



Biogenic Synthesis of Cao NPS Using Coriander Leaf Extract and Its Antibiofilm Activity Against Oral Pathogens.

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KEYWORDS

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ABSTRACT:

Introduction: The growing concern over environmental sustainability has encouraged the development of green synthesis methods for nanoparticles. Traditional chemical and physical approaches often involve toxic reagents and high energy consumption. In contrast, plant-mediated synthesis offers an eco-friendly, cost-effective, and sustainable alternative. Calcium oxide (CaO) nanoparticles have attracted significant attention due to their wide range of applications in catalysis, environmental remediation, and biomedical fields. In this study, Coriander (*Coriandrum sativum*) leaf extract was utilized as a natural reducing and stabilizing agent for the green synthesis of CaO nanoparticles.

Objectives: The main objectives of this study were: To synthesize calcium oxide (CaO) nanoparticles using *Coriandrum sativum* (coriander) leaf extract through a green synthesis approach, To characterize the synthesized nanoparticles using FTIR, XRD, and SEM analyses, To evaluate the antioxidant and antibacterial activity of the synthesized CaO nanoparticles against selected oral pathogens.

Methods: Calcium oxide nanoparticles were synthesized using *Coriandrum sativum* leaf extract as a biogenic reducing agent. The structural and morphological characteristics of the synthesized CaO nanoparticles were analyzed using various techniques: **FTIR Analysis:** Identified functional groups responsible for nanoparticle stabilization, showing peaks at 3781.88 cm^{-1} and 3321.46 cm^{-1} corresponding to hydroxyl group stretching vibrations. **XRD Analysis:** Determined the crystalline structure of CaO nanoparticles, showing diffraction peaks at 25.51° , 31.77° , 37.94° , 45.48° , 48.24° , 54.41° , 55.24° , 68.95° , 70.51° , and 75.11° , confirming the cubic phase of CaO. **SEM Analysis:** Examined the surface morphology and particle size, revealing predominantly spherical, agglomerated nanoparticles with diameters ranging from 70 to 95 nm. The antibacterial activity of the synthesized CaO nanoparticles was assessed against four oral pathogens: *Streptococcus pyogenes*, *Lactobacillus* sp., *Escherichia coli*, and *Staphylococcus aureus*.

Results: FTIR analysis confirmed the presence of hydroxyl functional groups associated with the plant extract, suggesting successful stabilization of the nanoparticles. XRD patterns confirmed the crystalline nature of CaO with distinct cubic phase peaks. SEM images revealed agglomerated spherical particles within a nanoscale range (70–95 nm). Antibacterial assays showed significant inhibition zones against the tested pathogens, particularly *Streptococcus pyogenes* and *Lactobacillus* sp., indicating strong antimicrobial potential of the green-synthesized CaO nanoparticles.

Conclusions: The study successfully demonstrated an eco-friendly and cost-effective synthesis of calcium oxide nanoparticles using *Coriandrum sativum* leaf extract. The characterization results confirmed the formation of crystalline, nanosized CaO particles with promising antibacterial activity against common oral pathogens. These findings suggest that green-mediated CaO nanoparticles could serve as potential agents in the prevention and treatment of oral infections, especially those caused by *Streptococcus pyogenes* and *Lactobacillus* sp.



1. Introduction

Nanotechnology is one of the most advanced fields of the 21st century. Nanoparticles have garnered significant attention because of their unique and intriguing properties, leading to a wide range of applications (Abd-El salam KA et al). Nanoparticles may be prepared chemically, physically, or by a biological method (Karthik L et al). The unique characteristics of nanoparticles can be linked to their tiny size, ranging from 1 to 100 nm, which allows for a high surface area-to-volume ratio (Agrawal DC et al). The effect of engineered nanoparticles on plants depends on several factors, including the nanoparticles' composition, concentration, size, other chemical and physical characteristics, and the specific plant species involved (Ming BQK et al).

Coriander (*Coriandrum sativum* L.), part of the Apiaceae family, is a widely utilized medicinal plant known for its nutritional and therapeutic benefits (Grieve M et al). Coriander extracts and essential oils have demonstrated antibacterial, antioxidant, free radical-scavenging, antidiabetic, anticancer, and antimutagenic properties (Diederichsen A et al). The type of solvent used was identified as the key factor in extracting antioxidants and bioactive compounds from coriander. While all parts of coriander are edible, they vary in flavor and use (Kačániová M et al). Coriander (*Coriandrum sativum*) leaf extract can serve as a reducing and stabilizing agent in nanoparticle synthesis (*Medicinal Natural Products: A Disease-Focused Approach*. Academic Press; 2020).

Calcium oxide nanoparticles (CaO NPs) are known for their antibacterial properties and biocompatibility, making them suitable candidates for medical applications (*Colloidal Metal Oxide Nanoparticles: Synthesis, Characterization and Applications*. Elsevier; 2019). Also, Calcium oxide (CaO) nanoparticles have a range of applications, including catalysis, adsorption, water purification, and as antibacterial agents (Fedlheim DL et al). CaO is of particular interest as it is regarded as a safe material to human beings and animals (Grumezescu AM et al). There are many reports on preparation of Calcium oxide nanoparticles from chemical methods. However, only few biogenic synthesis are being reported in literature. The biogenic synthesis of nanoparticles using plant extracts offers an eco-friendly alternative to chemical methods.

Oral pathogens are microorganisms that inhabit the oral cavity and can contribute to various dental and periodontal diseases (Miller WD et al). It plays a crucial role in the development of oral conditions like tooth decay, gum disease, and oral candidiasis (*Arch Oral Biol.* 2023;152:105721). The most notable oral pathogens include *Streptococcus mutans*: This bacterium is a primary contributor to dental caries (cavities). It metabolizes sugars to produce acids that demineralize tooth enamel. *Pseudomonas aeruginosa* can also play a role in oral infections, especially in individuals with compromised immune systems or underlying conditions. Its ability to form biofilms allows it to adhere to oral tissues and dental surfaces, contributing to conditions like periodontal disease. *E. coli's* ability to form biofilms can enhance its survival in the oral environment, leading to its potential involvement in dental caries and infections (Gendron R et al).

2. Objectives

The objectives of this study were (i)The study investigates the antibiofilm efficacy of calcium oxide nanoparticles (CaO NPs) synthesized using Coriander leaf extract against common oral pathogens. The research focuses on assessing the effectiveness of these nanoparticles in inhibiting biofilm formation and disrupting existing biofilms.

3. Methods

In the present research, distilled water, Calcium acetate, Ammonia solution, and sodium borohydrate solution (Fisher Scientific, India) were used for the synthesis of CaO NPs based on the article ([Hemmami H et al](#)) .

Collection and Preparation of Plant Sample

Coriander leaf extract leaves were collected from different locations within Saveetha University, Chennai. The samples were cleaned using distilled water to eliminate unwanted substances and then shade-dried to maintain their natural integrity. After the leaves were completely shade-dried, they were ground into a fine powder with a mortar and pestle. Then, 10 g of powdered leaf material was combined with 200 ml of distilled water in a sterile conical flask. The mixture was heated and stirred for 24 h. Subsequently, the extract obtained was passed through Whatman No. 1 filter paper to filter out impurities. This procedure was carried out based on the reference of ([Proficient synthesis of zinc oxide nanoparticles from Tabernaemontana heyneana Wall](#)).



Synthesis of CaO NPs

Initially, distilled water was added to a precisely calibrated solution of calcium acetate (0.4 M), along with the filtrate obtained from the plant extract. The mixture was boiled while stirring with a magnetic agitator. Simultaneously, 1M Sodium borohydride solution and ammonia solution were gradually added until a characteristic blackish – brown colour developed, indicating the formation of CaO NPs. The mixture was centrifuged at 5000 rpm for 15 min. After centrifugation, the supernatant and pellet were separated. The supernatant liquid was discarded, and the pellet containing nanoparticles was gradually transferred into a petri dish. The nanoparticles were further dried in a hot air oven at 100 °C according to the procedure by (Vidyasagar et al). A detailed study has been carried out to determine the characteristics of the CaO NPs obtained using the extract of *Coriander leaf extract*.

Characterization of CaO NPs :

Spectral interpretation using Fourier-transform infrared (FTIR) spectroscopy was performed using a Bruker Alpha-II ATR instrument within the wavelength range 4000–400 cm⁻¹. XRD analysis was applied to the finely pulverized CaO NPs to investigate their crystal structure, utilizing Ca K α radiation. Spectral interpretation was carried out using a Bruker D8 Advance X-ray diffractometer operating at 30 kV and 15 mA. Measurements of The diffracted intensities were measured at a rate of 4°/min with a step size of 0.05°, covering 2 θ angles from 20 to 80 °. SEM analysis was performed using a Jeol JSM IT 800 instrument.

Antibacterial activity and minimum inhibitory concentration (MIC)

The antibacterial properties of *C. bonduc* extract were evaluated using bacterial specimens obtained from Saveetha Dental College's Microbiology Laboratory. Efficacy against *Streptococcus pyogenes*, *Lactobacillus sp.*, *Escherichia coli*, and *Staphylococcus aureus*. was evaluated using the protocols recommended by the Clinical and Laboratory Standards Institute (CLSI), as described by Padil et al. 2013. The experiment utilized Muller-Hinton agar made on sterile petri plates and was used as the growth medium. Aseptic cotton swabs were used to evenly distribute pure bacterial cultures onto the agar surface, resulting in a density of 90 colony-forming units per mL (CFU/ml). Subsequently, sterile well borers were used to produce wells with diameters of 8 mm. Various doses of CaO NPs (25, 50, and 100 μ g/ml) were

then added to the wells. The positive control in each trial was streptomycin at a concentration of 10 mg/ml.

The Petri dishes were subsequently placed in an incubator at 37 \pm 2 °C for 24 h and the conditions were carefully monitored. After the incubation period, the areas of bacterial growth inhibition surrounding each well were quantified. These measurements yielded valuable data regarding the antibacterial efficacy of CaO NPs against the bacterial strains under examination. For the MIC assay, the broth dilution method was utilized to identify the lowest concentration of CaO NPs needed to prevent the growth of a specific bacterial strain.

Antibiofilm activity

Biofilm inhibition assay using Microtiter plate method

The synthesized CaO NPs inhibited biofilm formation at MIC's using the microtiter plate method with Crystal Violet as a staining agent. Briefly, 100 μ L of fresh Luria – Bertani (LB) broth, with or without nanoparticles at sub-MIC, was added to each well of a 96 – well microtiter plate. Next, 1 % of the overnight cultures of the targeted bacteria. Place The microtiter plate in an incubator at 37 °C and incubate overnight. After incubation, the free – floating cells were removed. The wells were thoroughly washed three times with sterile phosphate-buffered saline (PBS). The plate was then allowed to sit at room temperature for 30 min. The biofilm was stained with a crystal violet dye for 10–15 min. The unattached stains were removed by rinsing with sterile PBS. Then, 200 μ L of 95 % ethanol to each well to remove the dye attached to the cells. Biofilm formation was quantified with a microplate reader set to a wavelength of 620 nm. The inhibition percentage of biofilm formation was calculated using the following formula:

$$\text{Percentage of inhibition} = \frac{(\text{Control OD}_{620\text{nm}} - \text{Test OD}_{620\text{nm}})}{(\text{Control OD}_{620\text{nm}})} \times 100$$

Biofilm inhibition by CaO NPs using Confocal Laser Scanning Microscopy (CLSM):

Additional biofilm inhibition analysis was conducted using CLSM, following the method outlined by Banas et al. 2001. A representative strain of the targeted bacteria was utilized in this study. To each well of a 12 – well microtiter plate containing glass coverslips, 100 μ L of the target bacteria was added. Two different concentrations of CaO NPs (50 μ L, low concentration and 100 μ L, high concentration) were used, whereas the control was targeted to live bacterial cells only. The



plates were incubated at 37 °C for 24 h. After incubation, the glass coverslips were rinsed with PBS and stained. Coverslips were treated with 50 µm Acridine Orange (AO) and propidium iodide (PI) for 15 min at room temperature. This procedure allowed for the identification of dead bacterial cells, which appeared red in colour. After 15 minutes of staining, the cells were rinsed with PBS and then treated with 50 µg/ml concanavalin A conjugated with fluorescein isothiocyanate (ConA-FITC) for an additional 15 minutes to stain the glycocalyx matrix green. PI was excited at 520 nm, with emission detected at 620 nm, while ConA-FITC was excited at 495 nm, with emission detected at 525 nm. The bacteria were visualized using CSLM, and images were taken from randomly selected areas.

4. Results

The experiments are typically conducted three times to ensure reliability, and the results of data collection are usually expressed as mean values with their comparable standard deviations. It was determined properly and ascertained with related rigor in the statistical analysis, involving the ANOVA and linear regression model, in order to provide the IC50 value in terms of concentration at which 50% inhibition occurred. The IC50 value is an important parameter for evaluating the biological functionality and potential applications of nanoparticles.

FTIR ANALYSIS

Calcium oxide nanoparticles (CaO NPs) synthesized using Coriander leaf extract exhibit distinct Fourier-transform infrared (FTIR) spectroscopy peaks, reflecting the unique chemical interactions and functional groups involved in their formation. The FTIR spectrum of these nanoparticles reveals significant peaks at 3781.88 cm⁻¹ and 3321.46 cm⁻¹, corresponding to O-H stretching vibrations, indicating the presence of hydroxyl groups likely from the plant extract. The peaks at 2336.50 cm⁻¹ and 1726.46 cm⁻¹ are attributed to CO₂ and C=O stretching vibrations, respectively, suggesting possible adsorption of atmospheric CO₂ and carbonyl compounds from the extract. The peaks at 1560.80 cm⁻¹ and 1406.73 cm⁻¹ indicate C=C and C-H bending vibrations, highlighting organic constituents in the extract. Additional peaks at 1247 cm⁻¹ and 1025.24 cm⁻¹ correspond to C-O stretching, while the lower frequency peaks at 669.13 cm⁻¹ and 615.65 cm⁻¹ are characteristic of Ca-O vibrations, confirming the formation of CaO nanoparticles.

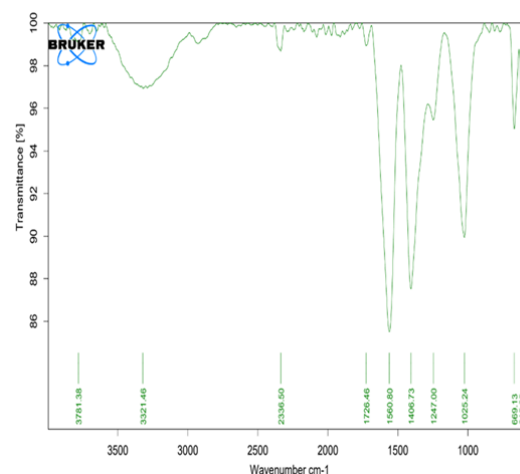


FIGURE: 1 Fourier-transform infrared (FTIR) spectrums

XRD (X-ray diffraction):

Calcium oxide nanoparticles (CaO NPs) synthesized from Coriander leaf extract exhibit a distinctive crystalline structure as revealed by X-ray diffraction (XRD) analysis. The XRD pattern shows prominent peaks at 25.51°, 31.77°, 37.94°, 45.48°, 48.24°, 54.41°, 55.24°, 68.95°, 70.51°, and 75.11°, which correspond to the specific planes of the cubic phase of CaO. These sharp and well-defined peaks indicate the high crystallinity and purity of the synthesized nanoparticles. The peak at 31.77° is particularly significant, which is a characteristic reflection of CaO, confirming the formation of the desired oxide. The presence of these peaks, matching well with the standard JCPDS (Joint Committee on Powder Diffraction Standards) card for CaO, underscores the efficacy of Coriander leaf extract as a biotemplate and reducing agent in the green synthesis process.

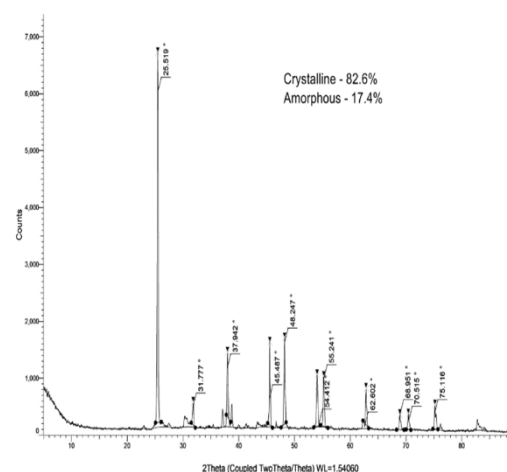


FIGURE : 2 X-ray diffraction (XRD) analysis



Scanning electron microscope (SEM):

A Scanning Electron Microscope (SEM) is a powerful imaging tool used to observe the surface structure and morphology of samples at a very high resolution. Unlike optical microscopes, which use light, SEM uses a focused beam of electrons to scan the surface of a sample. SEM provides detailed, three-dimensional images that reveal fine surface features, textures, and topographies of materials. It can magnify objects up to several million times their actual size, allowing scientists to examine very small details, such as the shape and size of particles or cells, down to the nanometer scale. These particles are well distributed with aggregation and greater numbers of the agglomerated spherical particles within the diameter range of 70 to 95 nm.

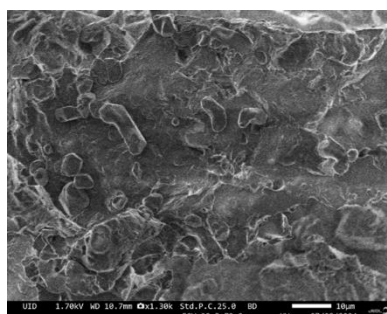
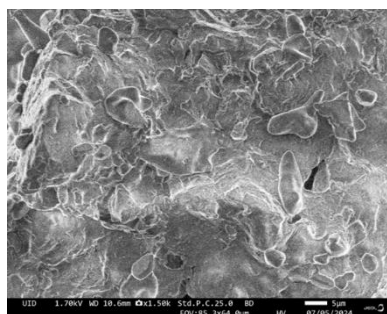


FIGURE 3 AND 4 shows agglomerated spherical particles indicating CaO NPs. All images taken at *50 magnification and acquired using ZEN software.

Antimicrobial activity:

The antibacterial activity of CaO oxide synthesized from Coriander leaf extract were performed for four organisms named *Streptococcus pyogenes*, *Lactobacillus* Sp., *Escherchia coli*, *Staphylococcus aureus* at four different concentrations like standard control, 50 μ l, 75 μ l and 100 μ l respectively. The *Streptococcus mutans* shows about 10.2 mm, 6.3 mm, 8.4 mm and 9.6 mm whereas *Lactobacillus* Sp. shows about 9.6 mm, 6.7 mm, 8.2 mm and 9.1 mm respectively. This results that *Streptococcus pyogenes* & *Lactobacillus* sp. is sensitive than other bacterial strains.

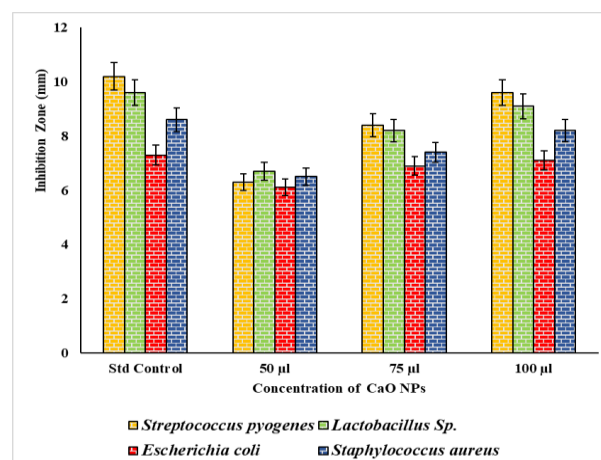


FIGURE: 5 The graph represents the antibacterial activity of CaO NPs against *Streptococcus pyogenes*, *Lactobacillus* Sp., *Escherchia coli*, *Staphylococcus aureus*

Antibiofilm activity:

Confocal microscopy images stained with Acridine Orange (AO) and Propidium Iodide (PI) demonstrate clear differentiation of cell viability. In panel (a), cells exhibit strong AO (green) fluorescence without PI (red), indicating predominantly viable cells with intact membranes. Panel (b) shows a mixture of green and red fluorescence, suggesting partial cell death with some intact and some compromised cell membranes. Panel (c) exhibits primarily PI staining, characterized by intense red fluorescence, indicative of extensive cell membrane damage and predominant cell death. Overall, the images illustrate a clear progression from high cell viability in (a) through intermediate viability in (b) to extensive cell death in (c).

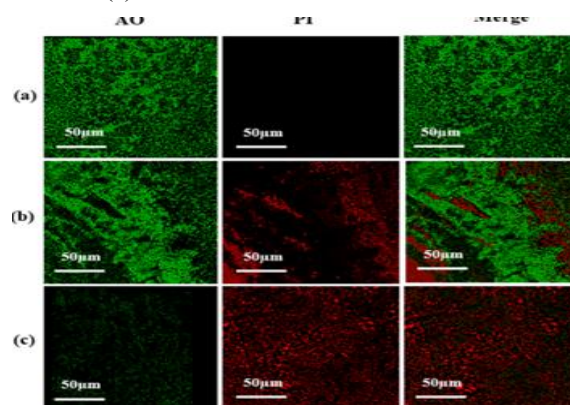


FIGURE : 6 represents Confocal laser scanning microscope (CLSM) analysis where Acridine Orange (AO) and propidium iodide (PI) are used to identify dead bacterial cells, which appeared red.



5. Discussion

The nanomaterial industry produces large amounts of metal-based nanomaterials for various technological and biomedical uses. However, this process also significantly harms the environment due to the use of toxic chemicals and the generation of hazardous waste. Furthermore, the toxic chemicals used can impact the biocompatibility of the resulting particles, significantly limiting their potential applications. For these reasons, biogenic synthesis methods have developed, providing eco-friendly, sustainable, and nature-based alternatives for production, which help reduce the environmental impact of the nanomaterial industry. Nanotechnology is a cutting-edge and rapidly developing field with a wide range of applications, including packaging, biomedical advancements, drug and gene delivery, tissue engineering, healthcare, the food industry, and the space sector²⁰. It is the field focused on manipulating matter at the atomic level, specifically with particles smaller than 100 nm²¹.

SEM images of CaO nanoparticles synthesized using *Coriander* leaf extract shown in Figure 3 and 4. The surface morphology and size of the nano-copper by this eco-friendly method showed the nearly monodisperse distribution of particle sizes. The average particle size of the CaO nanoparticles was observed around 70 to 95 nm. It shows mostly spherical CaO nanoparticles, as well as the number of aggregates, synthesized nanoparticles and some of them, show the undefined shape of nanoparticles. XRD spectrum provides an insight into the crystallinity of the nanoparticle represent. The CaO nanoparticles synthesized using leaf extract of *Coriander* was confirmed by X-ray diffraction analysis.

Green synthesis of nanoparticles is gaining popularity because plants serve as both reducing and capping agents, making the process environmentally friendly²⁵. The process typically involves mixing coriander leaf extract with a calcium precursor, often calcium chloride. The natural reducing agents in the extract lead to the formation of CaO NPs, which can be characterized using techniques like UV-Vis spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) to confirm their size, shape, and crystalline structure.

The antibiofilm activity of these biogenic CaO NPs against oral pathogens, such as *Streptococcus mutans* and *Lactobacillus sp.*, is particularly promising. Biofilms formed by these pathogens are a major contributor to dental caries and oral infections. Studies have indicated that CaO NPs exhibit significant antimicrobial

properties, potentially disrupting biofilm formation and promoting the death of bacterial cells. The mechanism may involve the generation of reactive oxygen species (ROS), which can damage microbial cell membranes and disrupt metabolic functions.

Overall, the use of coriander leaf extract for the synthesis of CaO NPs represents a sustainable approach with potential applications in dental therapeutics, addressing the growing challenge of antibiotic resistance and biofilm-related infections in oral health. Further research is necessary to optimize synthesis conditions and fully understand the mechanisms of action and efficacy of these nanoparticles in clinical settings.

This study concluded that CaO nanoparticles synthesized from Coriander leaf extract demonstrate significant antibiofilm and antibacterial properties oral pathogens. The green synthesis of the nanoparticles was initially confirmed by the FTIR band at 669.13 cm⁻¹ and 615.65 cm⁻¹ are characteristic of Ca-O vibrations, confirming the formation of CaO nanoparticles. The nanoparticles were found to have a spherical shape. The XRD spectrum indicated the presence of crystalline nanostructured CaO particles. The CaO nanoparticles exhibited enhanced antibacterial and antioxidant activities against oral pathogens like *Lactobacillus sp.* and *Streptococcus pyogenes*.

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