读书报告

汇报人：胡文攀

时　间：2018/7/7

SCIENTIFIC REPORTS

OPEN

Abrupt suspension of probiotics administration may increase host pathogen susceptibility by inducing gut dysbiosis

Received: 22 January 2016
Accepted: 29 February 2016
Published: 17 March 2016

Zhi Liu*, Wenshu Liu*, Chao Ran*, Jun Hu & Zhigang Zhou

4.122

目录

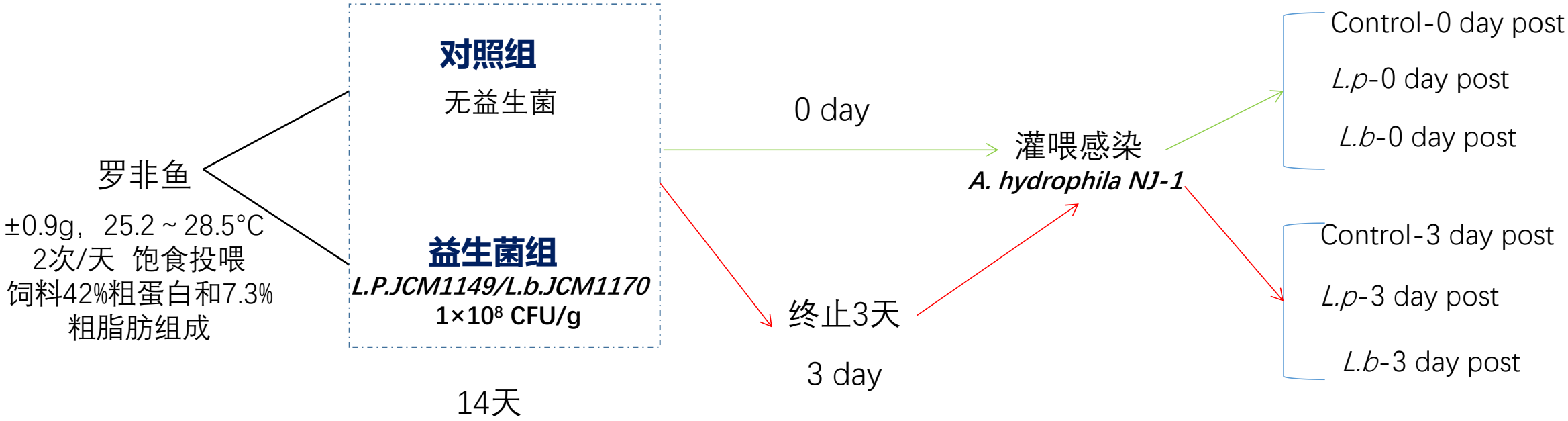
CONTENTS

1.研究背景

- **益生菌**（主要是乳酸杆菌和双歧杆菌）是天然共生细菌，通常它的使用被认为是**安全**的，研究表明一些特定菌株的使用还存在一些安全风险。例如，*Lactobacillus*（乳酸菌）可能引起免疫受损患者对于益生菌败血症的感染。目前关于益生菌使用的安全问题主要聚焦于免疫受损患者对于病原菌感染的风险。益生菌的使用涉及大量的活微生物，会建立新的肠道微生物群的平衡。**但益生菌使用的突然中止对于微生物群平衡的破坏却鲜有研究**。

- **罗非鱼**（脊椎动物）其环境适应性强，成本低，易于繁殖，且罗非鱼拥有低效的特异性免疫力，使用罗非鱼是为了模拟免疫缺陷群体。

2.材料与方法


罗非鱼
±0.9g，25.2 ~ 28.5°C
2次/天 饱食投喂
饲料42%粗蛋白和7.3%
粗脂肪组成
对照组
无益生菌
益生菌组
L.P.JCM1149/L.b.JCM1170
1×10⁸ CFU/g
14天
0 day
终止3天
3 day
灌喂感染
A. hydrophila NJ-1
Control-0 day post
L.p-0 day post
L.b-0 day post
Control-3 day post
L.p-3 day post
L.b-3 day post

3.研究结果

Fig 1：

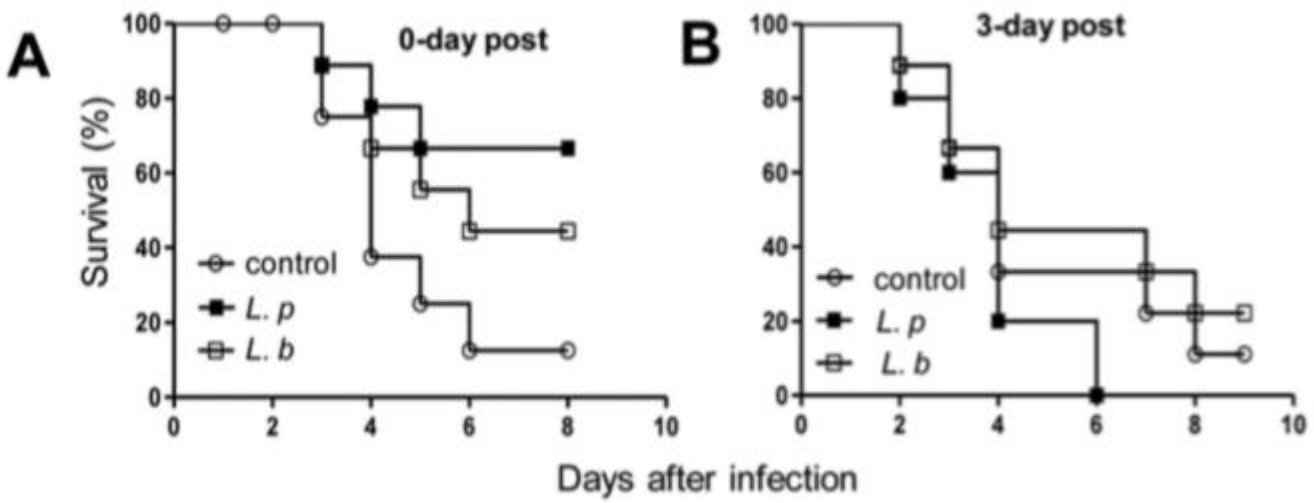


Figure 1. Probiotics feeding suspension increased tilapia mortality and morbidity. Survival curve of tilapia following A. hydrophila NJ-1 challenge: tilapias were continuously fed using an experimental diet without (control: empty circle), or with probiotics (L. p JCM1149: empty square, L. b JCM 1170: filled square). The tilapias were then subjected to a pathogen challenge at (A) 0-day post and (B) 3-days post probioticsfeeding suspension.

使用益生菌增加了宿主的抵抗力(图1A)，然而3天暂停后益生菌的保护作用被完全取消(图1B)。甚至L. p停用后引起了罗非鱼100%死亡(图1B)。

3.研究结果

Fig 1：

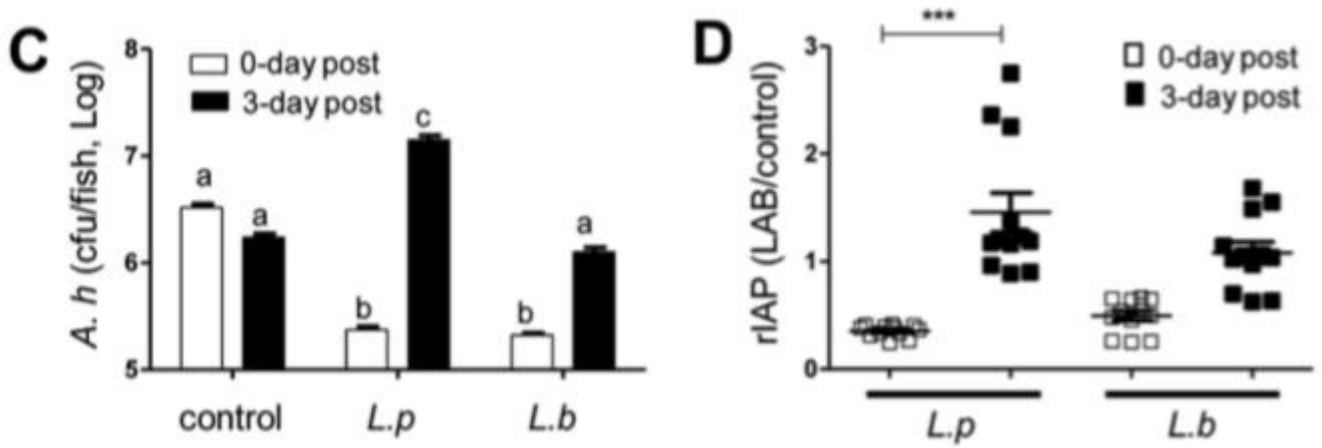


Figure 1. Probiotics feeding suspension increased tilapia mortality and morbidity. (C) A. hydrophila NJ-1 colonization in tilapias, values with different superscript letters are significantly different (P < 0.001). (D) Relative IAP (rIAP) activity in tilapias, three asterisks indicate significant difference (P < 0.001).

益生菌暂停使罗非鱼更易受病原菌的侵袭且导致宿主对感染的敏感性增加。L. p 施用比L. b造成了更严重的负面影响，将聚焦于L. p。

3.研究结果

Fig 2：L. p 喂养终止后A.*hydrophila* NJ-1在肠道中的黏附和增殖。

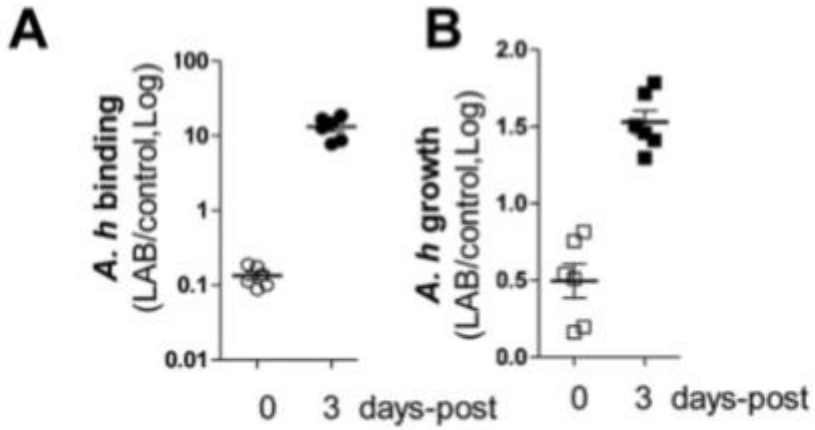
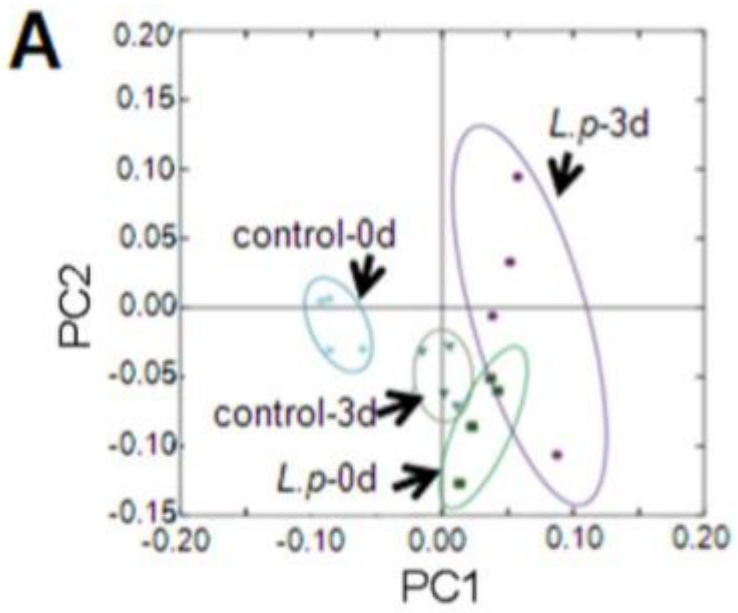


Figure 2. *A. hydrophila* NJ-1 binding (A) and proliferation (B) on tilapia intestinal tissue after *L.p* JCM1149 feeding suspension. After treatment, tilapia intestines were opened to expose the inner surface. *A. hydrophila* NJ-1 cells were mounted on the inner surface for binding and growth measurement as described in the materials and methods section.

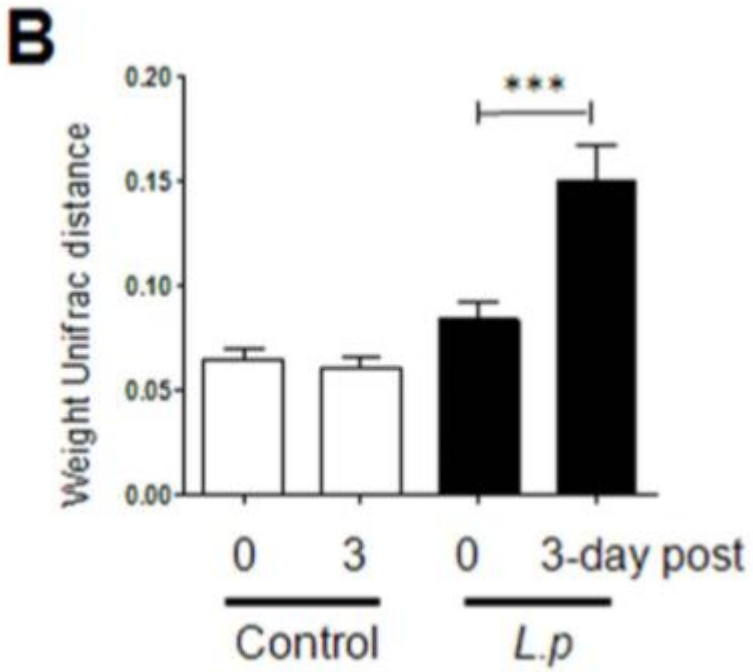
L.P施用中止促进病原菌在罗非鱼肠道内的黏附和增殖，提升了病原菌的感染效率。

3.研究结果

Fig 3：

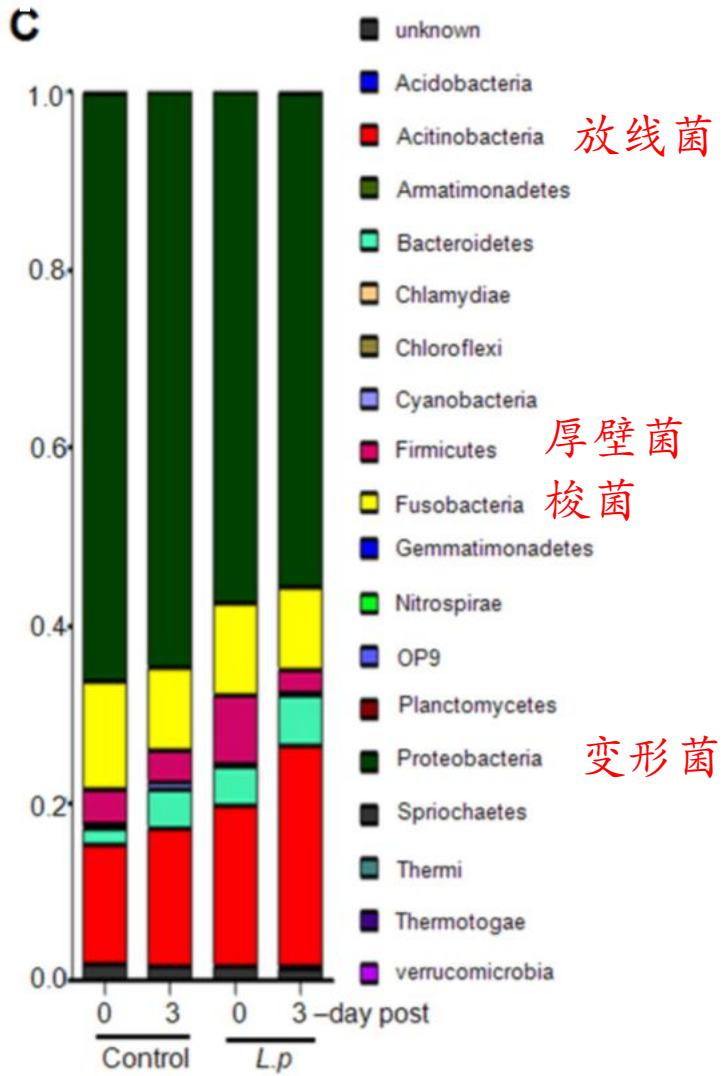


在二维主坐标分析(PCoA)图中，L.*p*-3d（益生菌终止） 紊乱分散。



利用UniFrac加权距离进一步分析，与对照组相比，L. p 差异性明显高于对照组(图3B)。

3.研究结果



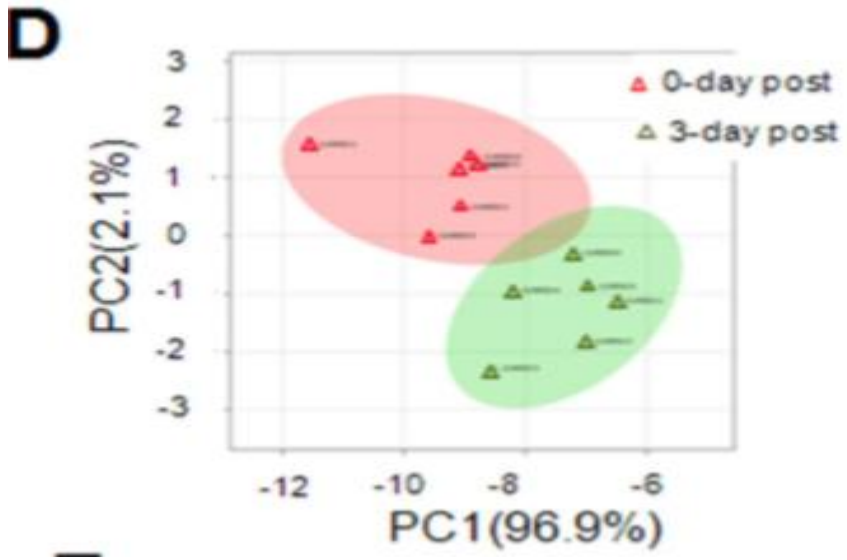
放线菌

厚壁菌

梭菌

变形菌

对照组前后差异不大，实验组厚壁菌减少，放线菌增多。

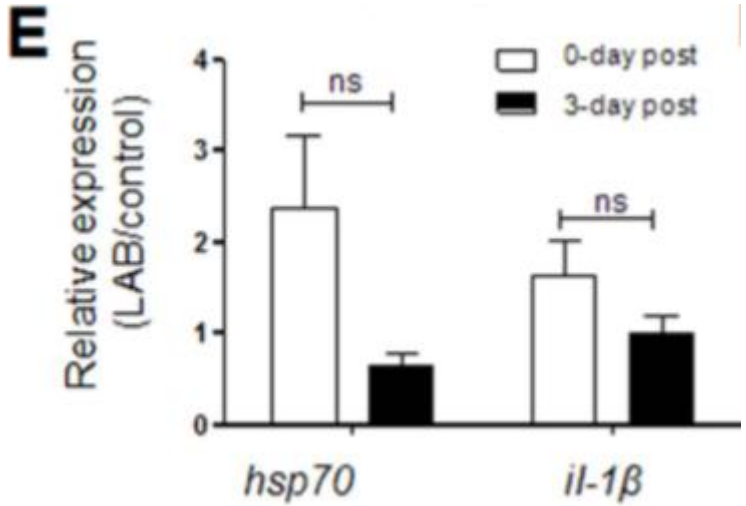


主成分分析(PCA)(图3D)

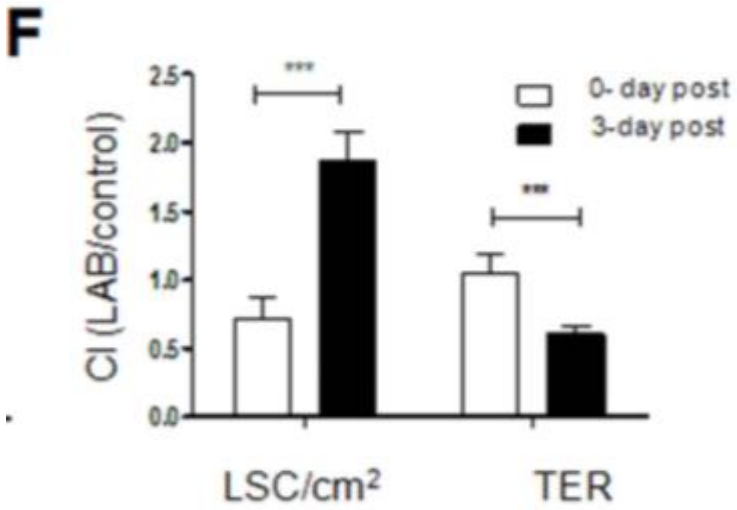
FIG2.A-D，L. p 施用中止破坏了罗非鱼体内微生物原有的稳态。

Fig 3：

3.研究结果



在对照组以及L. p JCM1149前后，热休克蛋白基因hsp70和炎症相关基因il-1β的表达没有显著差异 (图3 E)。这表明宿主的免疫反应并不是增强嗜水细胞感染易感性的主要原因。



利用Ussing Chamber检测肠组织短路电流。在L. p 前后罗非鱼肠组织存在显著差异(图3F)，说明在给予益生菌后，罗非鱼肠上皮的物理结构发生了改变。

Fig 4：

3.研究结果

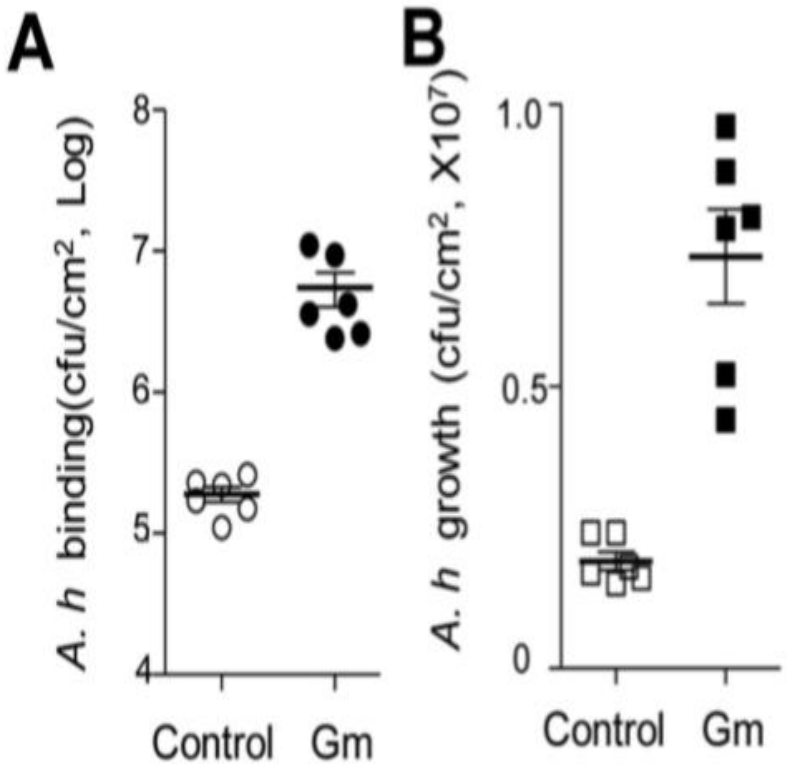


Figure 4. Influence of antibiotics treatment on *A. hydrophila* NJ-1 binding (A) and proliferation (B) on intestinal surfaces. After antibiotic treatment, tilapia intestines were opened to expose the inner surface, and *A. hydrophila* NJ-1 cells were applied to the inner surface for binding and growth measurement as described in the materials and methods section.

为了进一步证实罗非鱼肠道菌群失调与病原体敏感性之间的关系，探究抗生素对NJ-1在肠内定植和增殖的影响。

与对照相比，NJ-1在庆大霉素(Gm)中具有更高的结合效率(图4A)和更大的增殖率(图4B)。

3.研究结果

Fig 5：罗非鱼肠内表面益生菌细胞的空间分布

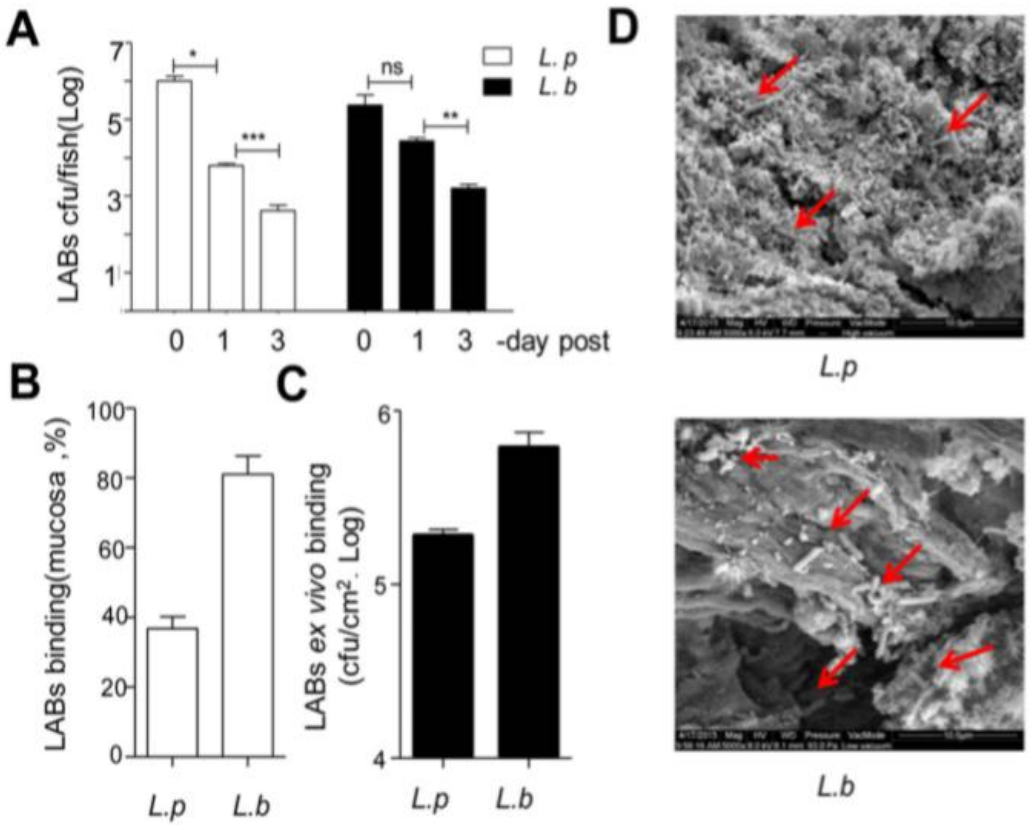


Figure 5. Dynamic kinetics and spatial distribution of probiotics in tilapia intestines. *L. p* JCM1149 and *L. b* JCM1170 populations in intestinal inner surface area (A) and intestinal mucosa (B) of tilapias. Probiotic binding on tilapia *ex vivo* intestinal tissue was visualized using cell counts (C) and SEM (D). One, two and three asterisks represent significant differences ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively).

我们观察到L. p大多位于粘液层，而L.b多粘膜区。这一结果与罗非鱼GIT中L. p的快速释放是一致的，因为与黏膜部分相比，益生菌细胞的黏膜结合部分被认为更加松散。

Fig 6：

3.研究结果

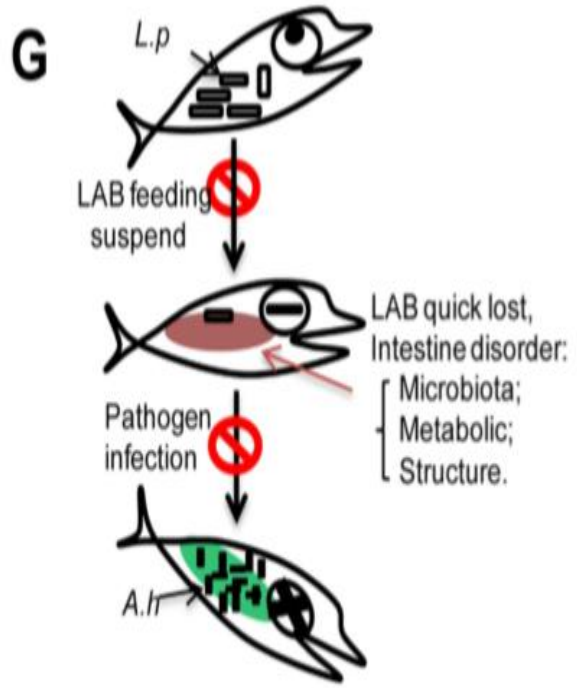
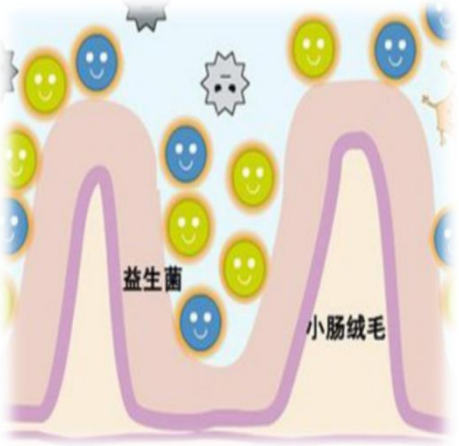


Figure 6. Working model of the risk of probiotics administration suspension. Under continuous feeding conditions, lactic acid bacteria reside in the host intestines and benefit host health. When probiotics administration is suspended, lactic acid bacteria are rapidly released, causing a host intestinal imbalance in the gut microbiota, gut metabolites, and intestinal physical structure. As a result of the gut dysbiosis condition, host pathogens (i.e. *A. hydrophila*) easily infect the host and cause host disease and mortality.

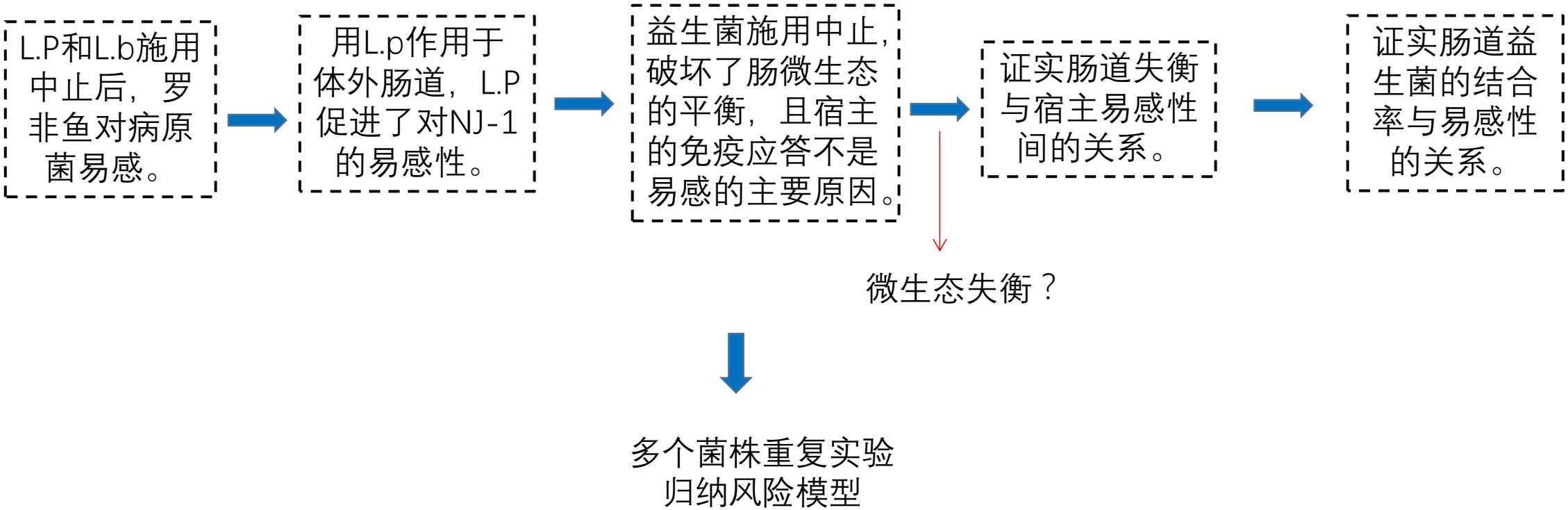
根据以上实验结果，我们提出了如下模型。在连续施用益生菌期间，大量益生菌进入宿主GIT中，促进宿主GIT的健康，抵御病原菌的侵袭。当停止使用益生菌时，益生菌细胞迅速释放，导致肠道菌群失调、肠道代谢产物、生理功能紊乱和肠上皮通透性的改变。由此产生的GIT环境更有利于潜在致病基因的粘附和增殖，从而导致疾病的发展(图6)。

4.讨论



在本研究中，提出了一种与益生菌使用相关的新的不良反应。即在连续施用益生菌后暂停，益生菌细胞迅速释放，导致肠道菌群失调和肠道代谢产物和生理功能紊乱。 新的风险模型，对未来益生菌菌株的研究和选择提供了参考。

整体思路


L.P和L.b施用中止后，罗非鱼对病原菌易感。
用L.p作用于体外肠道，L.P促进了对NJ-1的易感性。
益生菌施用中止，破坏了肠微生态的平衡，且宿主的免疫应答不是易感的主要原因。
证实肠道失衡与宿主易感性间的关系。
证实肠道益生菌的结合率与易感性的关系。
微生态失衡？
多个菌株重复实验
归纳风险模型