Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.121422

Effect of *Lactobacillus* isolated from Chinese fermented food on antibiotic induced intestinal microflora disorder in early life of mice

Ruokun YI[1], Tongji LIU[1], Rui XUE[1], Zhennai YANG[1]*

Abstract

This study investigated the effect of *Lactobacillus* isolated from Chinese fermented food on antibiotic-induced intestinal microflora disorder during the early life of mice. The experimental strain *Lactobacillus fermentum* (LF) CQPC04 was isolated from naturally fermented pickles in Chongqing, China. *Lactobacillus plantarum* (LP) KFY02 was isolated from naturally fermented yogurt from Korla in the Xinjiang Uygur Autonomous Region. The results showed that LF-CQPC04 and LP-KFY02 alleviated the decrease in bacterial diversity caused by the antibiotics, maintained the abundance of beneficial bacteria, and reduced the abundance of harmful bacteria. These results suggest that LF-CQPC04 and LP-KFY02 can be used as probiotics to alleviate antibiotic-induced intestinal microfloral disorder.

Keywords: *Lactobacillus*; Chinese fermented food; intestinal microflora disorder; mice.

Practical Application: This study investigated the effect of *Lactobacillus* isolated from Chinese fermented food on antibiotic-induced intestinal microflora disorder during the early life stages of mice. We provided a theoretical basis for the research of probiotic resources in food to improve antibiotic-induced intestinal microflora disorder.

1 Introduction

The gastrointestinal tract plays an important role in metabolism and immune defense. Intestinal microbes are closely related to health and affect maturation of the infant's intestinal epithelium (Becattini et al., 2021). The use of antibiotics is an important factor affecting the steady state of the intestinal flora. Antibiotics can cause microfloral disorder for a long time (Becattini et al., 2016). For example, beneficial *Lactobacillus* and *Bifidobacterium* decrease, and pathogenic bacteria, such as *Enterobacter*, which are usually resistant to β-lactam antibiotics (Hao et al., 2020).

Studies have shown that antibiotics are more commonly used in newborns, particularly preterm infants and low birth weight infants (Lebeaux et al., 2022). Statistics also show that ampicillin and gentamicin are used more than twice as often as other drugs in the neonatal care unit (Schwartz et al., 2020). Antibiotics are often used preventively in high-risk infants to prevent early neonatal streptococcal infections (Reyman et al., 2022). At the same time, premature low-birth-weight infants are susceptible to necrotizing enterocolitis, late-onset sepsis, and respiratory distress syndrome, which require immediate treatment with antibiotics (Aversa et al., 2021). One study reported that preterm infants treated with antibiotics have lower gut microbiota diversity (Zhou et al., 2021). Antibiotics delay the colonization time of various probiotics in the gut, leading to an increase in opportunistic bacteria, which makes the intestinal flora more likely to be disturbed (Maier et al., 2018). The duration of antibiotic use further affects the diversity of the intestinal flora (de Gunzburg et al., 2018). Exposure to antibiotics during early life increases the risk of obesity, allergies, and inflammatory bowel disease in adulthood (Vallianou et al., 2021).

The intestinal flora of infants is unstable due to incomplete maturity and is easily damaged by exogenous factors, such as antibiotics. It is worth understanding how to alleviate or restore disorder of the intestinal flora during early life caused by antibiotics.

Probiotics are being increasingly used. Studies have shown that probiotics enhance intestinal wall barrier function, balance the intestinal microecological environment, prevent pathogens from invading the intestinal wall through adhesion to the intestinal mucosal surface, and reduce the permeability of the intestinal wall (Wieërs et al., 2020; Almegrin et al., 2022). Additionally, other studies have shown that probiotic supplementation increases *Bifidobacteria* and *Lactobacillus*, and reduces the frequency of opportunistic pathogens, such as *E. coli*, *Enterococcus*, and *Klebsiella* (Lebeaux et al., 2021). These opportunistic pathogens often carry resistance genes (Shamsaddini et al., 2021). Therefore, increasing the colonization of probiotics in the gut reduces the resistance of the intestinal flora to antibiotics (Li et al., 2020).

China has a long history of eating fermented foods, the most representative of which is Paocai (fermented vegetables) in Chongqing and Sichuan Province as well as naturally fermented yogurt in ethnic minority areas. These fermented foods are prepared using natural inoculation. The fermentation process is a relatively open system, involving a wide variety of microorganisms (Zhao et al., 2022a). A relatively stable microflora gradually

Received 30 Oct., 2022
Accepted 22 Dec., 2022
[1] *Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University, Beijing, China*
*Corresponding author: yangzhennai@th.btbu.edu.cn

forms during long-term fermentation and domestication, and excellent probiotic resources are retained (Zhao et al., 2022b).

Therefore, this study investigated the effect of *Lactobacillus* isolated from Chinese fermented food on antibiotic-induced intestinal microflora disorder during the early life stages of mice. We also explored probiotic resources in food to improve antibiotic-induced intestinal microflora disorder.

2 Materials and methods

2.1 Experimental strains

The experimental strains were preserved at the Chinese General Microbiological Culture Collection Center (CGMCC, Beijing, China). *Lactobacillus fermentum* (LF) CQPC04 (CGMCC preservation no. 14493) was isolated from naturally fermented pickles in Chongqing, China. *Lactobacillus plantarum* (LP) KFY02 was isolated from naturally fermented yogurt in Korla from the Xinjiang Uygur Autonomous Region (CGMCC preservation no. 15638).

2.2 Animal experiment and sample collection

Forty 3-week-old male Balb/c mice were obtained from Hunan Slike Jingda Laboratory Animal Co., Ltd. [Animal Qualification License Number: SCXK (Xiang) 2019-0004]. The mice were maintained in a climate-controlled room (25 ± 2 °C, relative humidity 50 ± 5%) under a 12 h light/dark cycle with *ad libitum* access to standard chow and water. After 7 days of acclimatization, the mice were randomly and equally divided into 4 groups of 10 mice per group, including the normal, model, CQPC04, and KFY02 groups. As shown in Figure 1, the experiment lasted 4 weeks. The mice were administered 40 mg/kg ceftriaxone/day during the first week, except the normal group. The CQPC04 and the KFY02 groups were given 1×10^9 CFU/mL of the LF-CQPC04 and LP-KFY02 bacterial suspensions, respectively 2 hours later. The normal and model groups were administered saline (0.2 mL/mouse). The antibiotic intervention was stopped after the second week. The CQPC04 and KFY02 groups continued to receive the bacterial suspension, and the normal and model groups were given saline for 3 weeks.

The body weights of the mice were measured weekly. Feces were collected in individual sterile microcentrifuge tubes on days 7 and 28 and stored at −80 °C for further microbial analysis. This study was approved by the Ethics Committee of the Collaborative Innovation Center for Child Nutrition and Health Development, Chongqing University of Education, and followed the Collaborative Innovation Center for Child Nutrition and Health Development laboratory animal guidelines for ethical review of animal welfare.

2.3 Fecal DNA extraction and 16S rRNA sequencing

Bacterial genomic DNA was extracted from the feces using the FastPrep DNA extraction kit (QBIOGENE, Carlsbad, CA, USA). The polymerase chain reaction (PCR) amplification primers were universal primers (338F/806R) of the 16S rRNA gene V3–V4 region. The bacterial primer set included the forward primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR reaction system included 5 μL of 5× reaction buffer, 5 μL of 5× GC buffer, 2 μL of dNTP (2.5 mmol/L), 1 μL of the forward primer (10 μmol/L), 1 μL of the reverse primer (10 μmol/L), 2 μL of template, 0.25 μL of Q5 DNA polymerase, and 8.75 μL of ddH_2O. The amplification conditions were predenaturation at 98 °C for 2 min, denaturation at 98°C for 15 s, annealing at 55 °C for 30 s, 30 cycles of extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The amplified

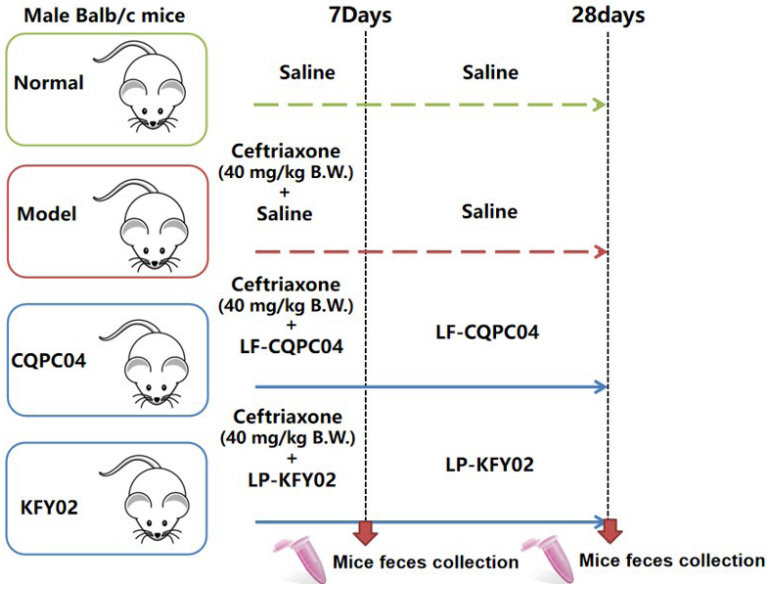

Figure 1. Animal experiment process. The mice were administered 40 mg/kg ceftriaxone/day during the first week, except the normal group (n = 10 mice/group). CQPC04: mice administered with 1×10^9 CFU/mL LF-CQPC04, KFY02: mice administered with 1×10^9 CFU/mL LP-KFY02. The normal and model groups were administered saline (0.2 mL/mouse).

products were analyzed by 2% agarose gel electrophoresis. The V3 and V4 regions of the PCR products were sequenced on the Illumina MiSeq PE 300 platform (Illumina, San Diego, CA, USA). The 16S rRNA sequence data were analyzed using the QIIME2-pipeline on the Majorbio online platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China).

2.4 Statistical analysis

Data are presented as mean ± standard deviation. Differences between mean values of individual groups were assessed with one-way analysis of variance and Duncan's multiple range test using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered significant.

3 Results and discussion

3.1 Changes in body weight of the mice

As shown in Figure 2, the body weights of the mice in the model group, the CQPC04 group, and the KFY02 group with the antibiotic intervention were significantly lower than those in the normal group during the first week ($p < 0.05$). The antibiotic intervention was discontinued after the second week, and the body weights of all mice gradually increased. The body weights of the mice in the CQPC04 and the KFY02 groups were similar to the normal group during the last week. The loss of body weight was observed immediately after administering the antibiotic, suggesting that exposure to an antibiotic early in life has an inhibitory effect on short-term weight gain. Our results suggest that the use of LF-CQPC04 and LP-KFY02 after antibiotic exposure may contribute to normal body weight gain.

Figure 2. Body weight changes of mice. Data are means ± standard deviations. CQPC04: mice administered with 1×10⁹ CFU/mL LF-CQPC04, KFY02: mice administered with 1 × 10⁹ CFU/mL LP-KFY02.

3.2 Microbial alpha diversity in mice feces

The alpha diversity of the mice feces is shown in Tables 1 and 2. A series of alpha diversity indices were evaluated to obtain the richness and diversity of the species in the mice feces. Among these, community richness was determined by the Ace index and the Chao index, bacterial diversity was determined by the Shannon and the Simpson indices, and community coverage was determined by the coverage data.

The Ace, Chao, and Shannon indices of the normal group increased significantly on day 7, and the Simpson index of the normal group decreased significantly compared with the other experimental groups (Table 1, $p < 0.05$). According to the alpha diversity results, community richness and bacterial diversity decreased significantly in the model group, the CQPC04 group, and the KFY02 group compared with the normal group after the antibiotic intervention ($p < 0.05$).

On day 28, community richness and bacterial diversity of all experimental groups increased significantly compared with day 7 (Table 2). However, the Ace, Chao, and Shannon indices of the CQPC04 group and the KFY02 group increased significantly compared with the model group ($p < 0.05$). The Shannon indices of the KFY02 group increased significantly compared with the normal group and the other experimental groups ($p < 0.05$). The Simpson index of the KFY02 group was similar to the

Table 1. The alpha diversity in mice feces on day 7.

Sample	Ace	Chao	Shannon	Simpson	Coverage
Normal	662.12 ± 93.85^{a}	624.44 ± 102.58^{a}	4.24 ± 0.90^{a}	0.13 ± 0.04^{b}	0.99930
Model	152.56 ± 23.89^{c}	151.18 ± 44.52^{c}	2.56 ± 0.56^{d}	0.43 ± 0.10^{a}	0.99969
CQPC04	450.77 ± 75.28^{b}	479.65 ± 73.53^{b}	3.92 ± 0.71^{c}	0.10 ± 0.09^{b}	0.99966
KFY02	446.38 ± 52.09^{b}	475.83 ± 91.55^{b}	4.02 ± 0.12^{b}	0.11 ± 0.02^{b}	0.99949

Data are means ± standard deviations. $^{a-d}$ Different superscript letters correspond to significant differences ($p < 0.05$). CQPC04: mice administered with 1 × 10⁹ CFU/kg LF-CQPC04, KFY02: mice administered with 1 × 10⁹ CFU/kg LP-KFY02.

Table 2. The alpha diversity in mice feces on day 28.

Sample	Ace	Chao	Shannon	Simpson	Coverage
Normal	752.20 ± 124.15^{a}	780.86 ± 126.26^{a}	4.83 ± 0.99^{b}	0.23 ± 0.09^{b}	0.99923
Model	212.83 ± 45.71^{d}	202.15 ± 52.66^{d}	1.31 ± 0.46^{d}	0.51 ± 0.11^{a}	0.99935
CQPC04	553.20 ± 83.51^{c}	671.75 ± 65.60^{b}	4.54 ± 1.01^{c}	0.19 ± 0.10^{c}	0.99975
KFY02	573.86 ± 84.97^{b}	624.44 ± 75.21^{c}	4.99 ± 0.78^{a}	0.23 ± 0.09^{b}	0.99932

Data are means ± standard deviations. $^{a-d}$ Different superscript letters correspond to significant differences ($p < 0.05$). CQPC04: mice administered with 1 × 10⁹ CFU/kg LF-CQPC04, KFY02: mice administered with 1 × 10⁹ CFU/kg LP-KFY02.

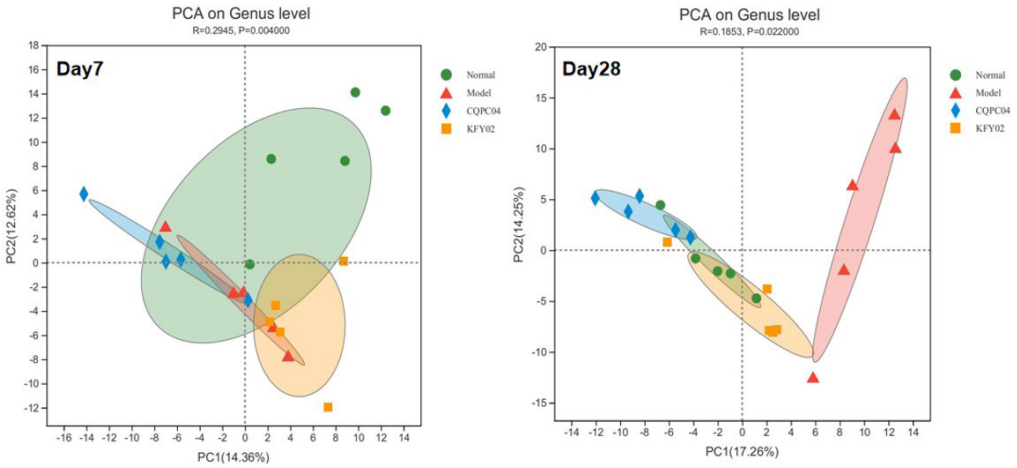

Figure 3. Principal component analysis (PCA) of mice feces. Each colored symbol represents the composition of fecal microbiota of one mice. CQPC04: mice administered with 1 ×10^9 CFU/mL LF-CQPC04, KFY02: mice administered with 1×10^9 CFU/mL LP-KFY02.

normal group, the Simpson index of the CQPC04 group was decreased significantly ($p < 0.05$).

Although the community diversity of the intestinal flora had self-recovery ability after being damaged by antibiotics, the composition of the microflora is difficult to return to normal. Therefore, it is suggested that antibiotics break the balance of the original flora and induce the growth of harmful bacteria in large numbers, resulting in increased community diversity indices (Zhou et al., 2021). In addition, the LF-CQPC04 and LP-KFY02 treatments helped recover the diversity of the bacterial community.

3.3 Microbial beta diversity in mice feces

The use of antibiotics leads to the disturbance of intestinal flora and changes in the type, number and proportion of normal intestinal flora, thus deviating from the normal physiological combination and transforming into a state of pathological combination (Li et al., 2020).

The beta diversity analysis compared the diversity between the groups to reflect whether there were significant differences in the microbial communities between samples. Beta diversity was determined by principal component analysis. In Figure 3, the CQPC04 and KFY02 group cluster was similar to the model group on day 7 but relatively separated from the normal group, indicating that the diversity of the gut microbiota in the antibiotic-induced mice tended to intestinal microflora disorder. On day 28 after the LF-CQPC04 and LP-KFY02 treatment, the CQPC04 and KFY02 group clusters were similar to the normal group but relatively separated from the model group, indicating that the diversity of the mice gut microbiota in the CQPC04 and KFY02 groups tended to be normal.

3.4 The relative abundance of the microbiota in mice feces on day 7

The gut microbiota flora composition on day 7 is shown in Figure 4. At the phylum level, the most dominant phyla in the normal group were *Firmicutes* and *Bacteroidetes*, followed by *Actinobacteria* and *Desulfobacterota*. The most dominant phyla in the CQPC04 and KFY02 groups were *Firmicutes* and *Bacteroidetes*, followed by *Proteobacteria* and *Actinobacteria*. At the same time, *Proteobacteria* and *Actinobacteria* significantly

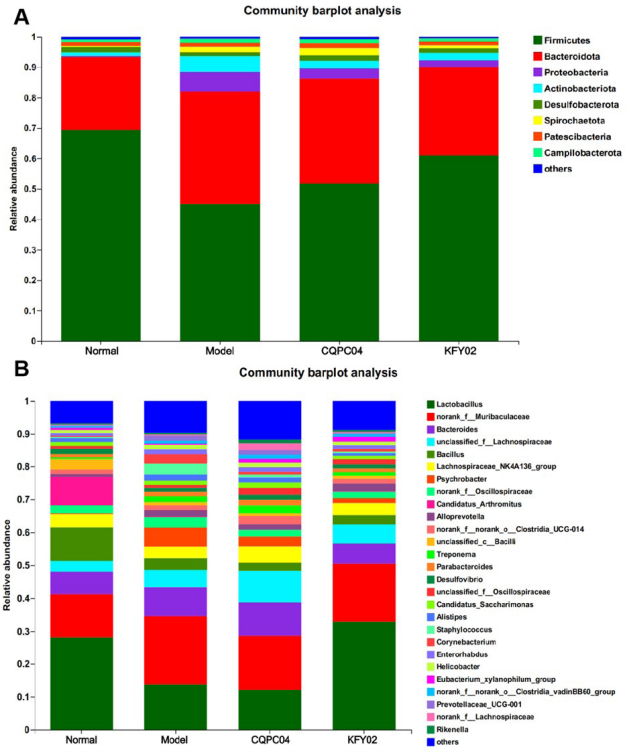

Figure 4. Effect of *Lactobacillus* on gut microbiota constipation of mice on day 7. (A) Relative abundance of microbiota at the phylum level; (B) relative abundance of microbiota at the genus level. CQPC04: mice administered with 1 × 10^9 CFU/mL LF-CQPC04, KFY02: mice administered with 1 × 10^9 CFU/mL LP-KFY02.

increased in the model group ($p < 0.05$), which included many pathogenic bacteria, such as *E. coli*, *Salmonella*, *Vibrio cholerae*, and *Helicobacter pylori*.

At the genus level, the abundance of *Lactobacillus* significantly decreased in the model and CQPC04 groups compared with the normal group ($p < 0.05$). The intestinal microecology of animals is unstable during early life and can be easily damaged by exogenous factors, such as antibiotics. Harmful bacteria increased in the antibiotic-induced groups, while beneficial bacteria decreased.

Antibiotics are usually divided into bactericidal (inhibits cell wall synthesis) and bacteriostatic (inhibits protein synthesis) types, but both inhibit or kill beneficial and pathogenic bacteria indiscriminately. This disruption of the flora can affect the functionality of the intestinal flora (Weersma et al., 2020).

The study of microbial species and colony structure, as well as the important role of microbial metabolism on host immunity, suggests that a relatively stable microbial community is important for the construction and response of the immune system. In contrast, the use of antibiotics leads to an increased chance of host infection with pathogenic bacteria and abnormal immune regulation, as a relatively healthy and stable gut prevents colonization and proliferation of exogenous pathogenic bacteria (Amoroso et al., 2020).

3.5 Relative abundance of microbiota in mice feces on day 28

The gut microfloral composition on day 28 is shown in Figure 5. After the LF-CQPC04 and LP-KFY02 treatments, at the phylum level, the CQPC04 and the KFY02 groups had a similar composition of gut flora as the normal groups. However, the abundance of *Firmicutes* and *Desulfobacterota* significantly increased, the abundance of *Bacteroidetes* significantly decreased in the model group ($p < 0.05$).

At the genus level, the abundance of *Desulfovibrio* significantly increased in the model group compared with the other experimental groups ($p < 0.05$). This genus is toxic to the intestinal epithelia and causes gastrointestinal disease (Amoroso et al., 2020). On the other hand, the composition of the gut flora in the CQPC04 and the KFY02 groups was close to that observed in the normal group. The abundance of *Muribaculaceae*, which plays a beneficial role in the energy metabolism of the intestine significantly increased in the CQPC04 and the KFY02 groups compared with the model group ($p < 0.05$). These results indicate that LF-CQPC04 and LP-KFY02 balanced the gut microbiota of the mice after it was damaged by antibiotics.

Compared to drug therapy, *Lactobacillus* preparations have great potential for research and application because they avoid the dysbiosis, proliferation of resistant strains and side effects of drugs caused by the use of antibiotics (Mantegazza et al., 2018).

As a Generally Recognized as Safe (GRAS) food-grade microorganism, *Lactobacillus* spp. is approved as a potential probiotic by the Chinese Ministry of Health, the U.S. Food and Drug Administration (FDA), and the European Union's Ministry of Food Safety and Health (EFSA) (Wieërs et al., 2021).

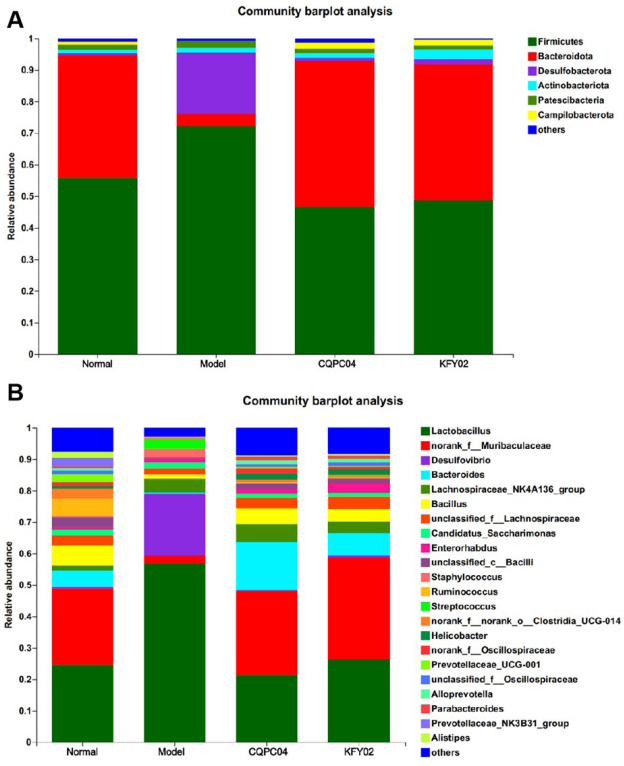

Figure 5. Effect of *Lactobacillus* on gut microbiota constipation of mice on day 28. (A) Relative abundance of microbiota at the phylum level; (B) relative abundance of microbiota at the genus level. CQPC04: mice administered with 1×10^9 CFU/mL LF-CQPC04, KFY02: mice administered with 1×10^9 CFU/mL LP-KFY02.

Lactobacillus proliferates and produces acid, which can limit the growth of other bacteria or impede the contact of pathogenic intestinal microorganisms and their toxins with the intestinal mucosal epithelium (Riehl et al., 2019). It can also antagonize other microorganisms such as conditionally pathogenic bacteria by producing bacteriocins, extracellular enzymes, short-chain fatty acids and competing for nutrients (Kim et al., 2019). The beneficial metabolites of *Lactobacillus* spp. include lactic acid, acetic acid, butyric acid and other organic acids, which can improve the biochemical and biophysical environment of the intestinal habitat, promote the growth of specific flora, optimize the intestinal flora, thus restore the dysregulated microecological environment to a normal state (Miles, 2020).

4 Conclusions

Exposure to antibiotics early in life inhibited weight gain, reduced intestinal floral diversity, inhibited the growth of beneficial gut bacteria, and promoted the growth of harmful gut bacteria. The use of LF-CQPC04 and LP-KFY02 alleviated the decrease in bacterial diversity caused by antibiotics, maintained the abundance of beneficial bacteria, and reduced the abundance of harmful bacteria. These results suggest that LF-CQPC04 and LP-KFY02 are useful probiotics to alleviate antibiotic-induced intestinal microfloral disorders.

Funding

This research was funded by the National Natural Science Foundation of China (31871823).

References

Almegrin, W. A., Yehia, H. M., Korany, S. M., Alkhateeb, M. A., Alahdal, H., Sonbol, H., Alkhuriji, A. F., & Elkhadragy, M. F. (2022). *In vitro* and *in vivo* evaluation of probiotic as immunomodulatory and anti-Campylobacter agent. *Food Science and Technology (Campinas)*, 42, e20322. http://dx.doi.org/10.1590/fst.20322.

Amoroso, C., Perillo, F., Strati, F., Fantini, M. C., Caprioli, F., & Facciotti, F. (2020). The role of gut microbiota biomodulators on mucosal immunity and intestinal inflammation. *Cells*, 9(5), 1234. http://dx.doi.org/10.3390/cells9051234. PMid:32429359.

Aversa, Z., Atkinson, E. J., Schafer, M. J., Theiler, R. N., Rocca, W. A., Blaser, M. J., & LeBrasseur, N. K. (2021). Association of infant antibiotic exposure with childhood health outcomes. *Mayo Clinic Proceedings*, 96(1), 66-77. http://dx.doi.org/10.1016/j.mayocp.2020.07.019. PMid:33208243.

Becattini, S., Sorbara, M. T., Kim, S. G., Littmann, E. L., Dong, Q., Walsh, G., Wright, R., Amoretti, L., Fontana, E., Hohl, T. M., & Pamer, E. G. (2021). Rapid transcriptional and metabolic adaptation of intestinal microbes to host immune activation. *Cell Host & Microbe*, 29(3), 378-393.e5. http://dx.doi.org/10.1016/j.chom.2021.01.003. PMid:33539766.

Becattini, S., Taur, Y., & Pamer, E. G. (2016). Antibiotic-Induced Changes in the Intestinal Microbiota and Disease. *Trends in Molecular Medicine*, 22(6), 458-478. http://dx.doi.org/10.1016/j.molmed.2016.04.003. PMid:27178527.

de Gunzburg, J., Ghozlane, A., Ducher, A., Le Chatelier, E., Duval, X., Ruppé, E., Armand-Lefevre, L., Sablier-Gallis, F., Burdet, C., Alavoine, L., Chachaty, E., Augustin, V., Varastet, M., Levenez, F., Kennedy, S., Pons, N., Mentré, F., & Andremont, A. (2018). Protection of the human gut microbiome from antibiotics. *The Journal of Infectious Diseases*, 217(4), 628-636. http://dx.doi.org/10.1093/infdis/jix604. PMid:29186529.

Hao, W. Z., Li, X. J., Zhang, P. W., & Chen, J. X. (2020). A review of antibiotics, depression, and the gut microbiome. *Psychiatry Research*, 284, 112691. http://dx.doi.org/10.1016/j.psychres.2019.112691. PMid:31791704.

Kim, S. K., Guevarra, R. B., Kim, Y. T., Kwon, J., Kim, H., Cho, J. H., Kim, H. B., & Lee, J. H. (2019). Role of probiotics in human gut microbiome-associated diseases. *Journal of Microbiology and Biotechnology*, 29(9), 1335-1340. http://dx.doi.org/10.4014/jmb.1906.06064. PMid:31434172.

Lebeaux, R. M., Coker, M. O., Dade, E. F., Palys, T. J., Morrison, H. G., Ross, B. D., Baker, E. R., Karagas, M. R., Madan, J. C., & Hoen, A. G. (2021). The infant gut resistome is associated with E. coli and early-life exposures. *BMC Microbiology*, 21(1), 201. http://dx.doi.org/10.1186/s12866-021-02129-x. PMid:34215179.

Lebeaux, R. M., Karalis, D. B., Lee, J., Whitehouse, H. C., Madan, J. C., Karagas, M. R., & Hoen, A. G. (2022). The association between early life antibiotic exposure and the gut resistome of young children: a systematic review. *Gut Microbes*, 14(1), 2120743. http://dx.doi.org/10.1080/19490976.2022.2120743. PMid:36289062.

Li, T., Teng, D., Mao, R., Hao, Y., Wang, X., & Wang, J. (2020). A critical review of antibiotic resistance in probiotic bacteria. *Food Research International*, 136, 109571. http://dx.doi.org/10.1016/j.foodres.2020.109571. PMid:32846610.

Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E. E., Brochado, A. R., Fernandez, K. C., Dose, H., Mori, H., Patil, K. R., Bork, P., & Typas, A. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, 555(7698), 623-628. http://dx.doi.org/10.1038/nature25979. PMid:29555994.

Mantegazza, C., Molinari, P., D'Auria, E., Sonnino, M., Morelli, L., & Zuccotti, G. V. (2018). Probiotics and antibiotic-associated diarrhea in children: A review and new evidence on *Lactobacillus rhamnosus* GG during and after antibiotic treatment. *Pharmacological Research*, 128, 63-72. http://dx.doi.org/10.1016/j.phrs.2017.08.001. PMid:28827186.

Miles, M. P. (2020). Probiotics and gut health in Athletes. *Current Nutrition Reports*, 9(3), 129-136. http://dx.doi.org/10.1007/s13668-020-00316-2. PMid:32451960.

Reyman, M., van Houten, M. A., Watson, R. L., Chu, M., Arp, K., de Waal, W. J., Schiering, I., Plötz, F. B., Willems, R. J. L., van Schaik, W., Sanders, E. A. M., & Bogaert, D. (2022). Effects of early-life antibiotics on the developing infant gut microbiome and resistome: a randomized trial. *Nature Communications*, 13(1), 893. http://dx.doi.org/10.1038/s41467-022-28525-z. PMid:35173154.

Riehl, T. E., Alvarado, D., Ee, X., Zuckerman, A., Foster, L., Kapoor, V., Thotala, D., Ciorba, M. A., & Stenson, W. F. (2019). *Lactobacillus rhamnosus* GG protects the intestinal epithelium from radiation injury through release of lipoteichoic acid, macrophage activation and the migration of mesenchymal stem cells. *Gut*, 68(6), 1003-1013. http://dx.doi.org/10.1136/gutjnl-2018-316226. PMid:29934438.

Schwartz, D. J., Langdon, A. E., & Dantas, G. (2020). Understanding the impact of antibiotic perturbation on the human microbiome. *Genome Medicine*, 12(1), 82. http://dx.doi.org/10.1186/s13073-020-00782-x. PMid:32988391.

Shamsaddini, A., Gillevet, P. M., Acharya, C., Fagan, A., Gavis, E., Sikaroodi, M., McGeorge, S., Khoruts, A., Albhaisi, S., Fuchs, M., Sterling, R. K., & Bajaj, J. S. (2021). Impact of antibiotic resistance genes in gut microbiome of patients with cirrhosis. *Gastroenterology*, 161(2), 508-521.e7. http://dx.doi.org/10.1053/j.gastro.2021.04.013. PMid:33857456.

Vallianou, N., Dalamaga, M., Stratigou, T., Karampela, I., & Tsigalou, C. (2021). Do Antibiotics cause obesity through long-term alterations in the gut microbiome? A review of current evidence. *Current Obesity Reports*, 10(3), 244-262. http://dx.doi.org/10.1007/s13679-021-00438-w. PMid:33945146.

Weersma, R. K., Zhernakova, A., & Fu, J. (2020). Interaction between drugs and the gut microbiome. *Gut*, 69(8), 1510-1519. http://dx.doi.org/10.1136/gutjnl-2019-320204. PMid:32409589.

Wieërs, G., Belkhir, L., Enaud, R., Leclercq, S., Philippart de Foy, J. M., Dequenne, I., de Timary, P., & Cani, P. D. (2020). How probiotics affect the microbiota. *Frontiers in Cellular and Infection Microbiology*, 9, 454. http://dx.doi.org/10.3389/fcimb.2019.00454. PMid:32010640.

Wieërs, G., Verbelen, V., Van Den Driessche, M., Melnik, E., Vanheule, G., Marot, J. C., & Cani, P. D. (2021). Do probiotics during in-hospital antibiotic treatment prevent colonization of gut microbiota with multi-drug-resistant bacteria? A randomized placebo-controlled trial comparing saccharomyces to a mixture of *Lactobacillus, Bifidobacterium*, and *Saccharomyces. Frontiers in Public Health*, 8, 578089. http://dx.doi.org/10.3389/fpubh.2020.578089. PMid:33763399.

Zhao, X., Hu, R., He, Y., Li, S., Yang, J., Zhang, J., Zhou, J., & Xue, T. (2022a). Screening of isolated potential probiotic lactic acid bacteria from Sichuan pickle for cholesterol lowering property and triglycerides lowering activity. *Food Science and Technology (Campinas)*, 42, e09122. http://dx.doi.org/10.1590/fst.09122.

Zhao, X., Mu, J., & Yi, R. (2022b). Research progress of naturally fermented yogurt with lactic acid bacteria in Xinjiang: a review of anti-constipation probiotics. *Food Science and Technology (Campinas)*, 42, e23722. http://dx.doi.org/10.1590/fst.23722.

Zhou, Y., Ma, W., Zeng, Y., Yan, C., Zhao, Y., Wang, P., Shi, H., Lu, W., & Zhang, Y. (2021). Intrauterine antibiotic exposure affected neonatal gut bacteria and infant growth speed. *Environmental Pollution*, 289, 117901. http://dx.doi.org/10.1016/j.envpol.2021.117901. PMid:34371267.