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ISOLATION AND CHARACTERIZATION OF BACTERIOCIN PRODUCING BACTERIA ISOLATED FROM ORAL CAVITY

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ABSTRACT

The mouth is a diverse ecosphere that houses a huge range of microorganisms such as fungi, viruses and bacteria. Oral cavity consists of a variety of habitats, each with its own intrinsic, particular mini-environment, such as tonsils, hard and soft palates, tongue, cheeks, and gingival sulcus. Plaque biofilms on dental surfaces and planktonic phase organisms floating in saliva are also examples of oral microbiome organisms. The majority of oral bacteria have not yet been cultured; however, culture-independent techniques have been effective in providing a through description of the mouth bacterial ecology. The dental flora is constantly altering because its interaction with the surroundings and produces bacteriocins and competes for nutrients within this tiny ecosystem. The objective of the current research was to evaluate and contrast the microbial flora of both healthy and unhealthy people. In this study, the isolation and identification of bacteriocin-producing bacteria was carried out. The samples were collected from Parul University Master's students in Vadodara, Gujarat, India. The bacteria

were extracted and identified using microscopy, gram staining, and biochemical analysis. The study resulted in the isolation of 5 different bacterial strains, of which 2 were gram negative, whereas three were gram positive. The very dominant species belonged to the *Streptococcus* and *Escherichia* species as revealed according to Gram staining and biochemical tests. Many other bacteria were also isolated such as *Staphylococcus, enterococcus, Bacillus subtilis* family etc. It was found that the *Enterobacter* and *Lactobacillus* species produced bacteriocin against *Escherichia coli*. Some of these microbes have been connected to the emergence of multiple infectious illnesses.

Keywords: Bacteriocin, fungi, virus, bacteria, *Streptococcus pneumoniae, Escherichia coli, Staphylococcus, enterococcus, Lactobacillus*

INTRODUCTION

An integral part within the physical body and a little ecosystem is the mouth cavity. The likelihood of the residing organisms enduring in the environment and being harmful to hosts would be reduced. Microbes are typically found on the human skin's external tissues, such as the mouth cavity **[1]**. Microorganisms, including viruses, protozoa, fungus, archaea, and bacteria, are widely present in the human mouth **[2]**. The teeth, one of the most important structures in the oral cavity, is essential for both speaking and eating. The most prevalent diseases globally are dental caries in permanent teeth, according to the Global Burden of Disease Study 2017 of the WHO **[3]**. The bacteria that are most common within the mouth are *Streptococci*. It is a gram-positive, catalase-negative, facultative anaerobic cocci. There are five main categories of oral *Streptococci*: mutans, salivary, anginous, oralis and mitis **[4]**. *Streptococcus* and *Enterococcus* are two significant oral bacteria because, when they enter the oral tissue and bloodstream, they can change from being beneficial microflora on the surface of the mouth cavity and oropharynx to destructive pathogens **[5]**. The bacteriocins eliminate bacteria that are genetically similar, but not the producing strain **[6]**. The WHO evaluate that dental caries causes pain and discomfort in 60–90% of school-aged children and nearly 100% of adults globally **[7]**. Saliva aids in the removal of bacteria from the mouth, but it also provides colonizing bacteria with nutrition by breaking down dietary proteins, lipids, and starches as well as by allowing bacteria to break down salivary components such glycoproteins **[8]**. The most frequent organisms in the mouth include several species of the genera *Enterococcus, Staphylococcus, Corynebacterium, Veillonella, Lactobacillus, Streptococcus*, and *Bacteroids* **[5]**. More than 700 bacterial species or phylotypes have been discovered

within the mouth, more than half of which have never been cultured **[9]**. Bacteriocin are cationic, hydrophobic, secreted anti-microbial peptides (AMP) with a length of 20-60 amino acids produced by the ribosome. They can inhibit both Gram-positive and Gram-negative pathogenic bacteria **[10]**. The lactic acid bacteria (LAB) that produce bacteriocins are promising natural food preservatives **[11, 12]**. The two most common types of bacteriocins are nisin and pediocin **[13]** This is especially useful when preserving food; however, for safety applications, it has implications for the creation of beneficial flora in fermented foods **[14]**. Bacteriocins show antibacterial action and a particular form of protection against strains of the producing bacterium **[15]**. LAB (Lactic Acid Bacteria) exhibits a wide range of antimicrobial activities in fermented foods **[16]**.

In the present research the bacteriocin producing bacteria were isolated from the oral cavities of different people so as to identify the infectious organisms of oral cavity and method to eliminate them. There are multiple normal microflora present in human oral cavity and to eliminate them from drugs will affect the various metabolic reaction of body which may develop the adverse effects in body. The method of removal of such bacteria by bacteriocin producers will be mostly suitable and harmless to human body. Therefore, the oral infections can be treated without the use of drugs in future.

MATERIAL AND METHOD

Collection of samples from oral cavity

Teeth and saliva samples were collected from adult healthy students at Parul University (22.2887◦N, 73.3634◦E). Samples were collected in sterile Eppendorf tubes containing nutrient broth. The various bacteria were isolated from the collected samples and analysed for bacteriocin production ability.

Bacteriocin production assay

Isolated strains were stabbed into THY agar plates with help of sterile tooth picks. After that the bacteria were grown in anaerobic condition by the use of candle jar method and incubated for 24 hours. After incubation, 24 hr old indicator strain was dispersed into the Petri dish.

Identification of bacteria

The Gram staining and biochemical characterization of the isolates was carried out in accordance with Bergey's manual of determinative bacteriology. For colony morphological characterization, the microbes were grown on Nutrient agar medium at 37°C for 24 h. To identify the microscopic pattern of isolates the Gram's staining was done. However, to unfold the metabolic properties different Biochemical analyses were conducted.

Effect of pH on growth of bacteriocin producing bacteria

The bacteria were inoculated into different pH-containing nutrient broth flasks and the neutral pH was set using tris HCL buffer (pH- 7.5). The acidic pH was set using sodium acetate buffer (pH- 5) while, the alkaline pH was set using Tris base buffer (pH- 10). The inoculation of bacterial samples was carried out in all the flasks which was followed by incubation of flasks at 37 °C for 24 hours. The bacterial growth was examined by measuring optical density at 600 nm by spectrophotometer.

Effect of temperature on growth of bacteriocin producing bacteria

The bacterial isolates were inoculated into Nutrient broth medium and incubated at various temperature 25℃,37℃,55℃ for 24hr and the bacterial growth was measured by the spectrophotometer at 600nm.

Growth curve of bacterial strains

The bacterial isolates were inoculated in the sterile nutrient broth flask and incubate at 37 °C for 72 hours. The optical density was measured in every 6 hour for 72 hours to check the bacterial growth pattern.

Inoculum size of bacteria

The different inoculum samples were prepared from 1% to 3% and inoculated in the sterile broth flask, which was incubated at 37°C for 24 hours. The optical density of all the flasks were measured after 24 hours at 600nm.

RESULTS AND DISCUSSION

Isolation of oral cavity bacteria

A total 5 bacteria were isolated from oral cavity samples. Among these strains only 2 bacteria were screened to have antimicrobial activity. As shows in **Figure 1**, only two bacterial isolates were able to produce bacteriocin which were named as Strain DR1 and Strain DR2.

Identification of bacterial isolates

The microscopic observation showed that the cells of Strain DR1 were Gram-positive, spherical or ovoid arranged in pairs or chain. While, Strain DR2 was Gram-positive, coccoid-shaped bacteria **(Figure 2)**.

Biochemical characterization

The isolate Strain DR2 was more potent to produce bacteriocin therefore, to identify it's metabolic properties the biochemical tests were performed. It was observed that isolate was found positive for the Indole test, Urease test, and MR-VP test but negative for the Citrate utilisation test and the catalase test. In carbohydrates fermentation test, the isolated bacteria were able to ferment lactose and unable to ferment sucrose and dextrose sugars **(Figure 3)**.

Bacteriocin production assay

Bacteriocin is produced by some specific bacteria, which kills the harmful bacteria and protect the beneficial bacteria, but not all bacteria are able to produce bacteriocin. Therefore, As shown in **Figure 4 A and B**, bacterial isolate Strain DR1 and Strain DR2 did not produce bacteriocin in unhealthy person may be due to genetic deficiency.

While as shown in **Figure 4 C and D**, bacterial isolates Strain DR1 and Strain DR2 produced bacteriocin which marked the presence of bacteriocin producing genes in organism which also made the person healthy.

Optimization of growth conditions of isolate Strain DR1 and Strain DR2

Effect of pH on Strain DR1 and Strain DR2

The graphs shown in **Figure 5** revealed that the Strain DR1which was grown at various pH levels, such as acidic, basic, and neutral had the highest growth under neutral pH 7 and the lowest growth was observed in a basic condition i.e., pH 10. However, the Strain DR2 had highest growth in acidic conditions i.e., pH 5 and the lowest growth under neutral conditions i.e., 7. This variation reveals that the isolates belong to the different habitat and possess different genetic makeup.

Effect of temperatures on Strain DR1 and Strain DR2

The graphs shown in **Figure 6** revealed that the Strain had the highest growth under temperature 25℃ and the lowest growth was observed at 55℃ temperature. However, the Strain DR2 had highest growth in 25℃ temperature and the lowest growth under 55℃ temperature. This might be possible due to difference in metabolic reactions of bacteria.

Growth curve of Strain DR1 and Strain DR2

The graphs shown **Figure 7** revealed that the Strain DR1 was entered into the log phase between 0 and 24 hr and its exponential phase was observed in 24 hr to 26 hr, and after that bacteria were entered into the stationary phase. The Strain DR2 was entered into the log phase between 0 and 24 hr after which it entered the exponential phase between 24 and 26-time duration, and then it was entered into the stationary phase.

Effect of Inoculum size on Strain DR1 and Strain DR2

The graph shown in **Figure 8**, revealed that Strain DR1 had the highest growth at 3% of inoculum size and the lowest growth at 1%, whereas Strain DR2 had the highest growth at 3% of inoculum size and the lowest growth at 1% and 2%, respectively.

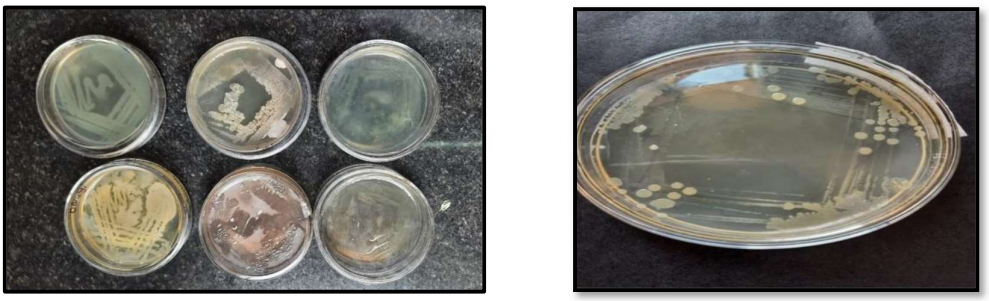

Figure 1: Isolation of Bacteriocin producing bacteria

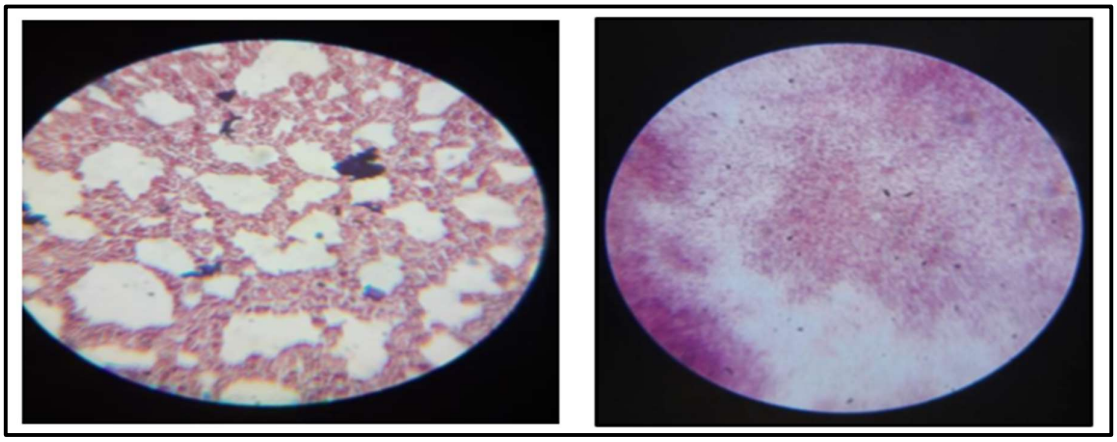

Figure 2: Gram's staining of Bacteriocin producing isolates Strain DR1 and Strain DR2

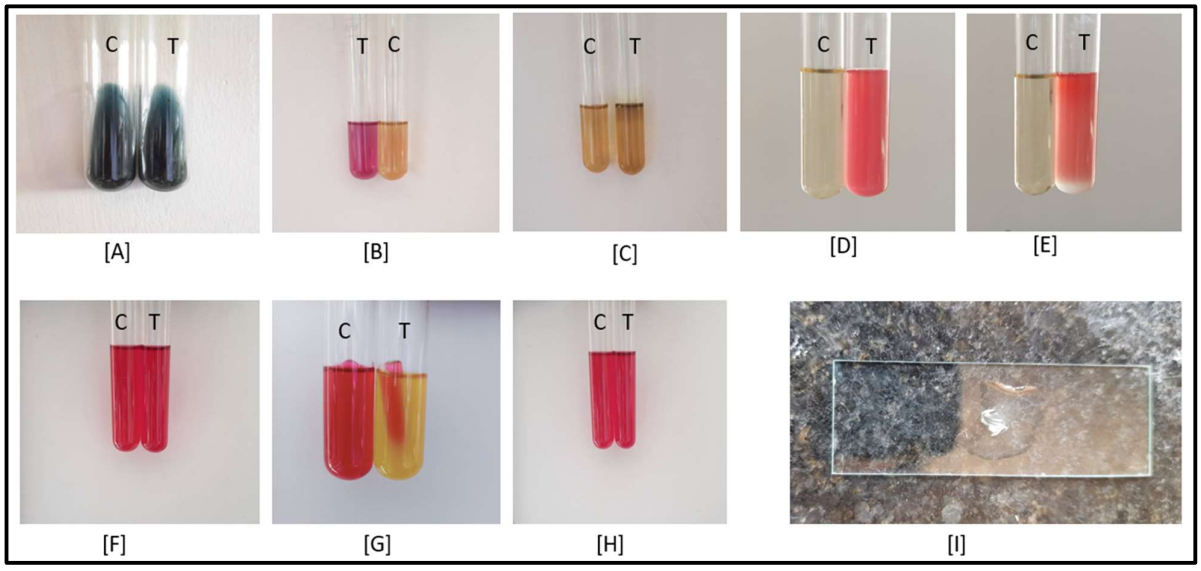

Figure 3: Biochemical Characterization of Strain DR2 (A) Citrate utilization test, (B) Urease test, (C) Indole test, (D) MR test, (E) VP test, (F) Sucrose test (G)Lactose test (H) Dextrose test (I) Citrate utilis test

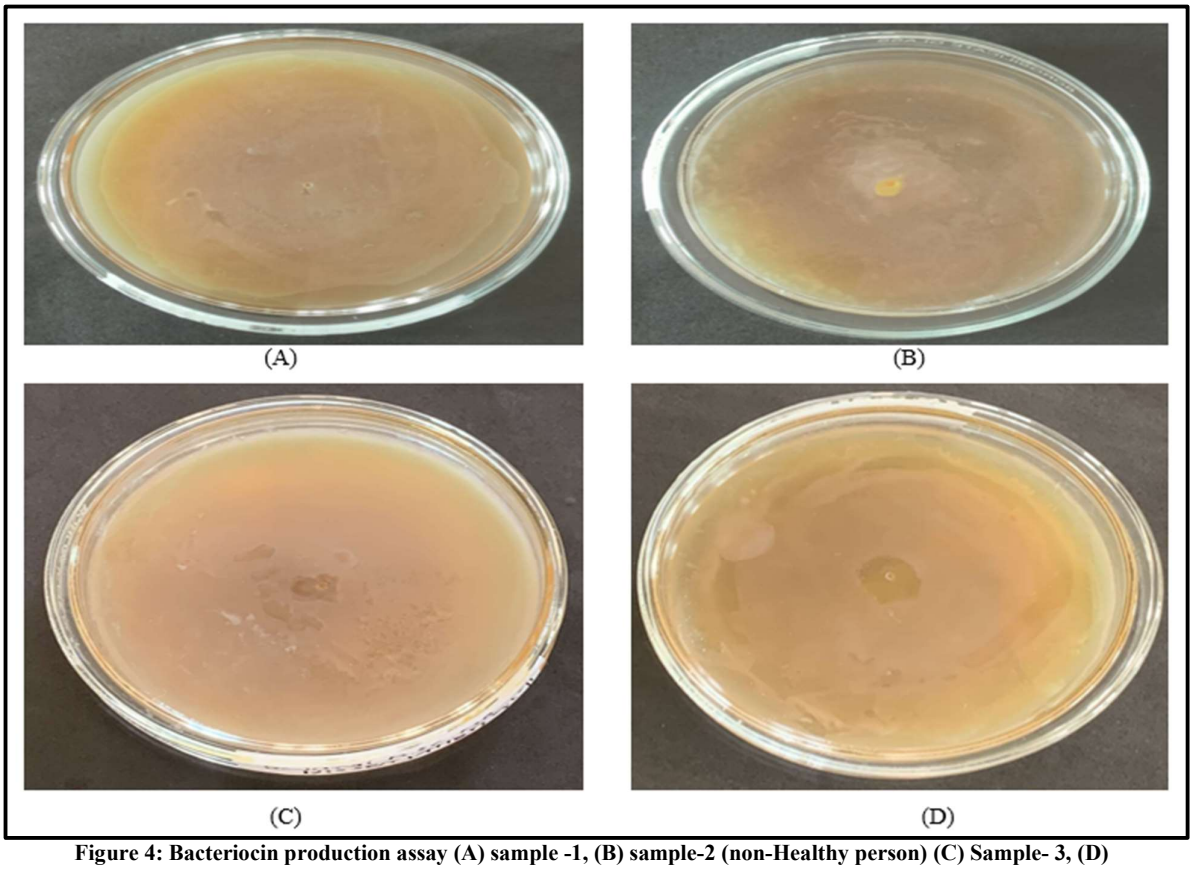

Figure 4: Bacteriocin production assay (A) sample -1, (B) sample-2 (non-Healthy person) (C) Sample- 3, (D) Sample-4 (Healthy person)

Table 1: Result of bacteriocin production

Samples	Zone of inhibition (diameter)
Sample 1	-
Sample 2	-
Sample 3	1 cm
Sample 4	1.9 cm

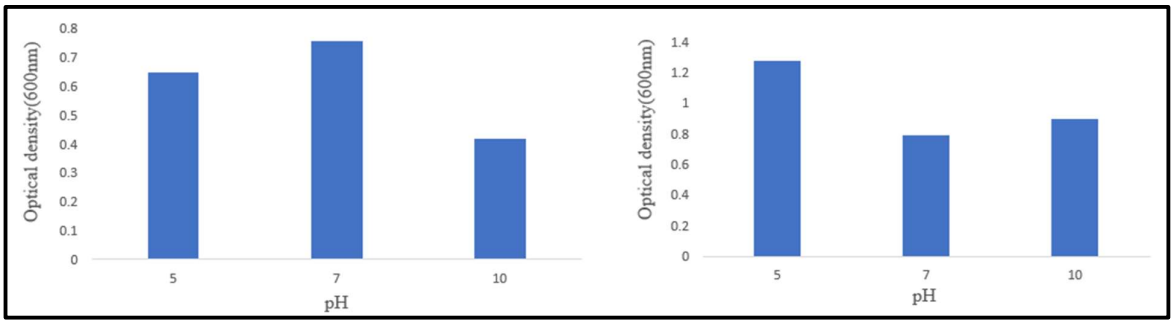

Figure 5: Effect of pH on the growth of Strain DR1 and Strain DR2

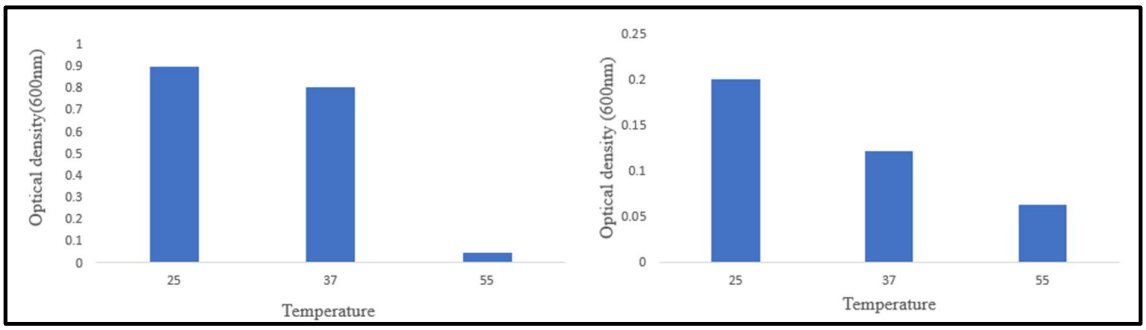

Figure 6: Effect of Temperature on the growth of Strain DR1 and Strain DR2

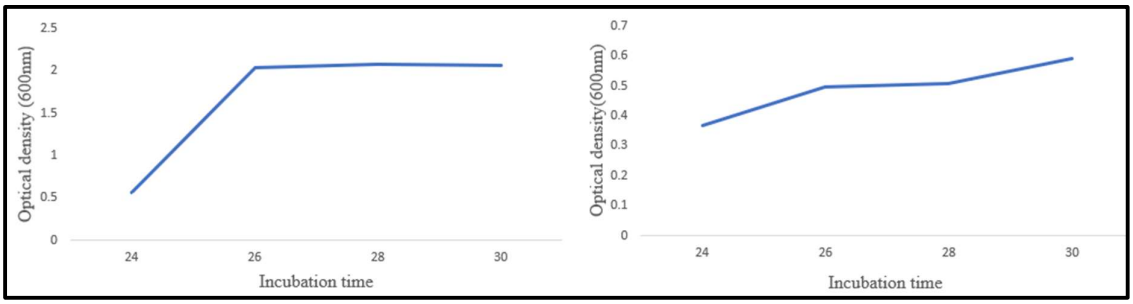

Figure 7: Growth curve of isolates: Strain DR1 and Strain DR2

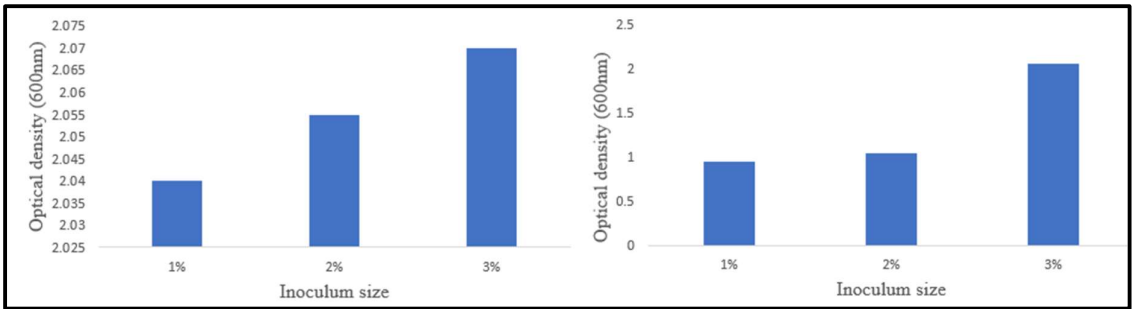

Figure 8: Effect of inoculum size on the growth of Strain DR1 and Strain DR2

CONCLUSION

To protect the mouth cavity communities there is a need for research capable of isolating and identifying a wide range of oral bacteria which can produce bacteriocins and kill the pathogens. For a very long time, bacteriocins have been suggested as a possible cure for food rotting issues and infections in the field of the food industry. In this research, two bacteriocin producing bacterial strains were isolated and characterized. Additionally, their antimicrobial activity was assessed that finally indicate that these isolates may be used for bacteriocin based treatment of oral diseases.

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REFERENCES

[1] Alghamdi, S. (2022). Isolation and identification of the oral bacteria and their characterization for

bacteriocin production in the oral cavity. Saudi Journal of Biological Sciences, 29(1), 318-323.

[2] Wade, W. G. (2013). The oral microbiome in health and disease. Pharmacological research, 69(1), 137-143.

[3] Li, Y., Tang, H., Liu, Y., Qiao, Y., Xia, H., & Zhou, J. (2022). Oral wearable sensors: health management based on the oral cavity. Biosensors and Bioelectronics: X, 10, 100135.

[4] Rostamifar, S., Azad, A., Bazrafkan, A., Modaresi, F., Atashpour, S., & Jahromi, Z. K. (2021). New Strategy of Reducing Biofilm Forming Bacteria in Oral Cavity by Bismuth Nanoparticles. BioMed Research International, 2021.

[5] Rahman, M., Islam, M. N., Islam, M. N., & Hossain, M. S. (2015). Isolation and identification of oral bacteria and characterization for bacteriocin production and antimicrobial sensitivity. Dhaka University Journal of Pharmaceutical Sciences, 14(1), 103-109.

[6] Wang, J. W., Derilo, R. C., Lagitnay, R. B. J. S., Wu, H. P., Chen, K. I., & Chuang, D. Y. (2020). Identification and characterization of the bacteriocin Carocin S3 from the multiple bacteriocin producing strain of Pectobacterium carotovorum subsp. carotovorum. BMC microbiology, 20, 1-13.

[7] Petrov, T., Pecheva, E., Walmsley, A. D., & Dimov, S. (2018). Femtosecond laser ablation of dentin and enamel for fast and more precise dental cavity preparation. Materials Science and Engineering: C, 90, 433-438.

[8] Pedersen, A. M. L., & Belstrøm, D. (2019). The role of natural salivary defences in maintaining a healthy oral microbiota. Journal of dentistry, 80, S3-S12.

[9] Yamashita, Y., & Takeshita, T. (2017). The oral microbiome and human health. Journal of oral science, 59(2), 201-206.

[10] Kumariya, R., Garsa, A. K., Rajput, Y. S., Sood, S. K., Akhtar, N., & Patel, S. (2019). Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. Microbial pathogenesis, 128, 171-177.

[11] Dai, J., Fang, L., Zhang, M., Deng, H., Cheng, X., Yao, M., & Huang, L. (2022). Isolation and identification of new source of

bacteriocin-producing Lactobacillus plantarum C010 and growth kinetics of its batch fermentation. World Journal of Microbiology and Biotechnology, 38(4), 67.

[12] Henning, C., Vijayakumar, P., Adhikari, R., Jagannathan, B., Gautam, D., & Muriana, P. M. (2015). Isolation and taxonomic identity of bacteriocin-producing lactic acid bacteria from retail foods and animal sources. *Microorganisms*, *3*(1), 80-93.

[13] Sidhu, P. K., & Nehra, K. (2019). Bacteriocin-nanoconjugates as emerging compounds for enhancing antimicrobial activity of bacteriocins. *Journal of King Saud University-Science*, *31*(4), 758-767.

[14] Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology*, *3*(10), 777-788.

[15] Darbandi, A., Asadi, A., Mahdizade Ari, M., Ohadi, E., Talebi, M., Halaj Zadeh, M., & Kakanj, M. (2022). Bacteriocins: Properties and potential use as antimicrobials. *Journal of Clinical Laboratory Analysis*, *36*(1), e24093.

[16] De Vuyst, L., & Leroy, F. (2007). Bacteriocins from lactic acid bacteria: production, purification, and food applications. *Microbial Physiology*, *13*(4), 194-199.

[17] Radaic, A., de Jesus, M. B., & Kapila, Y. L. (2020). Bacterial anti-microbial peptides and nano-sized drug delivery systems: The state of the art toward improved bacteriocins. *Journal of Controlled Release*, *321*, 100-118.