Isolation, Identification, Fermentation and Antimicrobial Activity of Streptococcus SP in Soil Sample

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Abstract: **- *Streptococcus* bacteria are ubiquitous in various environments, including soil, where they play crucial roles in nutrient cycling and ecosystem health. However, their presence and dynamics in soil ecosystems remain poorly understood. This study aims to comprehensively evaluate the abundance, diversity, and ecological significance of *Streptococcus* species in soil sources. Using advanced molecular techniques, including next-generation sequencing and bioinformatics analysis, we will characterize the *Streptococcus* community in soil samples collected from diverse geographic locations. Additionally, we will investigate the potential factors influencing *Streptococcus* distribution and abundance, such as soil pH, moisture content, and organic matter content. Furthermore, the functional roles of *Streptococcus* strains will be explored through metagenomic analysis, shedding light on their contributions to soil processes, such as carbon and nitrogen cycling. The findings of this study will advance our understanding of *Streptococcus* ecology in soil ecosystems and provide insights into their ecological functions and potential applications in agriculture and environmental management.**

Keywords: **- Isolation, Identification, Fermentation, Antibiotic susceptibility testing.**

1. INTRODUCTION

1.1 *Streptococcus* Bacteria: -

Soil-extracted *Streptococcus* bacteria were associated serologically and in other respects with typical *Streptococcus* pneumoniae bacteria. The non-capsulated microorganisms were linked to a substantial number of the capsulated bacteria; autolysis and animal passage eliminated some capsular types and temporarily improved laboratory growth conditions. Strains of the microorganism were associated serologically with untypable pneumococcus-like bacteria. Possible ways of testing the roles of soil Streptococcus in pneumonia epidemiology are discussed.

Structure - Streptococci are Gram-positive, nonmotile, nonsporeforming, catalase-negative cocci that occur in pairs or chains. Older cultures may lose their Gram-positive character. Most streptococci are facultative anaerobes, and some are obligate (strict) anaerobes. Most require enriched media blood agar. [1]

1.2 Overview of *Streptococcus* Taxonomy

Streptococcus species are often difficult to isolate from environmental samples. Some soils have not yet been found to contain naturally occurring *Streptococcus.* Natural isolate repositories obtain their strains from biological, soil, and other environments, which help to demonstrate the presence of *Streptococcus* in these environmental sources and environments. In general, there is some correlation between S. thermophilus and *Streptococcus* isolates because S. thermophilus lyses efficiently in a viral infection, but this partitioning is not reliable. Pulsed-field gel electrophoresis is very widely accepted as a robust profiling method for S. thermophilus identification, separation-based due to its apparent lack of unique gene markers and high genetic diversity. *Streptococcus* is a genus of gram-positive coccus bacteria. The infecting species are a part of the normal commensal human and animal microflora. In addition to *Streptococcus,* there are presently approximately 20 additional described genera in the family Streptococcaceae. This figure represents almost 100 officially recognized species. *Streptococcus* contains numerous species, mostly obligately fermentative forms, but also some facultative anaerobic forms. This genus has been consistently identified in diverse environments due to its contributions to processes like cheese ripening, silage fermentation, as well as its causative roles in

bovine mastitis and necrotizing fasciitis.

2. LITERATUREREVIEW

1)H.J.Kibbey et.al - The survival, recovery, and identification of *Streptococcus* isolates from soilwas investigated by (i) examination of survival in soil under different moistureand temperature conditions, (ii) evaluation of media combinations for recoveringfecal streptococci from soil, and (iii) partial identification of isolates from diversehabitats. Cool, moist conditions prolonged the survival of *Streptococcus* faecalisin soil for at least 12 weeks, whereas freezing was lethal, with the populationsbeing reduced up to 95% when several freeze-thaw treatments occurred. Mediaevaluations indicated that both the efficiency of recovery and enumeration of thefecal streptococci from soil can be influenced by the combination of media used.Taxonomic data revealed a need to develop procedures to differentiate betweenisolates of fecal origin and plant-derived streptococci that possess many of thecultural reactions of S. faecalis. It was found that recent fecal isolates exhibiteda much greater incidence of multiple antibiotic resistance than soil or vegetationisolates, and this characteristic, coupled with the use of enterococci as indicatorsof fecal contamination in soil systems, is discussed.[9]

2)TJ Beveridge et.al -The Gram stain differentiates bacteria into two fundamental varieties of cells. Bacteria that retain the initial crystal violet stain (purple) are said to be ``Gram-positive,'' whereas those that are decolorized and stain red with carol fuchsin (or safranin) are said to be ``Gram-negative.'' This staining response is based on the chemical and structural makeup of the cell walls of both varieties of bacteria. Gram-positives have a thick, relatively impermeable wall that resists decolorization and is composed of peptidoglycan and secondary polymers. Gram-negatives have a thin peptidoglycan layer plus an overlying lipid-protein bilayer known as the outer membrane, which can be disrupted by decolorization. Some bacteria have walls of intermediate structure and, although they are officially classed as Gram-positives because of their linage, they stain in a variable manner. One prokaryote domain, the Archaea, have such variability of wall structure that the Gram stain is not a useful differentiating tool [10].

3)ROBERT K. ALICO et.al -Several selective media were evaluated for the primary isolation and enumeration of Staphylococcus aureus from halogenated indoor swimming pool waters. Standard plate counts of the viable population and total coliform densities were also determined to ascertain their value as indicator systems. All studies were done with membrane filters. The most selective, accurate, and reliable medium was Vogel-Johnson (VJ) medium supplemented with 0.5% pyruvate. This medium recovered two times more typical colonies than VJ medium alone, and subsequent identification of these well-defined black colonies proved that approximately 80% were S. aureus. The S. aureus recoveries correlated well with halogen levels and bather density use also. In contrst, VJ medium alone was 60% selective for S. aureus, and VJ medium supplemented with catalase did not increase either the percent recovery or the selectivity over that of VJ medium alone. Standard plate counts did not correlate with halogen levels, bather density, or total viable colonies. Coliforms were rarely recovered from indoor pool waters and were not considered to be useful indicators of water quality [9].

3. AIM AND OBJECTIVE

The main objectives of these systems are follows

- To collect the soil sample for microbial activity
- In detailed study of *streptococcus sp*
- To optimize the process of antimicrobial activity
- To evaluate and prepared report of result

4. NEED OF WORK

- Isolation of *Streptococcus sp* from soils.
- To purify sample and identify the isolates.
- To perform identification test of isolated Bacteria.
- To obtain extract from fermented broth.

5. PLAN OF WORK

6. MATERIALS USED

Ingredients	Supplier
Nutrient Growth Agar	AMIP, Ambap
Glucose Broth	AMIP, Ambap
Sterile Water	AMIP, Ambap
Soil	AMIP, Ambap
Alcohol	AMIP, Ambap
Ethyl Acetate	AMIP, Ambap

7.INSTRUMENT USED

Sr. No	Name of Instruments
1	Microscope
2	Autoclave
3	Centrifuge
4	UV Cabinet
5	Laminar Air Flow
6	Incubator

8. MATERIALS AND METHOD

8.1 Study area and sample location

The study was carried out in the town Maharashtra, Kolhapur, Ambap. In Ashokrao mane institute of Pharmacy, Ambap. The study area for evaluating *Streptococcus sp* in soil locations, including agricultural fields, forests, or urban areas.

8.2 Bacteria Isolation: -

 Collection of Soil Sample: Collect soil from the desired location using a sterile spatula or scoop. Take samples from different depths and locations to ensure a diverse microbial population.

 Preparation of Dilutions: Weigh out a specific amount of soil sample (usually 1 gram) and mix it with a known volume of sterile saline solution (such as 0.85% NaCl). Serial dilutions are then prepared by transferring aliquots of the soil suspension into tubes containing fresh sterile saline solution.

 Inoculation onto Agar Plates: Spread a small volume (usually 0.1 mL) of each dilution onto the surface of agar plates using a sterile spreader or glass rod. Commonly used agar media for bacterial isolation include nutrient agar, tryptic soy agar, and R2A agar.

 Incubation: Incubate the plates at the appropriate temperature (usually 25-37°C) for a specific period (typically 24-48 hours). This allows bacteria to grow and form visible colonies.

 Colonies Selection: After incubation, observe the plates for the presence of bacterial colonies. Select distinct colonies based on their morphology (size,

shape, color, texture) and transfer them onto fresh agar plates to obtain pure cultures.

 Subculture: Streak the selected colonies onto new agar plates to obtain isolated colonies. This helps ensure the purity of the cultures.

Identification: Perform biochemical tests, molecular techniques (such as PCR), or use commercial identification kits to identify the isolated bacteria. Biochemical tests may include catalase test, oxidase test, Gram staining, and various metabolic assays. (2)

Distinct colonies were isolated and stored on nutrient agar slants at 40C in a refrigerator.

9. PROCEDURE

For the water samples, 1ml each was pipetted into a sterile petri dish, thereafter,20ml of sterile nutrient agar was poured into it using pour plate method. It was then rocked gently to mix and allowed to set. Inoculated agar plate was afterwards opened briefly for 30 seconds in an incubator set at 370C, so as to remove condensed water on its surface. Thereafter, covered, inverted and inoculated at 370C for 24 to 48hrs in the same incubator. Using their colonial appearance such as size, shape, consistency, color, elevation and the differential characteristics such as pigmentation, suspected discrete colonies of S. aureus were sub-cultured by re-streaking on nutrient agar plates to obtain a pure culture according to guidelines.[3] Distinct well-separated yellow colonies and creamy white colonies on MSA and blood agar respectively were picked aseptically and stored on nutrient agar slants at 40C for further biochemical characterization. Each organism was subjected to some preliminary bio chemical tests such as gram staining, catalase, mannitol fermentation, blood hemolysis and slide coagulase test.[4]

10. IDENTIFICATION

10.1 Antimicrobial susceptibility testing (AST)

Susceptibility testing is used to determine which antimicrobials will inhibit the growth of the bacteria causing a specific infection.

10.2 Isolation and Identification

I. Sample Collection and Culturing:
- Clinical samples such as blood, wound swabs, or respiratory specimens are collected from the patient.
- The samples are cultured on selective media such as blood agar to isolate *Streptococcus sp*.

II. Identification:
- Colony morphology, hemolysis patterns, and biochemical tests like the coagulase test are used to confirm the identity of *Streptococcus* [5]

10.3 Antimicrobial Susceptibility Testing Methods

I. Disk Diffusion Method:

This method consists of inoculating the isolated bacteria onto a Mueller-Hinton agar plate, followed by placing antibiotic-impregnated paper disks on the surface of the agar. By incubating this plate, antibiotics will diffuse into the agar in a gradient; the antibiotic concentration will decrease as the distance from the disk increases. Antibiotic susceptibility is determined by measuring the diameter of the zones of bacterial inhibition around the antibiotic disks and comparing the diameter with disk diffusion interpretive criteria (Schwalbe, et al., 2007).(6)

II. Broth Dilution Method:
- Prepare serial dilutions of antibiotics in a broth medium.
- Inoculate each dilution with a standardized number of bacteria.
- Incubate the tubes at 35°C for 16-20 hours.
- Determine the minimum inhibitory concentration (MIC) as the lowest concentration that inhibits visible growth.
- Interpret the MIC values using CLSI or EUCAST breakpoints.

III. Automated Systems:
- Use automated systems like VITEK, Micro Scan, or BD Phoenix, which utilize cards or panels with multiple antibiotics.
- These systems provide rapid and standardized results.

IV. Methicillin-Resistant Streptococcus Testing:
- Use the cefoxitin disk diffusion test as a surrogate marker for mecA-mediated resistance.
- Perform PCR to detect the mecA gene directly, which confirms MRSA.

Antibiotics are commonly tested to determine the susceptibility of Streptococcus bacteria to them. Some of the antibiotics typically tested for streptococcal infections include:

a. Penicillin: Penicillin is one of the primary antibiotics used to treat streptococcal infections. Testing for penicillin susceptibility helps determine if the strain of Streptococcus is sensitive or resistant to this antibiotic.
b. Erythromycin: Erythromycin is a macrolide antibiotic often used as an alternative to penicillin, especially in individuals with penicillin allergies. Testing for erythromycin susceptibility helps guide antibiotic selection.
c. Clindamycin: Clindamycin is another antibiotic commonly tested for streptococcal infections, particularly for strains that are resistant to penicillin or erythromycin.
d. Ceftriaxone: Ceftriaxone is a cephalosporin antibiotic that may be used to treat severe or resistant streptococcal infections. Testing for ceftriaxone susceptibility helps determine its effectiveness against the specific strain of Streptococcus.
e. Vancomycin: Vancomycin is a potent antibiotic often reserved for treating serious infections caused by multidrug-resistant strains of bacteria, including some strains of Streptococcus.
f. Azithromycin: Azithromycin, another macrolide antibiotic, may be tested for
g. with crystal violet (primary stain) and let it stand for about
h. 1 minute. Susceptibility in cases where erythromycin is not suitable or effective.

10.4 Importance of Susceptibility testing

- The results from this test helps to determine which drugs are likely to be most effective in treating a person's infection.
- Aids in the evaluation of treatment services provided by hospitals, clinics and national programs for control and prevention of infectious diseases.
- Monitor for resistance patterns due to the mutations in bacterial DNA
- Ensures the selection of effective antibiotics, minimizing the use of broad-spectrum agents.
- Helps in monitoring resistance trends and informing public health strategies.
- Improves patient outcomes by tailoring antimicrobial therapy based on susceptibility results.[7]

10.5 The Gram stains: -

The general protocol for performing a Gram stain on bacteria obtained from soil:

- Prepare the Bacterial Smear: Take a small amount of soil sample and suspend
- it in a drop of sterile water on a clean glass slide. Spread the suspension thinly over the slide to create a bacterial smear. Allow it to air dry.
- Heat Fixation: Pass the slide through the flame of a Bunsen burner several times to heat fix the bacterial cells. This helps to adhere the cells to the slide and denatures their proteins, preventing them from washing away during staining.
- Gram Staining:
  - Cover the bacterial smear
  - Rinse the slide gently with water to remove excess stain.
  - Flood the smear with Gram's iodine (mordant) and let it stand for 1 minute. This helps to fix the crystal violet in the cell wall.
  - Decolorize the smear by gently rinsing it with ethanol or acetone until no more colour runs off (usually for about 10-20 seconds). This step is crucial as it differentiates between Gram-positive and Gram-negative bacteria.
  - Quickly rinse the smear with water to stop the decolorization process.
  - Counterstain the smear with safranin (counterstain) for about 30 seconds.
  - Rinse the slide gently with water and blot it dry with bibulous paper.

Microscopic Examination: Examine the stained smear under an oil immersion microscope (1000x magnification). Gram-positive bacteria will appear purple or blue, while Gram-negative bacteria will appear pink or red.

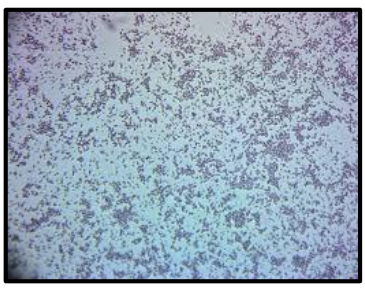


Fig 1: - Gram Staining of soil sample

11. FERMENTATION AND EXTRACTION

Based on the results from preliminary screening of potent antibacterial activity, one endophytic isolate *Streptococcus sp* was selected for further studies. e inoculum was prepared by inoculating 1cm2 nutrient agar plugs age 7 days into two 1000mL Erlenmeyer flasks, each containing 500mL of the SD broth medium. e cultures were cultivated at 28°C with speed of 150rpm. After three weeks of incubation, the fermented broth and fungal bio mass were separated out by centrifugation for 15min. Supernatant was then extracted thrice with equal volume of ethyl acetate (1:1, v/v). e upper organic phase was con contracted to dryness under reduced pressure to obtain the crude organic extract. (8)

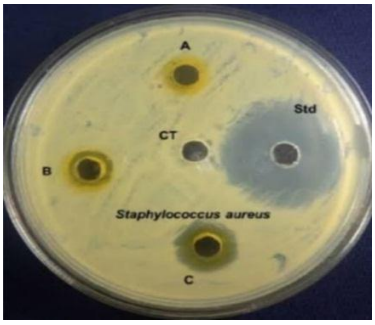
12. RESULT & DISCUSSION

Identification and Enumeration of *Streptococcus sp*: Describe the methods used for sampling soil and isolating *Streptococcus species*. Present the quantitative results, such as colony-forming units per gram (CFU/g) of soil. Discuss any variations in *Streptococcus species* diversity across different soil samples or locations.

Characterization of *Streptococcus sp* Isolates: Detail the biochemical and genetic characterization techniques used Present the identified species or strains of *Streptococcus* found in the soil samples. Discuss any novel or potentially pathogenic strains identified.

12.1 ANTIMICROBIAL ACTIVITY

Sr. no	Sample	Zone of Inhibition (mm) 1µl	Zone of Inhibition (mm) 2µl	Zone of Inhibition (mm) 3µl
1	Ampicillin (Standard)	20	19	21.5
2	*Streptococcus*	10	11	12



Fig; Antimicrobial Activity

13. CONCLUSION

Presence of *Streptococcus sp* in Soil: The study likely confirms the presence of *Streptococcus* bacteria in soil samples, indicating that soil serves as a reservoir for these microorganisms.

Variability in *Streptococcus* Species: The research may have identified various species or strains of *Streptococcus* within the soil samples, highlighting the diversity of these bacteria in different environments.

Implications for Human Health: If relevant, the study could discuss the implications of *Streptococcus sp* presence in soil for human health, such as the potential for soil as a reservoir for pathogenic species or the role of environmental factors in bacterial transmission.

Our findings highlight the critical need for stringent surveillance of water sources for AMR pathogens. The significant levels of antibiotic resistance in *Streptococcus sp isolated* from various soil environments suggest that water bodies play a crucial role in the dissemination and persistence of AMR. Implementing effective waste management practices, controlling antibiotic usage, and developing soil treatment protocols are essential strategies to mitigate the spread of AMR in the environment. Further research should focus on the molecular mechanisms underlying resistance in environmental isolates and the potential impact on public health.

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