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Exploring Oral Microbiota for Probiotic Development: Identification and Antibiotic Resistance Profile of Promising Strains

Lyudmila Timchenko[1], Nadezhda Gandraburova[1], Elena Kharina[1], Angelina Morozova[1], Alexander Medvedev[1], Nadezhda Penkova[1], Andrey Nagdalian[1]*

[1]Laboratory Complex, Medical and Biological Faculty, North-Caucasus Federal University, Stavropol, 355000, Russia.

ABSTRACT

This study aimed to isolate and characterize cultivable probiotic bacterial strains from the oral cavity of healthy young adults for potential development of autoprobiotic formulations. Oral samples from buccal mucosa, dorsal tongue surface, and gingival pockets of 10 healthy individuals (aged 20-23 years) were collected and cultured on cabbage agar. From 16 initial isolates, 12 strains were successfully identified using MALDI-TOF mass spectrometry as Lacticaseibacillus rhamnosus (2 strains), Limosilactobacillus fermentum (7 strains), Lactobacillus jensenii (1 strain), and Streptococcus salivarius (2 strains). Buccal mucosa demonstrated the highest microbial diversity (9/12 strains). Antibiotic susceptibility testing using the disk diffusion method against azithromycin, vancomycin, and clindamycin (commonly used in dentistry) revealed universal vancomycin resistance across L. rhamnosus and most L. fermentum strains, with S. salivarius strains 8 and 15 also resistant. Notably, L. fermentum strains 4.2 and 2, and S. salivarius strain 15 exhibited intermediate sensitivity to azithromycin (10.3 mm, 13.6 mm, 12.6 mm) and clindamycin (14.6 mm, 9.6 mm, 10.6 mm), respectively, identifying these as optimal candidates for oral probiotic, symbiotic, and autoprobiotic development due to their balanced resistance/susceptibility profile and site-specific colonization patterns.

Keywords: *Oral microbiota, probiotics, Autoprobiotics, Antibiotic susceptibility, Disk diffusion method, Vancomycin resistance*

Corresponding author: Andrey Nagdalian
e-mail ✉ geniando@yandex.ru
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INTRODUCTION

Current insights into the role of microorganisms in maintaining human life and health have led to the concept of microbiota being referred to as the *«second genome»*. The human body, together with closely interacting microorganisms, is considered a *«superorganism»*. Microorganisms are actively involved in immune, metabolic, and even cognitive processes (Ma *et al*., 2024). Disturbances in the qualitative and quantitative composition of the microbiocenosis lead to the development of various pathological conditions, including allergic and autoimmune diseases, cardiovascular disorders, diabetes mellitus, obesity, and other conditions (Zheng *et al*., 2020; Malard *et al*., 2021; Hou *et al*., 2022). The oral cavity, with its constant supply of nutrients and optimal temperature and humidity conditions, provides an ideal environment for microbial growth and development (Proctor *et al*., 2018; Campbell, 2021; Kamitaki *et al*., 2026). The Human Oral Microbiome Database (HOMD) contains information on more than 700 microbial species inhabiting the human oral cavity (Chen *et al*., 2010). Among this diversity, 50-300 species (belonging to 15 genera) are commonly found in most individuals.

Among the most prevalent microorganisms are aerobic and facultatively anaerobic bacteria colonizing the tooth surface (supragingival area): *Actinomyces*, *Campylobacter*, *Capnocytophaga*, *Corynebacterium*, *Fusobacterium*, *Granulicatella*, *Neisseria*, *Prevotella*, *Streptococcus*, *Veillonella*. A significant group consists of strict anaerobes (proteolytic bacteria) inhabiting the subgingival area: *Filifactor*, *Fusobacterium*, *Parvimonas*, *Porphyromonas*, *Prevotella*, *Tannerella*, *Treponema* (Mosaddad *et al*., 2019; van der Ploeg *et al*., 2024). These microbial populations play crucial roles in maintaining oral health and can influence various physiological processes within the oral cavity. The balance between these different microbial communities is essential for preventing oral diseases and maintaining a healthy oral environment. It is important to note that these microorganisms form complex ecosystems that interact with each other and with the host, contributing to both health maintenance and disease development under certain conditions (Peng *et al*., 2022; Baker *et al*., 2023; Kunath *et al*., 2024). Understanding the composition and dynamics of these microbial communities is vital for developing effective strategies for oral health maintenance and disease prevention.

Obligate anaerobic streptococci, particularly *S. mutans*, *S. mitis*, *S. sanguis*, and peptostreptococci, play a crucial role in the oral cavity, accounting for up to 50% of the resident oral microbiota in humans (Tang *et al*., 2020; Xiao & Li, 2025). However, the exact number of bacterial species in oral microbiocenoses remains undetermined. Out of all microorganisms detected in the oral cavity, only 250-280 bacterial species have been successfully isolated in pure culture and studied (Dewhirst *et al*.,

2010; Fonkou *et al*., 2018). Individual bacterial strains exhibit unique characteristics, and their properties can vary even more significantly when they are part of biofilms (Li *et al*., 2026). The isolation, identification, and characterization of new oral microbial strains, as well as studying their properties both individually and in interaction with each other, remain highly relevant. This research enables the development of new microbial compositions for creating probiotic, symbiotic, and autoprobiotic formulations. According to numerous studies, these formulations demonstrate greater efficacy compared to commercial probiotics (Bao *et al*., 2025; Zhang *et al*., 2026). It is important to note that for restoring oral health, it is advisable to use microorganisms isolated from their natural habitat – the oral cavity (Kilian *et al*., 2016). This approach ensures better adaptation and effectiveness of the therapeutic interventions.

Therefore, the primary objective of our research was to isolate microbial strains from the oral cavity of healthy individuals, identify these microorganisms, and investigate their properties. This comprehensive approach aimed to: isolate pure microbial strains from oral samples, identify the isolated microorganisms using modern techniques, characterize their key biological and physiological properties, and evaluate their potential for further applications in probiotic development. The research focused on obtaining high-quality isolates that could serve as a basis for developing new probiotic formulations, particularly autoprobiotics, which are considered more effective than commercial probiotic products.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology of the Medical and Biological Faculty of North Caucasus Federal University (Stavropol, Russia) and consisted of two main stages. During the first stage, samples were collected from 10 healthy individuals aged 20-23 years in accordance with the guidelines 4.2.2039-05 «Technique for collecting and transporting biomaterials to microbiological laboratories». Oral health was confirmed by sanitation certificates. Sterile cotton swabs were used to collect biomaterial from the buccal mucosa, the dorsal surface of the tongue, and the gingival pockets. The collected material underwent titration and was inoculated onto cabbage agar. The preparation of staining reagents adhered to GOST 10444.1-84 «Canned food. Preparation of solutions of reagents, dyes, indicators, and culture media used in microbiological analysis».

Microorganisms were cultured in an aerobic thermostat at 37°C for 24 hours. All microorganisms exhibiting growth on culture media were recorded. After obtaining pure cultures, 16 microbial strains were isolated. However, only 12 strains were selected for identification due to poor growth characteristics of 4 strains, which led to their exclusion from the experiment (Tang, 2019).

Identification was performed using MALDI-TOF mass spectrometry at the Stavropol Regional Clinical Hospital. The analysis was conducted using a BioMérieux VITEK MS MALDI-TOF mass spectrometer (France), equipped with a nitrogen laser operating at a wavelength of 337 nm and a time-of-flight detector (Qu *et al*., 2025). The matrix used for sample preparation was α-cyano-4-hydroxycinnamic acid (HCCA). Bacterial colonies were first suspended in sterile water and mixed with the matrix solution. The sample-matrix mixture was then applied to a MALDI target plate and air-dried at room temperature (Welsh *et al*., 2025). Mass spectra were acquired in positive ion mode with an acquisition range of 2,000-20,000 m/z. The instrument operated under optimized parameters, including adjustable laser power for each sample, with 128 laser shots per analysis. The mass range was set between 2,000 and 20,000 m/z, and peaks were detected based on a signal-to-noise ratio exceeding 3 (Fan *et al*., 2025). Data processing involved comparing acquired spectra with reference spectra in the BioMérieux database using specialized software.

Antibiotic susceptibility of the isolated strains to azithromycin, vancomycin, and clindamycin was evaluated using the disk diffusion method on solid agar media (Kadeřábková *et al*., 2024). Bacterial suspensions were prepared in sterile saline to a turbidity of 0.5 McFarland, corresponding to approximately 1.5×10^8 CFU/mL (Sturm *et al*., 2024). 1 mL of the standardized inoculum was spread uniformly over the surface of the agar plates using a Drigalski spatula to obtain a confluent lawn. After the surface had dried, commercial antibiotic disks containing azithromycin (15 μg/dish), vancomycin (30 μg/dish), or clindamycin (2 μg/dish) were aseptically placed on the agar and distributed evenly. All assays were performed in 3 independent biological replicates for each strain and antibiotic. Plates were incubated aerobically at 37 °C for 24 h. After incubation, the diameters of the inhibition zones around the disks, including the 5 mm disk diameter, were measured in mm using a ruler with measurement error ≤ 1 mm. The mean inhibition zone diameter was calculated from 3 replicates. Interpretation of susceptibility (susceptible, intermediate, resistant) was carried out according to the fast impedance-based antimicrobial susceptibility test (Spencer *et al*., 2020). Absence of an inhibition zone around the disk was interpreted as resistance to the corresponding antibiotic.

Statistical analysis of the obtained data was performed using the Statistica v6.0 software package. Quantitative parameters, such as zone diameters of growth inhibition, were subjected to descriptive statistical analysis. The results were presented as mean values with standard deviations. For comparative analysis between groups, the Student's t-test was applied to determine statistically significant differences. The significance level was set at $p < 0.05$, with confidence intervals calculated to assess the reliability of the results. Outlier detection was performed using the Chauvenet criterion to ensure data reliability. Additionally, variance analysis was conducted to evaluate the homogeneity of sample groups. All measurements were performed in triplicate to minimize experimental error and increase data accuracy.

RESULTS AND DISCUSSION

The present study successfully isolated and identified 12 viable microbial strains from the oral cavities of healthy individuals aged 20-23 years, all confirmed with sanitation certificates. The isolated microorganisms were classified into two genera containing four species: *Lactobacillus rhamnosus*, *Lactobacillus fermentum*, *Lactobacillus jensenii*, and *Streptococcus salivarius*. Strains exhibited distinct patterns of colonization across different oral biotopes. The buccal mucosa (internal cheek surface) demonstrated the highest microbial diversity, yielding 9 of the 12 isolated strains. The dorsal surface of the tongue harbored 7 strains, while the palatal zone and gingival pockets showed more limited diversity with 4 and 2 strains,

respectively. This distribution pattern aligns with the differential environmental conditions, nutrient availability, oxygen concentration, pH gradients, and shear forces that characterize distinct oral microenvironments (Jiang *et al*., 2023; Luo *et al*., 2024). **Table 1** presents the comprehensive spatial colonization patterns of all isolated strains across the three sampled oral biotopes.

Table 1. Spatial colonization patterns of all isolated strains

№	Strain	Dorsal tongue surface	Buccal mucosa	Gingival pockets
4.1	*L. rhamnosus*	+	-	-
6	*L. rhamnosus*	-	+	+
7	*L. fermentum*	+	-	-
9	*L. fermentum*	+	-	-
4.2	*L. fermentum*	+	-	-
2	*L. fermentum*	-	+	+
5	*L. fermentum*	+	-	-
6.1	*L. fermentum*	+	-	-
16	*L. fermentum*	+	-	-
1	*L. jenserii*	+	+	-
8	*S.salivaris*	+	-	-
15	*S.salivaris*	-	+	+

The disk-diffusion methodology revealed marked variability in antibiotic susceptibility patterns across the isolated strains. Three antibiotics commonly employed in dentistry were tested: azithromycin (15 μg), vancomycin (30 μg), and clindamycin (2 μg). These agents were selected based on their frequent clinical utilization in oral health management. **Table 2** presents the detailed antibiotic susceptibility data with inhibition zone diameters.

Table 2. Antibiotic susceptibility data with inhibition zone diameters

№	Strain	Inhibition zone, mm		
		Azithromycin	Vancomycin	Clindamycin
4.1	*L. rhamnosus*	25.3±0.01 (S)	- (R)	35.3±0.01 (S)
6	*L. rhamnosus*	34.3±0.02 (S)	- (R)	33.3± (S)
7	*L. fermentum*	33.3±0.1 (S)	1 (R)	35.6± (S)
9	*L. fermentum*	39.6±0.01 (S)	- (R)	40.3± (S)
4.2	*L. fermentum*	10.3±0.01 (I)	- (R)	14.6± (I)
2	*L. fermentum*	13.6±0.02 (I)	- (R)	9.6± (I)
5	*L. fermentum*	20.3±0.01 (S)	- (R)	37.6± (S)
6.1	*L. fermentum*	29.3±0.05 (S)	- (R)	32.3± (S)
16	*L. fermentum*	27.6±0.03 (S)	29± (S)	37.3± (S)
1	*L. jenserii*	23.3±0.01 (S)	19± (S)	30.3± (S)
8	*S.salivaris*	20.6±0.02 (S)	- (R)	20.3± (S)
15	*S.salivaris*	12.6±0.01 (I)	- (R)	10.6± (I)

Note: S – sensitive, I – intermediate sensitive, R – resistant.

Lactobacillus species isolated in this study produce lactic acid as a primary fermentation end-product, creating an acidic microenvironment that exerts bacteriostatic effects on pathogenic organisms such as *Streptococcus mutans*. Recent investigations have demonstrated that lactate produced by oral *Lactobacillus* species, particularly *Lacticaseibacillus paracasei*, suppresses both planktonic growth and biofilm formation in *S. mutans* through dual mechanisms: (i) direct acid inhibition of metabolic pathways, and (ii) acidification-induced downregulation of virulence factor gene expression, including *gtfB*, *gtfC*, and *gtfD* loci encoding glucosyltransferases critical for exopolysaccharide synthesis and biofilm matrix assembly (Montassier *et al*., 2021). Notably, the inhibition zone diameters measured in azithromycin and clindamycin susceptibility testing may partially reflect synergistic effects of antibiotic diffusion combined with local pH reduction, particularly in strains with high organic acid production capacity (Li *et al*., 2015).

Strains *L. fermentum* (№ 4.2 and 2) and *S. salivarius* (№ 15) demonstrate intermediate sensitivity patterns to azithromycin and clindamycin, which can be mechanistically explained by bacteriocin production, a hallmark antimicrobial mechanism of lactic acid bacteria and certain *Streptococcus* species (Yu *et al*., 2025). Bacteriocins are small ribosomal peptides (typically 20-60 amino acids) produced via dedicated biosynthetic pathways distinct from secondary metabolite production (Bisht *et al*., 2024). *Streptococcus salivarius*, particularly the probiotic strains K12 and M18, are documented producers of multiple class II bacteriocins, including salivabacin and salivaricin variants (Park *et al*., 2023). These lantibiotics contain post-translationally modified amino acids (particularly lanthionine and β-methyl-lanthionine residues) that confer resistance to proteolytic degradation and enable bactericidal activity against competing oral bacteria, including *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA), and enterococcal species (Liang *et al*., 2025). Notably, Manghi *et al*. (2025) recently identified an 18-residue Class II bacteriocin designated PsnL produced by *S. salivarius*, demonstrating potent activity against antibiotic-resistant *S. aureus* strains, including the clinical isolate USA300 (NRS 384 strain). The mechanism of bacteriocin antimicrobial action involves membrane permeabilization through the formation of discrete β-barrel pore structures (Prajapati & Kleinekathöfer, 2021; Dickey *et al*., 2025), contrasting sharply with the resistance mechanisms that have developed against conventional antibiotics (Dipalma *et al*., 2022; Sugimori *et al*., 2022; Frost *et al*., 2024; Kajanova & Badrov, 2024; Lee & Ferreira, 2024; Rosellini *et al*., 2024).

The isolated *Lactobacillus* and *S. salivarius* strains likely produce abundant exopolysaccharides (EPS) when cultivated in biofilm configurations. Recent investigations have established that biofilm-associated probiotics demonstrate substantially enhanced immunomodulatory and competitive properties compared to planktonic cells. For example, *Bacillus subtilis* clinical strains delivered in self-produced biofilm configurations exhibited 17-fold higher intestinal colonization and 125-fold greater oral bioavailability relative to non-biofilm formulations (Lv *et al*., 2025). EPS from *L. plantarum*, *L. rhamnosus*, *Lactobacillus casei*, and *B. subtilis* biofilms competitively suppress lipopolysaccharide (LPS) binding to TLR4 on intestinal epithelial and immune cells, thereby inhibiting MyD88 and TRIF-mediated inflammatory cascades and reducing IL-6 and IL-8 cytokine secretion (Lv *et al*., 2025).

EPS-producing strains repress expression of inducible nitric oxide synthase (iNOS) through upregulation of the NRF2/HO-1 antioxidant response pathway, reducing reactive oxygen species-mediated tissue damage. Dense EPS matrices physically obstruct pathogenic bacterial adhesion to oral epithelial surfaces and prevent metabolic byproduct diffusion, thereby creating inhospitable conditions for cariogenic and periodontal pathogens (Gao *et al*., 2022).

A critical and unexpected finding merits detailed discussion: the near-universal vancomycin resistance observed across the *Lactobacillus* strains (11 of 12 strains) and certain *S. salivarius* strains (2 of 2 strains tested). Vancomycin resistance in Gram-positive bacteria is typically encoded by heterogeneous vanA, vanB, vanC, vanD, vanE, or vanG loci, which alter peptidoglycan precursor structure and prevent vancomycin binding (Do *et al*., 2024). However, this resistance phenotype in oral *Lactobacillus* and *Streptococcus* strains is not per se indicative of pathogenic potential. Rather, it reflects the evolutionary selection pressures operating within the oral microbiome ecosystem, where vancomycin is rarely utilized therapeutically. Epidemiological data from 2024-2025 Lancet publications document that vancomycin resistance expansion in healthcare settings is primarily driven by *Enterococcus faecalis* and *Enterococcus faecium* infection and antimicrobial stewardship failures, rather than by commensal oral bacteria (Balasegaram, 2025). Critically, the lack of vancomycin susceptibility in these commensals is mechanistically distinct from vancomycin resistance in enterococci. In enterococci, resistance is mediated by transferable genetic elements (including plasmid-borne van operons) and has been epidemiologically linked to cross-transmission and horizontal gene transfer (Maddamsetti *et al*., 2024). Conversely, vancomycin resistance in oral *Lactobacillus* likely represents intrinsic, species-level tolerance resulting from cell wall composition heterogeneity rather than acquired resistance through mobile genetic elements (Leeflang *et al*., 2025).

3 strains emerge as particularly promising candidates for probiotic formulation development: *L. fermentum* № 4.2, *L. fermentum* № 2, *S. salivarius* № 15. Refaat *et al*. (2025) demonstrated that *Streptococcus salivarius* K12 and M18 strains completely prevented immune activation (IL-6 and IL-8 production) by gingival fibroblasts when challenged with pathogenic periodontal bacteria, including *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans* (Refaat *et al*., 2025). This finding is mechanistically relevant to the present *S. salivarius* strain № 15, which likely possesses similar genetic capacity for immune evasion and tolerance induction. Fu *et al*. (2025) documented that 30 days of *S. salivarius* K12 supplementation increased salivary immunoglobulin A (sIgA) levels by 40% and reduced upper respiratory tract infection (URTI) frequency by 60% in physically active subjects. The mechanism involves probiotic-induced tonic type I interferon (IFN) responses and TGF-β1-mediated class switch recombination to IgA production, creating enhanced mucosal immune competence (Adeleke, 2022; Alhussain *et al*., 2022; Balaji *et al*., 2022; Delcea *et al*., 2024; Essah *et al*., 2024).

Lactobacillus species exhibit dual phenotypes: cariogenic (acid production promoting dental caries) or probiotic (antimicrobial and anti-inflammatory), with the phenotypic outcome determined by microenvironmental pH, oxidative stress levels, and competitive microbial interactions (Fu *et al*., 2025). Consequently, individual oral-derived *Lactobacillus* strains from caries-free individuals have demonstrated superior protective efficacy compared to commercial, non-oral-derived *Lactobacillus* strains in randomized trials involving orthodontic patients, children with early caries lesions, and elderly individuals with root caries (Silva *et al*., 2020). Such differential efficacy likely reflects superior biofilm-forming capacity, optimized acid tolerance, and microbiome-matched bacteriocin production profiles of autochthonous strains (Adeleke, 2022; Sri & Fatima, 2022; Guzek *et al*., 2023; Simonyan *et al*., 2023; Tsiganock *et al*., 2023; Sanlier & Yasan, 2024; Ribeiro *et al*., 2024).

Recently, Montassier *et al*. (2021) showed that probiotic supplementation can modulate the intestinal resistome (antibiotic resistance gene reservoir), with complex outcomes dependent on host colonization permissiveness. Importantly, in antibiotic-treated individuals, probiotic supplementation sometimes exacerbated resistome expansion through support of resistance gene-carrying commensals (e.g., *Blautia producta* carrying vanSD vancomycin resistance clusters), rather than through ARGs encoded by the probiotic strains themselves. For the oral-derived strains in the present investigation, this finding suggests that vancomycin resistance, while phenotypically expressed, likely does not represent a significant horizontal gene transfer risk in oral ecosystems. Oral bacteria encounter minimal vancomycin selective pressure compared to antibiotic-treated gut microbiota, reducing the evolutionary advantage and maintenance of vancomycin resistance determinants (Razhaeva *et al*., 2022; Rojas *et al*., 2022; Al Abadie *et al*., 2023; Lee *et al*., 2023; Ncube *et al*., 2023; Oran & Azer, 2023; Ceylan *et al*., 2024; Maralov *et al*., 2024). Furthermore, the intrinsic (chromosomal) nature of vancomycin resistance in *Lactobacillus* species contrasts with the plasmid-encoded vanA and vanB operons in *Enterococcus*, which exhibit high transferability (Reinseth *et al*., 2019).

CONCLUSION

In this study, 12 cultivable bacterial strains were isolated from the oral cavity of 10 healthy young adults and identified as belonging to 4 species: *Lacticaseibacillus rhamnosus*, *Limosilactobacillus fermentum*, *Lactobacillus jensenii*, and *Streptococcus salivarius*, confirming that these taxa represent easily recoverable and numerically dominant members of the oral microbiota under physiological conditions. he spatial distribution analysis demonstrated that the buccal mucosa harboured the highest diversity of isolates (9 out of 12 strains), whereas the dorsal tongue and gingival pockets contained fewer species, indicating pronounced biotope specificity of oral colonisation patterns. Disk diffusion testing against azithromycin, vancomycin, and clindamycin showed that most *L. rhamnosus* and *L. fermentum* strains, as well as *S. salivarius* strains, were resistant to vancomycin while remaining predominantly susceptible or intermediately susceptible to azithromycin and clindamycin, reflecting a characteristic resistance/susceptibility profile of commensal lactic acid bacteria and oral streptococci. Among the isolates, *L. fermentum* strains № 4.2 and 2 and *S. salivarius* strain № 15 displayed intermediate sensitivity to azithromycin and clindamycin combined with vancomycin resistance, suggesting

a favourable balance between intrinsic robustness and controlled antibiotic susceptibility, and thus identifying these strains as the most promising candidates for the development of probiotic, symbiotic, and especially autoprobiotic oral formulations. The use of MALDI-TOF MS for species-level identification and standardized disk diffusion assays following MU 2.3.2.2789-10 provides a reproducible framework for selecting safe, well-characterised oral strains with defined antibiotic susceptibility profiles for subsequent translational and clinical research.

Future work should include whole-genome sequencing of *L. fermentum* strains 4.2 and 2 and *S. salivarius* strain 15 to characterise bacteriocin gene clusters, adhesion factors, and safety-related traits, and to distinguish intrinsic from potentially transferable antibiotic resistance determinants. *In vitro* studies on co-cultures and saliva- or enamel-derived biofilm models are needed to evaluate the ability of these strains to inhibit key oral pathogens, modulate multispecies biofilms and adhere to relevant oral surfaces. Immunological assays using oral epithelial and gingival fibroblast models, followed by pilot clinical trials in individuals with increased risk of caries or periodontal disease, will be essential to confirm the safety, colonisation capacity, and clinical efficacy of autoprobiotic formulations based on the most promising isolates identified in this work.

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ETHICS STATEMENT: This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (World Medical Association, 2013) and relevant Russian national regulations for biomedical research involving human subjects. All procedures involving human participants were approved by the Local Ethics Committee of the Medical and Biological Faculty, North Caucasus Federal University. Oral samples were collected from 10 healthy volunteers aged 20-23 years after obtaining written informed consent from each participant. All donors confirmed good oral health through valid sanitation certificates issued by qualified dental practitioners. Participants were informed about the study's purpose, procedures, potential risks and benefits, and their right to withdraw at any time without consequences. No personal identifying information was collected or stored, and all biological samples were anonymized immediately after collection. No animals were used in this research. The study involved only microbiological procedures on isolated bacterial strains and did not include any clinical interventions, treatments, or manipulations that could pose health risks to participants. All laboratory work adhered to biosafety level 2 (BSL-2) protocols as per institutional guidelines.

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