



## Antagonist effect of *Lactococcus* on *Pseudomonas aeruginosa* isolated from milk and curd

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### Abstract

*Lactococcus* is a Gram-positive bacteria generally used as a probiotic and its antimicrobial properties are well studied. *Pseudomonas* is one of the deadly pathogens for which vaccination was not available till now. *Lactococcus* is the nonpathogenic strain and hence the present study was with the antimicrobial activity of the genus with *Pseudomonas aeruginosa*, *Actinomyces*, *S.typhii*, and *paratyphii*, and *E.coli*. *Lactococcus* is shown to effective against *Pseudomonas aeruginosa* but not specific to gram-negative bacilli as the zone of inhibition is not observed with *E.coli*.

**Keywords** *Lactococcus*, *Lactobacillus*, Probiotic, Antimicrobial activity, and Antibiotics

### Introduction

*Lactococcus* is one of the lactic acid bacteria used as a probiotic. The effectiveness of *lactococcus* is due to its survival against the low PH of the stomach. The bacteria have to transverse the mucosal epithelium to enter into blood circulation. The strain did not produce carcinogenicity due to the lack of  $\beta$ - Glucuronidase enzyme. *Lactococcus* has regraded under GRAS by U.S food and Drug Administration in 1984 under sec 184 (Holzapfel et al., 2001; Felis and Dellaglio, 2007).

*Lactococcus* has no sensitivity against antibiotics over Nalidixic acid and has the highest sensitivity with antibiotics like Tetracycline, clindamycin, and Ampicillin. *Lactococcus* is a gram-positive, non-motile, non-sporulating, and cocc-shaped bacteria used in the dairy industry for curd production. Three species come under *Lactococcus* genus like *L.raffinolactis*, *L.lactis* subsp. and *L.lactis* subsp.lactis in which *L.lactis* is used as a probiotic (Yerlikaya, 2019; Boyle et al., 2006).

Probiotics are the live microorganisms applied for diary supplements to improve the health benefits of the organism. According to Food and Agriculture Organization of the United Nations, World Health Organization Probiotics are live

organisms when administered in adequate amounts promote health benefits on the host. *Lactococcus* and *Bifidobacterium* are the two genera used as probiotics spp. *Lactococcus lactis* is strictly not considered as a probiotic (Lee et al., 2015; Wessels et al., 2004; Jung et al., 2020).

The present study deals with the isolation of *Lactococcus* from milk and curd and the study of its antagonistic effect on five pathogenic strains of bacteria and one fungal strain *A.oryzae*.

### Methods

#### Assay of antimicrobial activity of *lactococcus* by plate assay

#### Isolation of *lactococcus* from milk and curd

Tirumala and Arokya milk products were tested for the bacterial strain used for the production of curd. Gram staining was used to know the structure and characteristics of the bacterial strain used.

#### Serial dilution

1ml of milk and curd was transferred to 10 ml autoclaved water and 1ml from the 10ml suspension is successively transferred for dilution of the number and  $10^{-8}$  and  $10^{-9}$

dilutions were used for spread plate and kept for 24 hr incubation and 48 hr incubation for the growth of bacteria and fungi. Nutrient Agar and Potato dextrose agar is used for the cultivation of bacteria and fungi (Dimri et al., 2018; Singh et al., 2012)

### Maintainance of cultures

After confirmation of bacterial spp and fungi in nutrient agar plates, slants of the pure culture were maintained in refridgerator and used for antimicrobial assay on pathogenic strains.

### Assay

Broth cultures of one-day-old lactococcus cultures were spread on the sterilized nutrient agar plates and spread evenly with the help of L-rod and wells are made with sterile autoclaved tips and each pure strain of 10µl was added and a zone of inhibition was observed after 24 hrs of incubation.

### Methylene blue staining for Yeast

Methylene blue staining of yeast was performed to extent of contamination in milk and curd. 0.01 g methylene is dissolved in 10ml of distilled water and 2g of sodium citrate is added and filtered. The filtered solution was made up of 100ml and used as a stain. Equal volumes of methylene blue and the cell culture were taken on the glass slide and a smear was made and examined under a light microscope.

### Results

From Fig.1 *Lactococcus* was isolated from the milk and curd. Gram staining of the bacteria confirmed *Lactococcus* and *Lactobacillus* as gram-positive bacteria and the milk and curd were found to be contaminated with yeasts to a minimum due to anaerobic conditions whereas the quality of milk was fair enough because of the absence of contamination by other bacteria and fungi.

Methylene blue staining was performed for detection of yeast and other fungal strains like *Fusarium spp.*, *Alternaria spp.*, *Aspergillus fumigatus*, *Candida albicans*, *non albicans Candida* and *Aspergillus flavus* (Moemen et al., 2015). But none of the strains were observed in milk or curd except yeast.

*Lactobacillus* are responsible for various diseases in immune-compromised people like Endocarditis, Neonatal meningitis, Bacteremia, Liver abscess, Pancreatic necrosis infection, Pulmonary infection, Dental caries, Intraabdominal abscesses etc. (Sherid et al., 2016) hence *Lactococcus* strain serves as one of the probiotics for pathogenic diseases caused by other bacterial species. *Lactococcus* is non-pathogenic and proved to be effective as a probiotic than *lactobacillus* even in immune-compromised patients.

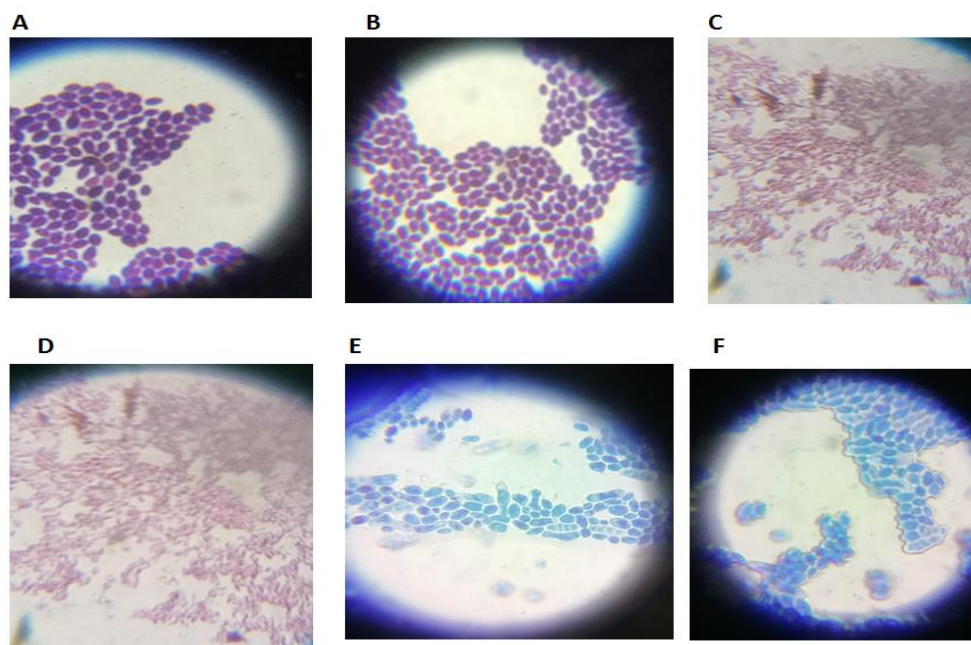
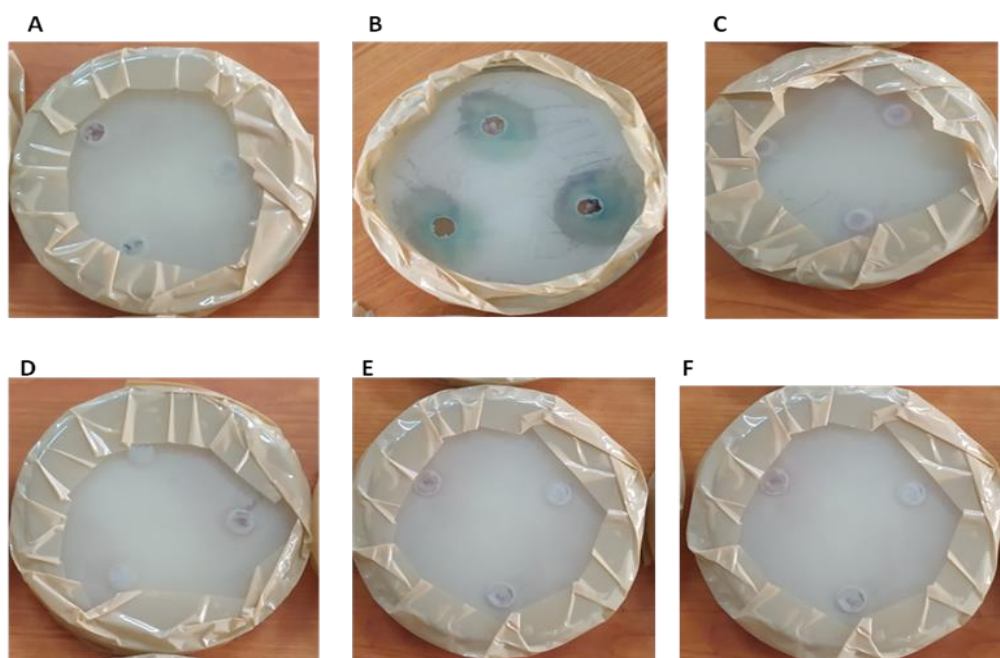


Fig. 1 Details and confirmation of bacteria and fungi in Milk and curd: Fig. 1A, 1B *Lactococcus* isolated from milk and curd, Fig. 1C and 1D *Lactobacillus* isolated from milk and curd. Gram staining was performed on the next day of inoculation and examined under light microscopy. Fig. 1E and 1F Methylene blue staining of yeast isolated from milk and curd.



**Fig. 2** Assay of the antagonistic effect of *Lactococcus* on various pathogenic strains of bacteria and fungi by plate method. Fig. 2A *Lactococcus*, 2B *Pseudomonas aeruginosa*, 2C *Actinomycetes*, 2D *Salmonella typhi*, 2E *Salmonella paratyphi*, 2 F *Aspergillus oryza*. Isolated lactococcus was spread on the plate and wells are made and inoculated with specific pathogenic strains and a zone of inhibition was observed on the next day.

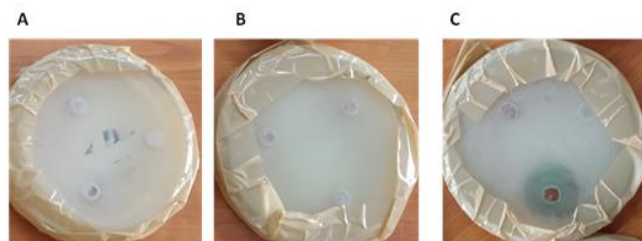
From Fig.2 *Lactococcus* was effective against *Pseudomonas A* compared to other bacterial strains like *E.coli*, *Actinomycetes*, *S.typhi*, *S.patyphi*, and *A.oryzae*. *Pseudomonas* is the gram-negative bacilli known to cause deadly diseases like urinary tract infections, respiratory tract infections, Bacteremia, Bone and joint infections, gastrointestinal disorders, and patients with severe burns, and persons with AIDS with immunosuppression (Clarke and Richman, 1975). Toxin A produced by *Pseudomonas Spp* is mainly responsible for the septic shock and bacteremia and it is one of the endotoxin produced by the bacteria and responsible for the infection (Woods and Iglewski, 1983).

Although strains of *Pseudomonas A* are susceptible to the antibiotics tobramycin, gentamycin, colistin and Amikacin however resistant strains of *pseudomonas* were frequently seen in mankind. A combination of Gentamycin and carbenicilin was frequently used to treat *Pseudomonas* infections but up to now so many vaccines were tested for the prevention of *Pseudomonas* infection but none of them found to be effective and available till now (Brown, 1975). So, instead of using antibiotics, we can use the *Lactococcus* as a probiotic to treat *Pseudomonas* infections as it is safe and economic.

To the gastrointestinal diseases caused by *pseudomonas* direct intake of curd containing *Lactococcus* instead of *Lactobacillus* may be effective for the treatment.

*Lactococcus* produce antimicrobial compounds like germacrene D and  $\beta$ - sesquiphellandrene and bacteriocin in addition to lactic acid the food preservative (Unlu et al.,

2016; Song et al., 2012; Song et al., 2012; Rajendran et al., 2016; Geldart et al., 2015; Nguyen et al., 2018).



**Fig. 3** Assay of antagonist effect of *lactococcus* on *E.coli*, *Salmonella typhi*, and *Pseudomonas* in the mixture. Fig. 3A *E.coli*, 3B *Lactococcus* against *S.Patyphi*, *Actinomycetes* and *S.typhi*. Fig. 3C *Lactococcus* against *E.coli*, *Pseudomonas*, and *S.typhi* on the same plate.

The antimicrobial compounds are effective against *Pseudomonas* but not for other strains. Coculturing of *E.coli*, *S.typhii*, and *Pseudomonas* on *lactococcus* found to be an effective antimicrobial probiotic against *Pseudomonas* but with the remaining strains.

### Discussion and conclusion

According to Gamal Enan et al., (2013) *lactococcus* secretes various antibacterial proteins like bacteriocin and the protein was stable at pH 2 to 7 and found to be heat resistant. The protein is produced in the mid to late exponential growth phase and has an inhibitory effect against pathogens *Listeria*, *Staphylococcus*, and *bacillus*.

From Montville and Chikindas, (2013) *Lactococcus* produced bacteriocins are regarded as safe considered by U.S Food and Drug Administration, and as for Johnson et. al., *Pediococcus acidilactici* bacteriocins are added to fresh milk to prevent the growth of *Listeria* in milk.

From Lin et al., *Lactobacillus plantarum* NTU102 was effective against *Vibrio parahemolyticus* and the antibacterial compound was a proteinaceous compound and is resistant to heat as proteolytic compounds destroyed the antibacterial activity of the compound.

*Pseudomonas aeruginosa* is a deadly pathogen and causes respiratory tract diseases, gastrointestinal infections, and bacteremia and it is an opportunistic pathogen in patients with AIDS, cancer, and severe burns. So, *Lactococcus* isolated from milk and curd products found to contain antibacterial compounds that inhibit the growth of *Pseudomonas* hence it is a good food preservative and excellent probiotic for patients suffering from pseudomonas infections.

### Consent for publication

The author declares that the work has consent for publication.

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### References

Boyle, R. J., Robins-Browne, R. M., & Tang, M. L. (2006). Probiotic use in clinical practice: what are the risks?. *The American journal of clinical nutrition*, 83(6), 1256-1264.

Brown, M. R. W. (ed): Resistance of a *Pseudomonas aeruginosa*. *John Wiley & Sons, New York*, 1975 .

Clarke, P. H., & Richman, M. N. (eds): Genetics and Biochemistry of *Pseudomonas*. *John Wiley & Sons, New York*, 1975 .

Dimri, A. G., Prasad, R., Chauhan, A., Aggarwal, M. L., & Varma, A. (2018). Characterization of soil actinomycete isolate against gram-positive and gram-negative food borne bacteria. *IJEP*, 38, 1004-1015.

Dimri, A. G., Prasad, R., Chauhan, A., Aggarwal, M. L., & Varma, A. (2018). Antibacterial activity of *Streptosporangium* sp., an Actinobacterium isolated from soil of Uttarakhand region, India. *Biochem Cell Arch*, 18(2), 1679-1683.

Enan, G., Abdel-Shafi, S., Ouda, S., & Negm, S. (2013). Novel antibacterial activity of *Lactococcus lactis* subspecies *lactis* z11 isolated from zabady. *International journal of biomedical science: IJBS*, 9(3), 174-180.

Felis, G. E., & Dellaglio, F. (2007). Taxonomy of lactobacilli and bifidobacteria. *Current issues in intestinal microbiology*, 8(2), 44-61.

Feng, J., Chang, X., Zhang, Y., Yan, X., Zhang, J., & Nie, G. (2019). Effects of *Lactococcus lactis* from *Cyprinus carpio* L. as probiotics on growth performance, innate immune response and disease resistance against *Aeromonas hydrophila*. *Fish & shellfish immunology*, 93, 73-81.

Geldart, K., Borrero, J., & Kaznessis, Y. N. (2015). Chloride-inducible expression vector for delivery of antimicrobial peptides targeting

antibiotic-resistant *Enterococcus faecium*. *Applied and environmental microbiology*, 81(11), 3889-3897.

Holzappel, W. H., Haberer, P., Geisen, R., Björkroth, J., & Schillinger, U. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. *The American journal of clinical nutrition*, 73(2), 365s-373s.

Hsing, T., Ming, & Pan, L. T. (2019). Characterization of an antimicrobial substance produced by *Lactobacillus plantarum* NTU 102. *Journal of Microbiology, Immunology and Infection*, 52, 409-417.

Jung, M. Y., Lee, C., Seo, M. J., Roh, S. W., & Lee, S. H. (2020). Characterization of a potential probiotic bacterium *Lactococcus raffinolactis* WiKim0068 isolated from fermented vegetable using genomic and in vitro analyses. *BMC microbiology*, 20, 1-10.

Lee, N. K., Han, K. J., Son, S. H., Eom, S. J., Lee, S. K., & Paik, H. D. (2015). Multifunctional effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi. *LWT-Food Science and Technology*, 64(2), 1036-1041.

Liu, M., Zhang, X., Hao, Y., Ding, J., Shen, J., Xue, Z., ... & Wang, N. (2019). Protective effects of a novel probiotic strain, *Lactococcus lactis* ML2018, in colitis: in vivo and in vitro evidence. *Food & function*, 10(2), 1132-1145.

Moemen, D., Bedir, T., Awad, E. A., & Ellayah, A. (2015). Fungal keratitis: Rapid diagnosis using methylene blue stain. *Egyptian Journal of Basic and Applied Sciences*, 2(4), 289-294.

Montville, T. J., & Chikindas, M. L. (2013). Biological control of foodborne bacteria. *Food Microbiology: Fundamentals and Frontiers*, 803-822.

Nguyen, T. L., Chun, W. K., Kim, A., Kim, N., Roh, H. J., Lee, Y., & Kim, D. H. (2018). Dietary probiotic effect of *Lactococcus lactis* WFLU12 on low-molecular-weight metabolites and growth of olive flounder (*Paralichthys olivaceus*). *Frontiers in microbiology*, 9, 2059.

Rajendran, V., Puvendran, K., Guru, B. R., & Jayaraman, G. (2016). Design of aqueous two-phase systems for purification of hyaluronic acid produced by metabolically engineered *Lactococcus lactis*. *Journal of separation science*, 39(4), 655-662.

Sherid, M., Samo, S., Sulaiman, S., Husein, H., Sifuentes, H., & Sridhar, S. (2016). Liver abscess and bacteremia caused by *Lactobacillus*: role of probiotics? Case report and review of the literature. *BMC gastroenterology*, 16(1), 1-6.

Song, A. A. L., Abdullah, J. O., Abdullah, M. P., Shafee, N., Othman, R., Tan, E. F., & Raha, A. R. (2012). Overexpressing 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) in the lactococcal mevalonate pathway for heterologous plant sesquiterpene production. *PLoS One*, 7(12), e52444.

Song, A. A. L., Abdullah, J. O., Abdullah, M. P., Shafee, N., & Rahim, R. A. (2012). Functional expression of an orchid fragrance gene in *Lactococcus lactis*. *International Journal of Molecular Sciences*, 13(2), 1582-1597.

Singh, D., Dimri, A. G., Goyal, P., Chauhan, A., Aggarwal, M. L., & Chacko, K. M. (2012). Microbiological evaluation of street-vended and restaurant's food items. *Curr Res Biol Pharm Sci*, 1(1), 25-30.

Ünlü, G., Nielsen, B., & Ionita, C. (2016). Inhibition of *Listeria monocytogenes* in hot dogs by surface application of freeze-dried bacteriocin-containing powders from lactic acid bacteria. *Probiotics and antimicrobial proteins*, 8(2), 102-110.

Wessels, S., Axelsson, L., Hansen, E. B., De Vuyst, L., Laulund, S., Lähteenmäki, L., & von Wright, A. (2004). The lactic acid bacteria, the food chain, and their regulation. *Trends in food science & technology*, 15(10), 498-505.

Woods, D. E., & Iglewski, B. H. (1983). Toxins of *Pseudomonas aeruginosa*: new perspectives. *Reviews of infectious diseases*, 5(Supplement\_4), S715-S722.

Yerlikaya, O. (2019). Probiotic potential and biochemical and technological properties of *Lactococcus lactis* ssp. *lactis* strains isolated from raw milk and kefir grains. *Journal of dairy science*, 102(1), 124-134

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