Rhizobium</i>	<i>Enhydrobacter</i>	<i>Burkholderia-Paraburkholderia</i>	<i>Acinetobacter</i>
11	<i>Meganema</i>	<i>Acinetobacter</i>	<i>Chryseobacterium</i>	<i>Weissella</i>

CK, basal diet; LR, basal diet with  $1 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136; LR + LL, basal diet with both  $0.5 \times 10^8$  cfu/g of *L. rhamnosus* JCM 1136 and  $0.5 \times 10^8$  cfu/g of *L. lactis* JCM 5805; LL, basal diet with  $1 \times 10^8$  cfu/g of *L. lactis* JCM 5805.

实验结果表明：益生菌喂食后，抑制有害菌的生长，促进有益菌的生长。





# THANK YOU

感谢聆听，批评指导