

Antagonistic Activities of *Pediococcus Pentosaceus* Against *Pseudomonas Aeruginosa* Growth

Yaoting Xiao^{1#}, Yuetong Li^{2#}, Fengxia Zhang², Zhengliang Chen², Liquan Tang¹, Jianzhou Li^{1,2,3}, Xiaohua Chen^{1,2,3*}

¹ Nanyue College of Hengyang Normal University, Hengyang, 421008, P.R. China

² College of Life Sciences and Environment, Hengyang Normal University, Hengyang, 421008, P.R. China

³ Hunan Key Laboratory for Conservation and Utilization of Biological Resources in the Nanyue Mountainous Region, 421008, P.R. China



Accepted Apr 15, 2021
Published Apr 18, 2021

*Corresponding Author:
Xiaohua Chen

These authors contributed
equally to this work.

DOI: <https://doi.org/10.5281/zenodo.4699390>

Pages: 130-137

Funding: Nanyue College of
Hengyang Normal University

Distributed under
Creative Commons CC BY 4.0

Copyright: © The Author(s)

How to cite this article (APA):
Xiao, Y., Li, Y., Zhang, F., Chen, Z., Tang, L., Li, J., & Chen, X.. (2021). Antagonistic Activities of *Pediococcus Pentosaceus* Against *Pseudomonas Aeruginosa* Growth. *North American Academic Research*, 4(4), 130-137. doi: <https://doi.org/10.5281/zenodo.4699390>

Conflicts of Interest

There are no conflicts to declare.

ABSTRACT

Pediococcus pentosaceus is a kind of beneficial lactic acid bacteria (LAB), which can inhibit foodborne pathogens, regulate the intestinal immune function, reduce cholesterol level, anti-tumor, inhibit microorganisms and other probiotic functions. In this study, *Pseudomonas aeruginosa* was used as the indicator bacteria to investigate the mechanism of the antagonistic effect of *P. pentosaceus* (Rsp5 and Rsp6) against *P. aeruginosa* by methods of test the antibacterial ability, pyocyanin expression, biofilm formation, hydrophobicity and auto-aggregation of *P. pentosaceus* and co-aggregation ability between the two strains. The results showed that two strains of *P. pentosaceus* (Rsp5 and Rsp6) had a significant antagonistic ability against *P. aeruginosa*, but the antagonistic mechanism was different. Rsp5 can inhibit the growth of *P. aeruginosa* (inhibition zone is 2.013 ± 0.194 cm), inhibit biofilm formation (inhibition rate is about 55%), and reduce the infectivity of *P. aeruginosa* through its own co-agglutination with *P. aeruginosa* PAO1. Rsp6 can inhibit the growth of *P. aeruginosa* (inhibition zone is 1.907 ± 0.272 cm), the expression of pyocyanin (inhibition rate is about 47%) and biofilm formation (inhibition rate is about 60%). This study will provide a research basis to prevent *P. aeruginosa* by LAB.

Keywords: *PEDIOCOCCUS PENTOSACEUS*, *PSEUDOMONAS AERUGINOSA*, ANTAGONISTIC, BIOFILM

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*), also known as *Bacillus aeruginosus*, is a typical opportunistic pathogen, easily leading to

respiratory and urinary tract infection and even septicaemia (KALIL, 2016). In the meantime, it is also a significant foodborne pathogen as well. Its virulent factors may cause food poisoning and drinking water pollution, resulting in dizziness, vomiting and diarrhea (Yan Fang, 2011; Shen Ying, 2010). Moreover, this pathogen often results in the development of multidrug resistance and the failure of antibiotic therapy (Ding ye,

2020). In 2017, the World Health Organization classified carbapenem-resistant *P. aeruginosa* as one of the bacteria among the bacteria that urgently need to develop new anti-bacterial drugs. Therefore, developing new antibiotics and looking for natural antibacterial replacements are ideal options for the treatment of *P. aeruginosa*. Probiotic lactic acid bacteria have the characteristics of high efficiency, non-toxicity, high temperature resistance, no residue and no drug resistance, and can inhibit a variety of bacteria, fungi and even viruses.

Pediococcus pentosaceus (*P. pentosaceus*) is a kind of lactic acid bacteria (LAB), which is Gram-positive bacteria and often used in fermented products. It is also a kind of probiotics with the functions of improving nutritional levels, breaking down toxins, inhibiting the growth of pathogenic bacteria (Cao Zhenhui, 2016). Currently, numerous researchers have been investigating the antagonistic effect of lactic acid bacteria on *P. aeruginosa*. For example, Zhu et al. determined the diameter of the inhibition zone depending on the growth conditions of the LAB strains, such as enzymatic treatment, initial pH, temperature, culture time, initial NaCl concentration, and so on, to investigate the antibacterial effect of LAB strains on *P. aeruginosa* (Zhu Yinglian, 2019). Shikha Rana et al. used the LAB strains (*Lactococcus lactis*, *Lactobacillus fermentum* and *Lactobacillus rhamnosus*) supernatant to inhibit the expression of AHL signal molecules produced by *P. aeruginosa* PAO1, thus inhibiting elastase activity and reducing biofilm formation of *P. aeruginosa* (RANA, 2020). Chappell discovered that engineered lactobacilli show anti-biofilm and growth suppression activities against *P. aeruginosa* (CHAPPELL, 2020). These studies have shown that LAB can inhibit *P. aeruginosa*, but the mechanism is unclear. Therefore, the aim of the present study was to explore the antagonistic mechanism of *P. pentosaceus* against *P. aeruginosa*.

Materials and methods

1.1 Bacterial strains and culture media

Two strains of *P. pentosaceus* (Rsp5 and Rsp6) were isolated from fermented soybean curd in Hunan province. *P. aeruginosa* PAO1 was preserved in Hunan Key Laboratory for Conservation and Utilization of Biological Resources in the Nanyue Mountainous Region, China. MRS medium (Qingdao Haibo, China) was used to culture LAB strains, and LB medium (Qingdao Haibo, China) was used to culture *P. aeruginosa* PAO1.

1.2 Bacterial growth conditions and preparation of Supernatants

The LAB strains Rsp5 and Rsp6 were inoculated in MRS liquid medium at 37°C for 18 h. *P. aeruginosa* PAO1 was inoculated in LB liquid medium at 37°C for 18 h. Subsequently, these cultures were centrifuged at 6,000 rpm for 10 min, then the supernatants were filtered through 0.22 µm sterile membrane filters and stored in an ultra-low temperature refrigerator at - 80°C until analysis.

1.3 Preparation of co-cultivation Supernatants

The supernatants of LAB strains, MRS medium, LB medium and *P. aeruginosa* were added to 10⁶ CFU/mL *P. aeruginosa* culture at a ratio of 1:1. After 24 h of culture, the supernatant was centrifuged at 6,000 rpm for 10 min and the supernatant was filtered with a 0.22 µm sterilized filter to obtain sterile co-culture supernatant, which was stored in an ultra-low temperature refrigerator at - 80°C until analysis.

1.4 Determination of antibacterial activities of LAB strains

The antibacterial activities of the LAB strains were tested using Chappell's method (Chappell, 2020). and slightly modified as follows: *P. aeruginosa* cultures were plated on fresh LB agar plates (10^6 CFU per plate), and 6 mm discs were put into the plates with *P. aeruginosa*. Depending on the experimental design, 200 μ L aliquots of fresh *Lactobacillus* strains cultures were suspended into the discs. Plates were incubated for 24 to 48 h under microaerophilic conditions at 37°C, and the diameters of inhibition zones around the wells were measured. Results were expressed as a mean diameter and standard error. MRS medium (pH 4.0) was used as negative control.

1.5 Determination of pyocyanin

Pyocyanin is a fat-soluble compound, which can be extracted with chloroform, acidified with hydrochloric acid, and then removed from the hydrochloric acid layer. The OD value measured at the maximum absorption wavelength can indicate the content of Pyocyanin (RANA, 2020; Wang Liangcai, 2017). With reference to Lou et al. (Lou, 2017), the details are as follows: Pyocyanin was extracted with chloroform (3 mL) from the freeze-dried supernatant (2 mL). Then, the chloroform layer was acidified with 0.2 mol/L HCl (1 mL). The acid layer that contained pyocyanin was separated and quantified by recording OD 520.

1.6 Determination of biofilm formation

The determination of biofilm was determined according to Zhang Lidong's method (Zhang Lidong, 2016) and slightly modified as follows: *P. aeruginosa* PAO1 was inoculated in LB medium at 1% and incubated at 37°C for 18 h. Then *P. aeruginosa* medium was diluted with LB medium at a ratio of 1:100 and fully shaken for standby. After the LAB supernatant was mixed with the diluted *P. aeruginosa* PAO1 inoculum at a volume ratio of 1:100, 200 μ L mixture incubated for 18 h at 37°C in 96-well flat bottom polystyrene plates. Then the bacterial liquid was poured out, washed with PBS, dried and stained with 0.1% crystal violet. After incubating for 30 min at room temperature, the stain was removed by washing thrice with 200 μ L PBS. The stain was solubilized by adding 95% ethanol to each well by incubating it with ethanol for 10-15 min at 4°C. The crystal violet/ethanol solution measured at OD 600 nm by multi-functional microplate reader. The experiment was carried out in triplicates. Coculture of MRS and *P. aeruginosa* was used as the positive control. The cell membrane inhibition rate was determined using the following formula:

$$\text{Biofilm inhibition rate (\%)} = (\text{OD}_{\text{MRS+PA}} - \text{OD}_{\text{LAB+PA}}) / \text{OD}_{\text{MRS+PA}} \times 100$$

$\text{OD}_{\text{MRS+PA}}$ indicates that the co- culture of MRS and *P. aeruginosa* is the measured at OD 600 nm;

$\text{OD}_{\text{LAB+PA}}$ indicates that co-culture of various LAB strains with *P. aeruginosa* is the measured at OD 600 nm.

1.7 Auto-aggregation and co-aggregation assay

After the LAB strains and *P. aeruginosa* were activated and cultured, centrifuged at 4000 rpm for 10 min, washed with PBS buffer, OD 600 nm was adjusted to 0.50 ± 0.05 in order to standardize the number of bacteria (10^7 - 10^8 CFU/ mL). The lactic acid bacteria and *P. aeruginosa* were mixed in equal amount, oscillated for 5

min, and were incubated at 37°C for 2 h. The OD 600 nm absorption value of the upper liquid at different time was determined.

$$\text{co-aggregation rate (\%)} = \frac{(A_x + A_y) - 2 \times A_t}{A_x + A_y} \times 100$$

A_x indicates the absorbance value of lactic acid bacteria at 0 h; A_y indicates the absorbance value of *P. aeruginosa* at 0 h; A_t indicates the absorbance value of the mixture after 2 h.

Auto-aggregation was measured as above. Briefly, cells from an 18 h culture of the LAB strains washed by PBS and OD 600 was adjusted to 0.50 ± 0.05 . Then, the lactic acid bacteria suspensions were incubated in aliquots at 37°C for 1 h and absorbance value of the upper liquid was measured at OD 600 nm.

$$\text{auto-aggregation rate (\%)} = \frac{A_0 - A_t}{A_0} \times 100 \%$$

A_0 indicates the absorbance value at 0 h; A_t indicates the absorbance value at 1 h.

1.8 Hydrophobicity

Cells were washed once with PBS and resuspended in the same buffer to an OD 600 nm of approximately 0.50 ± 0.05 to standardize, and an equal volume of xylene was added. The 2-phase system was thoroughly mixed by vortexing for 5 min. The aqueous phase was removed after 1 h of incubation at room temperature and its OD 600 nm was measured. Affinity to hydrocarbons (hydrophobicity) was reported as the adhesion percentage according to the following formula:

$$\text{Hydrophobicity (\%)} = \frac{A_0 - A_t}{A_0} \times 100 \%$$

A_0 indicates the absorbance value at 0 h; A_t indicates the absorbance value at 1 h.

1.9 Statistical analysis

All analyses were conducted in triplicate and the results were statistically analyzed by computing means and standard deviations of the mean. Differences between means of the test and control groups were evaluated by the Dunnett's test, and $P < 0.05$ was considered significantly different by one-way ANOV and SPSS 20 (SPSS Inc., Chicago, IL).

Results and Analysis

2.1 Inhibition growth of *P. aeruginosa*

The metabolites of LAB have antibacterial effect, because hydrogen oxide, acetic acid, lactic acid, other organic acids, bacteriocin and other substances that have antibacterial effect may be produced in the metabolic process of LAB. Liu Jun et al.(2015) isolated *P. pentosaceus* from the intestines of fish and shrimp, which has different degrees of inhibition on *Bioluminobacter mermaidens*, *Escherichia coli*, *Vibrio alginolyticus*, *P. aeruginosa*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, etc. Zhu et al. (2019) isolated 6 strains of LAB from fermented food, among which 3 strains showed good inhibitory effect against *Pseudomonas*, and the lactobacillin produced had good heat resistance and showed good antibacterial effect under acidic conditions. In order to exclude the influence of acid on antibacterial effect, this study took the antibacterial effect of MRS at pH 4.0 as negative control. It can be seen from Figure 1 that the supernants of the two strains of *P. pentosaceus* had

significant inhibitory effect on *P. aeruginosa*, indicating that the metabolites of the two strains may have substances with antibacterial effect other than acid.

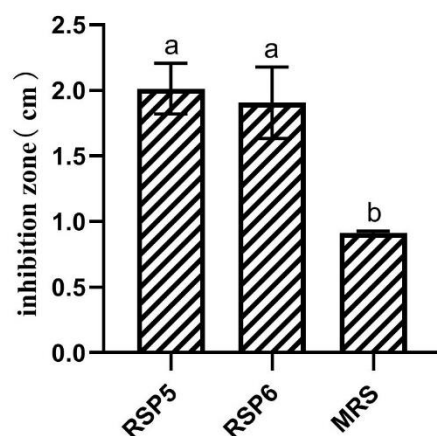


Fig. 1 Inhibition of *P. aeruginosa* growth by *P. pentosaceus*

2.2 Effect of *P. pentosaceus* on pyocyanin production

Pyocyanin is an important virulence factor of *P. aeruginosa*, which is closely related to the pathogenicity and infectivity of *P. aeruginosa* (Chen Yang, 2009). Pyocyanin is an active secondary metabolite, which can penetrate the biofilm, interrupt the respiratory chain, produce a large number of oxygen free radicals and hydrogen peroxide, and lead to cell death (Chai Wenshu, 2008). Tang (2017) and other studies have shown that different concentrations of pyocyanin can promote the growth of *P. aeruginosa* to a certain extent. Inhibition of pyocyanin production can inhibit the growth of *P. aeruginosa*, so as to reduce the toxicity of *P. aeruginosa* and inhibit its infection. Figure 2 showed that Rsp6 can significantly inhibit the expression of *P. aeruginosa*, while Rsp5 can upregulate the expression of *P. aeruginosa*, and MRS had little effect on the expression of *P. aeruginosa*.

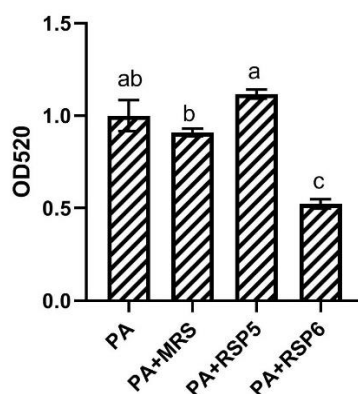


Fig. 2 Inhibition of pyocyanin expression by *P. pentosaceus*

2.3 Effect of LAB on *P. aeruginosa* biofilm

The formation of *P. aeruginosa* biofilm is closely related to its drug resistance. Biofilm can prevent *P. aeruginosa* from direct contact with antibiotics, thus improving *P. aeruginosa* resistance to antibiotics (GROSSOVA, 2017). In this study, coculture of MRS and *P. aeruginosa* was used as the positive control. Figure 3 showed that both strains of *P. pentosaceus* can inhibit the biofilm formation of *P. aeruginosa*, but there

is no significant difference.

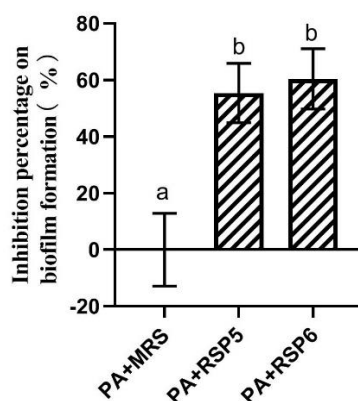


Fig. 3 Inhibition of *P. aeruginosa* biofilm formation by *P. pentosaceus*

2.4 Analysis of hydrophobicity, auto-aggregation and co-aggregation

The LAB adhere to and colonize the surface of intestinal epithelial cells and cause morphological, functional, physiological and biochemical changes, such as forming biofilm with other anaerobic bacteria to protect intestinal mucosa from the invasion of pathogenic bacteria. Meanwhile, the LAB inhibit the growth and reproduction of pathogenic bacteria by producing organic acid hydrogen peroxide so as to play the role of biological barrier and affect cell adhesion. The main factors affecting cell adhesion and colonization are hydrophobicity and auto-aggregation (Gong Hong, 2016). The auto-aggregation of bacterial strains refers to the phenomenon that the same bacteria aggregation each other to form multicellular clusters. The aggregation of LAB can form a barrier to prevent the colonization and infection of pathogens. Table 1 showed that RSP5 has a stronger aggregation ability than RSP6, while its hydrophobicity is weaker than RSP6.

Some products of *P. aeruginosa* can lead to cell adhesion and interact with virulence factors to increase the virulence and pathogenicity during infection (GORDON, 1984). If LAB can reduce the adhesion of *P. aeruginosa* to some extent through the co-aggregation between the bacteria and *P. aeruginosa*, it can reduce the infection of *P. aeruginosa*. Therefore, co-aggregation between probiotics and pathogens is considered to be a method to exclude pathogenic bacteria (RMLING, 2013). As can be seen from Table 1, the co-aggregation ability of RSP5 is stronger than that of RSP6. This result indicated that co-aggregation ability of RSP5 can improved its antagonistic against *P. aeruginosa*.

Table 1 Analysis of hydrophobicity, auto- aggregation and co-aggregation ability of the LAB with *P. aeruginosa*

strains	co- aggregation rate (%)	auto- aggregation rate (%)	hydrophobicity (%)
RSP5	15.80±1.91 ^a	8.62±0.32 ^a	5.48±0.21 ^b
RSP6	7.3±0.36 ^b	2.30±1.98 ^b	11.05±2.22 ^a

Conclusion

Both *P. pentosaceus* RSP5 and RSP6 could significantly inhibit the growth of *P. aeruginosa*, but the antagonistic mechanisms were different among strains. Rsp5 can inhibit the growth of *P. aeruginosa*, inhibit the biofilm formation of *P. aeruginosa*, and reduce the infectivity of *P. aeruginosa* by co-aggregation, but cannot inhibit the expression of pyocyanin. Rsp6 can inhibit the growth of *P. aeruginosa*, inhibit the

expression of pyocyanin and biofilm formation of *P. aeruginosa*. This study will provide theoretical support for the application of *P. pentosaceus* as probiotics in the prevention of *P. aeruginosa*.

Acknowledgements

This research was partly supported by Hunan Provincial National science Foundation of China (No.2019JJ50014), National Natural science foundation of China (No. 31601444), Scientific Research Fund of Hunan Provincial Education Department (No.15B034, 18C0667), Hunan Provincial University Students' Research Study and Innovative Experiment Plan Project (Xiangjiaotong [2018] 255:776), Hengyang Normal University Extracurricular Academic Technology Innovation Fund Project (Xiaokeyi 2019, No. 2-8) , Hengyang Normal University Innovation and Entrepreneurship Training Program Project (cxxy1934), The research supported by Hengyang science and technology project (2014KN54), The Open Fund Project of Key Laboratory in Hunan Universities, and Application-Oriented Characterized Disciplines, Double First-Class University Project of Hunan Province (Xiangjiaotong [2018] 469).

References

- KALIL A C, METERSKY M L, KLOMPAS M, et al. 2016. *Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society*. Clin Infect Dis, 63(5): e61-e111.
- Yan Fang, Sui Yingjie, Sun Jing, et al. 2011. *Investigation and analysis of food poisoning in students caused by the pollution of barreled drinking water*. Chinese Journal of health laboratory technology, 21(8): 2082-2084.
- Shen Ying, Yang Zhenglin, Yue Feng. 2010. *Detection of Pseudomonas aeruginosa in food poisoning samples*. Zhejiang preventive medicine, 22 (03): 45-46.
- Ding ye, Dai Lu, Yu Juan. 2020. *Progress of research on treatment of Pseudomonas aeruginosa infection*. Chinese Journal of nosocomial infection, 30:955-960.
- Cao Zhenhui, Pan Hongbin, Zhang Xianyan, et al. 2016. *The probiotic function of P. pentosaceus and its application in food science*. Journal of Anhui agricultural sciences, 44 (09): 106-108.
- Zhu Yinglian, Wang Peng, Sun Jingxin, et al. 2019. *Antibacterial effect of Lactobacillus on Pseudomonas aeruginosa*. Journal of Qingdao Agricultural University (Natural Science), 36:142-146.
- RANA S, BHAWAL S, KUMARI A, et al. 2020. *pH-dependent inhibition of AHL-mediated quorum sensing by cell-free supernatant of lactic acid bacteria in Pseudomonas aeruginosa PAO1*. Microb Pathog, 142: 104105.
- CHAPPELL T C, NAIR N U. 2020. *Engineered lactobacilli display anti-biofilm and growth suppressing activities against Pseudomonas aeruginosa*. NPJ Biofilms Microbiomes, 6(1): 48.
- Wang Liangcai, Jiang liming, Kang Ziteng, et al. 2014. *The Research of Expression Pattern and Function of Lactobacillus plantarum AY01 luxS Gene*. Life sciences research, 18:199-204.

- LOU Z, WANG H, TANG Y, et al. 2017. *The effect of burdock leaf fraction on adhesion, biofilm formation, quorum sensing and virulence factors of Pseudomonas aeruginosa*. J Appl Microbiol, 122(3): 615-624.
- Zhang Lidong, Dang Fangfang, Zhao Siyu, et al. 2016. *Study of the influence of factors for Lactobacillus biofilm formation*. China dairy industry, 44 (10): 4-6, 14.
- Liu Jun, Lin Junfang, Guo Liqiong, et al. 2015. *Diversity and antimicrobial activity of lactic acid bacteria isolated from aquaculture*. Fisheries Science, 34 (06): 351-357.
- Chen Yang. 2009. *The garlic extracts block the quorum sensing-controlled virulence factor production in Pseudomonas aeruginosa*. Huazhong University of Science and Technology.
- Chai Wenshu. 2008. *Study on Pseudomonas aeruginosa and pyocyanin induced immunity reaction of human monocytic U937 cells and lung infection in rates*. China Medical University.
- Tang Yunpeng. 2017. *Effects of pyocyanin on the growth and biofilm formation of foodborne bacteria: a preliminary study*. Central South University of Forestry and Technology.
- GROSSOVA M, RYSAVKA P, MAROVA I. 2017. *Probiotic biofilm on carrier surface: A novel promising application for food industry*. Acta Alimentaria, 46(4): 439-448.
- Gong Hong, Wang Haixia, Ma ZhengTu, et al. 2016. *Biofilm hydrophobicity and auto-agglutination properties of five Lactobacillus strains*. Chinese Journal of Microbiology, 28 (09): 1026-1028.
- GORDON A S, MILLERO F J. 1984. *Electrolyte Effects on Attachment of an Estuarine Bacterium*. Applied and Environmental Microbiology, 47(3): 495-499.
- RMLING U, GALPERIN M Y, GOMELSKY M. 2013. *Cyclic di-GMP: the First 25 Years of a Universal Bacterial Second Messenger*. Microbiology & Molecular Biology Reviews, 77(1): 1-52.



© 2021 by the authors. Author/authors are fully responsible for the text, figure, data in above pages. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>)

