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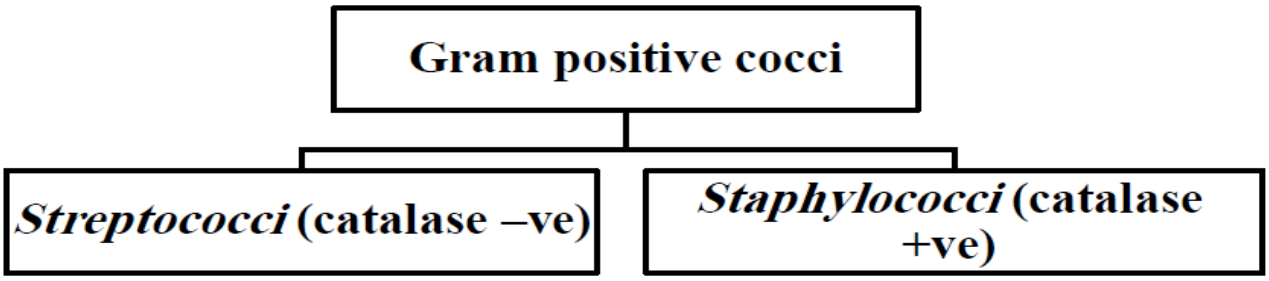
# GRAM-POSITIVE BACTERIA



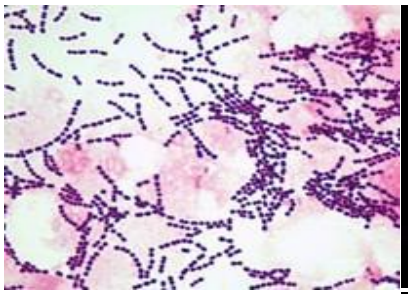

# Streptococci

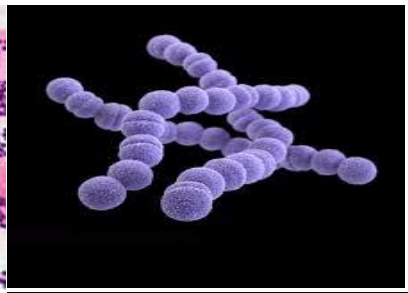
## INTRODUCTION

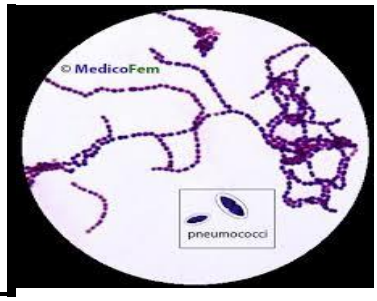
Streptococci are Gram-positive cocci arranged in chains or pairs. They are part of the normal flora of humans and animals. Some of them are human pathogens. The most important of them is *Streptococcus pyogenes* causing pyogenic infections, with a characteristic tendency to spread, as opposed to staphylococcal lesions, which are typically localized. It is also responsible for the nonsuppurative lesions, acute rheumatic fever and glomerulonephritis which occur as sequelae to infection.

## Characters of Streptococci

**facultative anaerobe**, **Gram-positive**, **Cocci or ovoid ( Spherical) bacteria** , **usually chains (sometimes pairs)**, **Some are members normal flora , others associated with important human diseases , Some capsulated (polysaccharide)such as pnemococci. (Hyaluronic acid ,Group A,B,C) in young culture ,Protection against phagocytosis , Cell wall contain M,T,R protein,carbohydrate and peptedoglycan ,pili (attachment).**







Streptococci in oral cavity

In the human oral cavity the Streptococci constitute the most numerous group of bacteria and cause oral infections .The Streptococci have a critical role in the dental caries ,and the periodontitis.

**Classification of Streptococci based on :-**

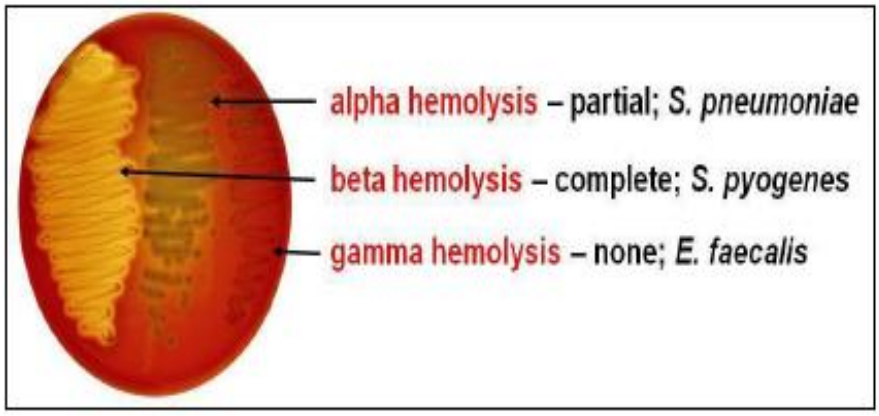
**(I) - Hemolysis reactions on blood agar) (Brown in 1903)**
The type of hemolytic reaction on blood agar has long been used to classify the streptococci.

**1. Alpha (α) hemolytic streptococci**

Alpha (α) hemolytic streptococci produce a greenish discolouration with partial hemolysis around the colonies known as 'viridans streptococci' or Streptococcus viridians (from 'viridis' meaning green). The alpha streptococci are normal commensals in the throat, but may cause opportunist infections rarely. Pneumococcus (***Streptococcus pneumonia***) is also an alpha hemolytic streptococcus.

**2. Beta (β) hemolytic streptococci**
Beta (β) hemolytic streptococci produce a sharply defined, clear, colourless zone of hemolysis, 2-4mm wide, within which red cells are completely lysed. Most pathogenic streptococci belong to this group **e.g. *Streptococcus pyogenes.***

**3. Gamma (γ) or nonhemolytic streptococci**
Gamma (γ) or nonhemolytic streptococci produce no change in the medium. They include the fecal streptococci ***(enterococci, Str faecalis)*** and related species (enterococcus group).



**(II) - Antigenic types of carbohydrate (Serology)**

Hemolytic streptococci were classified by Lancefield serologically into groups based on the nature of a carbohydrate (C) antigen on the cell wall. These are known as Lancefield groups, twenty of which have been identified so far and named A-V (without I and J). The great majority of hemolytic streptococci that produce human infections belong to group A. Hemolytic streptococci of group A are known as *Streptococcus pyogenes*. These may be further subdivided into types based on the protein (M, T and R) antigens present on the cell surface (Griffith typing). About eighty types of Str pyogenes have been recognized as far (types 1,2,3 and so on).

**(III) -Capsular polysaccharides** :classified ***Streptococcus pneumoniae*** into 84 types and to type the group B streptococci (***Streptococcus agalactiae)***.

**(V)-Biochemical reaction :**
Sugar fermentation.
Presence enzymes .
Susceptibility test or resistance to certain chemical agents .

**Antigenic structure**

1)-**Group –specific cell wall antigen**

**Carbohydrate in the cell wall** (**Lancefield groups) .A-H and K-U**

**2) -M protein**

Virulence factor of Group A ***S. pyogenes*** M protein hair-like projections of cell wall . -M-like proteins: binds IgM, IgG and α 2-macroglobulin; resist phagocytosis.

**3)-T substance** acid labile and heat labile differentiation of certain types of streptococci by agglutination with specific antisera .another antigen R protein .

4)-**Nucleoproteins** (P substance) make up most of the streptococci cell body.

Streptococci of Particular Medical Interest

***STREPTOCOCCUS PYOGENES***

**Morphology :** The individual cocci varies in size , they are spherical and oval in shape. They are arranged in chains. Streptococcus has been classified as Str. longus (long chain) & Str. breyis (short chain.). Streptococcus are gram positive, non-motive, non-sporing and capsulated.

**Toxins, Enzymes & other virulence factors :** Streptococcus *pyogenes* produces several types of exotoxins & enzymes those act as virulence factors. Also young protein act as a virulence factor by inhibiting phagocytosis. The C polysaccharide has been shown to have a toxic effect on connective tissue in experimental animals.

**A-Hemolysins :** Streptococci produce two hemolysins, streploysin 'O and 'S'.
Streptolysin O is so called because it is oxygen labile. It is inactive in the oxidized form but may be reactivated by treatment with mild reducing agents. On blood agar, streptolysin O activity is seen only in pour plates and not in surface cultures. It may be obtained in the active state by growing streptococci in broth containing reducing agents such as sodium hydrosulphite. It is also heat labile. It appears to be important in contributing to virulence. It is lethal on intravenous injection into animals and has specific cardiotoxic activity. It has leucotoxic activity also. In its biological action, streptolysin O resembles the oxygen labile hemolysins of Cl. *perfringens*, Cl. *tetani* and the *pneumococcus*.
Streptolysin O is antigenic and antistreptolysin O appears in serum after streptococcal infection, which is very important in diagnosis. Streptolysin O combines with ASO (an antibody that appear in humans following infection with any streptococci produce streptolysin O. block hemolysis .If (antistreptolysin O) titer >160-200 units suggests

I- recent infection II- exaggerated immune response to an earlier exposure in a hypersensitive persone .

Streptolysin S is so called because it is soluble in serum. It shows stability with oxygen, dry heat. It is responsible for hemolysis seen on the surface of the blood agar plates. It also has leucocidal activity.

**B-Streptokinase (fibrinolysin )**
-Produce by Group A beta –hemolytic
-Plasminogen---------- plasmin
-Active proteolytic enzyme digests fibrin and other protein this process
-Interfered with antistreptokinase
-Can lyses blood clots and may be responsible for the rapid spread of the organism.
-Used (IV injection) for treatment of pulmonary emboli, coronary artery thrombosis and venous thrombosis

**C-Deoxyribonuclease (streptodornase, DNAase) :** It is an enzyme which causes depolymerisation of DNA also showing diagnostic significance as Streptokinase. It shows biological significance because pyogenic exudates contain large amount of DNA. DNAase causes liquefaction of pus & its serus character. It also liquefy thick pus in empyema which

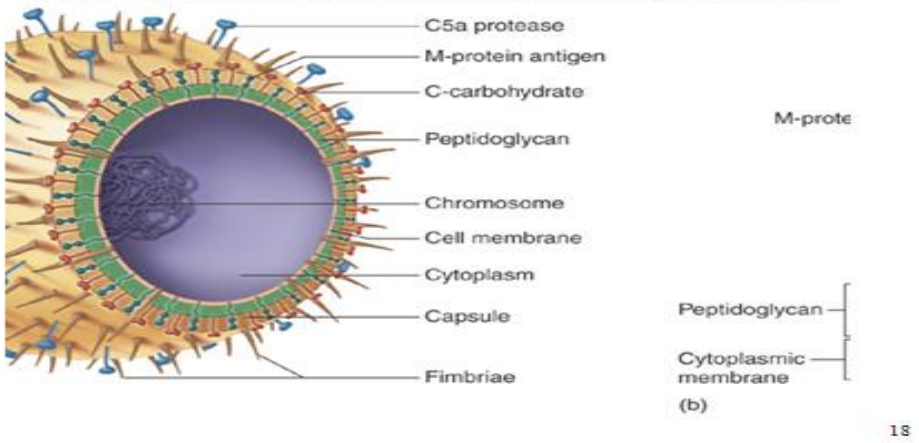
is a therapeutically important. Mixture of Streptodornase & streptokinase used in enzymatic debridement help to liquefy exudates and facilitate removal of pus and necrotic tissue.

D-Pyrogenic exotoxins (erythrogenic toxin) .associated with toxic shock syndrome and scarlet fever . is a toxin named erythrogenic because its intradermal inoculation in susceptible individual causes erythmatous reaction. This test is known as "dick test'. This test is used to identify the children susceptible to scarlet fever so, named as "Scarletinal toxin". This toxin induces fever so named as streptococcal pyrogenic exotoxin.

**E-Hyaluronidase** : It is a enzyme which breaks down the hyaluronic acid of tissue. It is a biological significance it helps in spread of infection.
F-Diphosphopyridin nucleotide : kill leukocytes , another enzyme proteinases and amylase .

**Antigenic Structure :** The capsule when present inhibitis phagcocytosis. It is not antigenic in human beings. The cell wall is composed of an outer layer of protein and lipoteichoic acid, a middle layer of group-specific carbohydrate and in inner layer of peptidoglycan. The peptidoglycan (mucoprotein) is responsible for cell was rigidity. It also has some biological properties such as pyrogenic and thrombolytic activity. Serological grouping of streptococci depends on the C carbohydrate. Str pyogenes belongs to group A. As this antigen is an integral part of the cell wall, it has to be extracted for grouping by a precipitation test with group antisera. Several protein antigens have been identified in the outer part of the cell wall. Str pyogenes can be typed based on the surface proteins M, T and R. The M Protein is the most important of these. It acts as a virulence factor by inhibiting phagocytosis. It is antigenic. The antibody to the M protein promotes phagocytosis of the coccus and is therefore protective. The M protein is heat and acid stable but susceptible to tryptic digestion. It can be extracted by the Lancefield acid extraction method and typing is done with type-specific sera. The T protein is an acid labile, trypsin resistant antigen present in many serotypes of Str pyogenes. It may be specific but many different M types possess the same T antigen. It is usually demonstrated by the slide agglutination test using trypsintreated whole streptococci. Hair-like pili (fimbria) project through the capsule of group A streptococci. The pili consist partly of M protein and are covered with lipoteichoic acid which is important in the attachment of streptococci to epithelial cells.

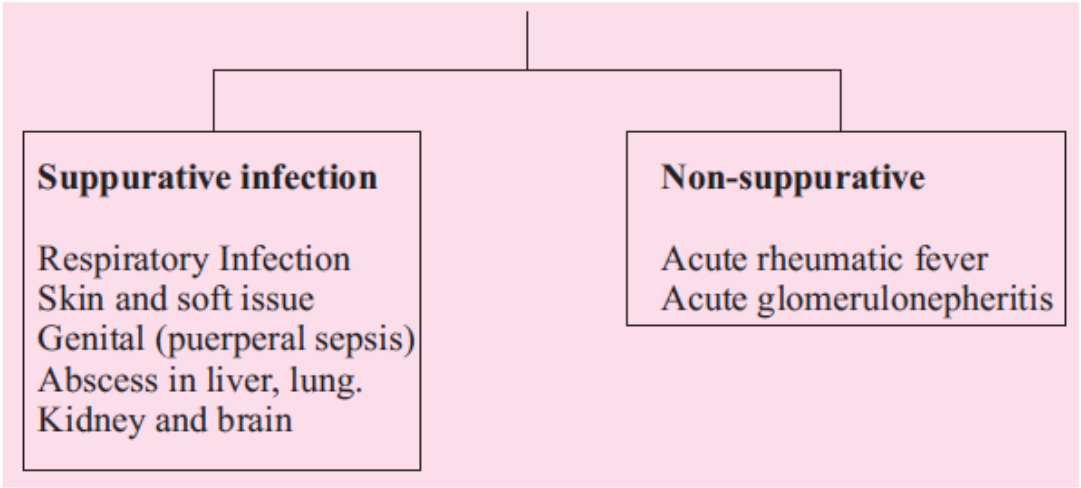




***Streptococcus pyogenes*** (Group A) .

**Pathogenesis of *Strep. pyogenes***

It is a major success pathogen to its ability to colonize, rapidly multiply and spread in host while evading phagocytosis and confusing the immune system. It is found usually in the respiratory tract, without signs of disease. *Strep. pyogenes* can infect when defenses are compromised or when its able to penetrate the constitutive defenses. Streptococcal diseases are associated in **respiratory tract (pharyngitis or tonsillitis)**, **bloodstream**, or the **skin (pyoderma)**. **Summary of diseases caused by *Strep. pyogenes***




™ **A-Respiratory Infection**
™ - Tonsillitis and pharyngitis
™ - Peritonsillar abscess .
™ -Mastoiditis & sinusitis .
™ -Otitis media.

**B-Skin and soft tissue infections**

**Erysipelas :** It is a diffuse infection involving the superficial lymphatics.
**Impetigo** : (superficial) infection of epidermal layers of skin.
**Cellulitis:** (deep) occurs when the infection spreads subcutaneous tissues.
**Wound infection** leading to cellulitis and lymphangitis.
**Genital Infections :** Both aerobic & anerobic streptococci are normal inhabitants of female genital tract. They are important causative organism of puerperal sepsis.

™

## Systemic infection

**Scarlet fever** (rash) :It caused by **erythrogenic toxin**.
**Toxic shock;** caused by a few strains that produce a toxic shock toxin (Superantigens).
Invasive, toxigenic streptococci cause **joint or bone infections**, and **myositis**, **meningitis** and **endocarditis, Bacteremia.**

**Other suppurative infections :** Str pyogenes may cause abscesses in internal organs such as the brain, lungs, liver and kidneys, and also septicemia and pyemia.

**Nonsuppurative complications : Non-suppurative Sequelae:**

**1-Rheumatic fever. M protein** cross reacts with sarcolemma.- Antibodies cross-react with heart tissue, fix complement, and cause damage.
**2-Glomerulonephritis.** Antigen- antibody complexes may be deposited in kidney, fix complement, and damage glomeruli- Only a few M-types are nephritogenic.

**Laboratory diagnosis of streptococci :** In acute infections, diagnosis is established by culture, while in the nonsuppurative complications, diagnosis is mainly based on the demonstration of antibodies. The sample collection require for acute conditions are throat swab, pus or blood for isolation of Str. Pyogen & Vaginal Swab, blood, CSF, ear swab for Str. Agalactiae, Urine & blood for enterococci. There are different methods of demonstrating organisms in direct method of organisms. In Microscopy Gram Staining is done for Gram Positive Cocci which formed chain and non motile which is indicative of Streptococci. Microscopy don't have any value in throat & genetal infections because these streptococci are part of resident flora.

**Host defenses against *Strep. pyogenes* infections**

In the normal human the **skin** is an effective barrier against invasive streptococci, and **nonspecific defense** mechanisms prevent the bacteria from penetrating the superficial epithelium of the **upper respiratory tract** (cilia movement, coughing, sneezing and epiglottal reflexes).
The **host phagocytic system** is a **second line** of defense against streptococcal invasion. Organisms can be opsonized by activation of the **complement** pathway and by **anti-streptococcal antibodies** in the serum.
*Strep. pyogenes* is rapidly killed following phagocytosis enhanced by specific antibody.
The bacteria do not produce **catalase** or significant amounts of **superoxide dismutase** to inactivate the oxygen metabolites (hydrogen peroxide, superoxide) produced by the oxygen-dependent mechanisms of the phagocyte. Therefore, they are quickly killed after engulfment by phagocytes. The streptococcal defense must be one to stay out of phagocytes.
In **immune individuals**, **IgG antibodies** reactive with **M protein** promote phagocytosis which results in killing of the organism. This is the major mechanism to terminate Group A streptococcal infections.
Antibody against M protein antigen is the only effective protective antibody, but there are more than 50 different M types (antigenic variation), and subsequent infections may occur with a different M serotype

**Group B (Str. agalactiae)**

-Habitat and Transmission .
- Group B ,In human vagina and anorectal
-Baby acquire infection from mother during delivery or during nursing

Characteristics

-Gram posetive cocci in chain

-B-haemolytic colonies on blood agar large than ***Stre.pyogene***s

Pathogenicity
-Cause neonatal meningitis and septicaemia -septic abortion and gynaecological sepsis

**Group C**
Streptococci of this group are mainly animal pathogen & divided into four species. Group C pathogens from human sources are mainly str. quisimilis species. It causes upper Respiratory tract infection as well as deep infection. It differs from Str. pyrogen that it ferments ribose It is commercial source of thrombolytic therapy.

**Lancefield Group D**
They mainly of two types Entercocci (E.faecalis) & Non-enterococci ( Str. bovis, Str. equines). Normal colonists of human large intestine
Causes; **Nosocomial Infections, opportunistic urinary**, wound and skin infections
Grow in the presence of 6.5% NaCl. **Grow on MacConkey agar .**
Usually non-hemolytic or α hemolytic.
**Naturally high levels of antibiotic resistance** , Sensitivity testing needed for enterococci .
Treatment ( Penicillin+ Gentamycin).
No vaccines available.

**Group D**
They mainly of two types Entercocci (E.faecalis) & Non-enterococci ( Str. bovis, Str. equines). E faecalls is most commond species isolated from human. It can be indentified by its ability to ferment mannitol, sincrose, sorbitol and aesculin & to grow on tellurite blood agar producing black colony. It mainly causes UTI, Wound infection & endocarditis. Non-enterococci are inhibited by 6.5% sodium chloride & bile they case UTI & endocarditis.

## OTHER HEMOLYTIC STREPTOCOCCI

***Streptococcus pneumoniae (Diplococcus pneumoniae*)**

**Gram-positive, cocci. Usually, pairs of cocci (diplococci) alpha hemolytic**, cultured in media that contain blood (**fastidious)**. **Possess a capsule of polysaccharide that permits typing with specific antisera.**Young colonies resemble dew-drops due to capsule-spontaneous autolysis of older bacteria. **Fermentative aerotolerant anaerobe** special tests such as inulin fermentation, bile solubility, Quelling reacion, optochin antibiotic. **Like other streptococci, they lack catalase and ferment glucose to lactic acid. Do not display C- substrate of cell wall composition.**
normal inhabitant of the human upper respiratory tract can cause **pneumonia**, sinusitis, otitis media, meningitis- It also causes osteomyelitis, septic arthritis, endocarditis, cellulitis and brain abscesses. usually secondary to one of the former infections. ***Strep. pneumoniae* is currently the leading cause of invasive bacterial disease in children and the elderly.** do not form spores. non-motile. sensitivity must be routinely employed to differentiate the pneumococcus from *Strep. viridans.* **Pneumonia** is a disease of the lung that is caused by a variety of bacteria including *Streptococcus, Staphylococcus, Pseudomonas, Haemophilus, Chlamydia & Mycoplasma,* several viruses, and certain fungi and protozoans. The disease may be divided into two forms, **bronchial pneumonia** and **lobar pneumonia**.**Bronchial pneumonia:** most prevalent in **infants**, **young children** and **aged adults**. It is caused by **various bacteria, including *Strep. pneumoniae***. produces Patchy Pneumonic Consolidation **Lobar pneumonia:** occur in **younger adults**. A majority **(more than 80%)** of the cases of lobar pneumonia are caused by ***Strep. pneumoniae***. causes consolidation of whole lobe.

## THE VIRIDANS GROUP

This group, formerly called Streptococcus viridians, is a miscellany of streptococci normally resident in the mouth and upper respiratory tract, and typically producing greening (alpha lysis) on blood suger – hence the name viridians. Some of them may be nonlytic. They cannot be categorized under the Lancefield antigenic groups. They are ordinarily nonpathogenic but can on occasion cause disease. In persons with preexisting cardiac lesions, they may cause bacterial endocarditis, Str sanguis being most often responsible. Following tooth extraction or other dental procedures, they cause transient bacterremia and get implanted on damaged or prosthetic valves or in a congenitally diseased heart, and grow to form vegetation. Prophylactic antibiotic cover is advisable in such persons before tooth extraction or similar procedures. While viridians streptococci are generally penicillin sensitive, some strains may be resistant. It is therefore essential that in endocarditis.
Str mutans (so called because it assumes a bacillay form in acid environments) is important in the causation of dental caries. It breaks down dietary sucrose,

producing acid and a tough adhesive dextran. The acid damages dentine and the dextrans bind together food debris, epithelial cells, mucus and bacteria to form dental plaques, which lead to caries.

Oral streptococci divided into four main species :
1-Strep.mutans

2-Strep. Salivarius

3-Strep. Milleri

4-Strep. oralis

Habitat and Transmission
Common inhabitants oral cavity
-One –quarter of the total cultivable flora from supragingival and gingival plague .
-One- half of the isolates from the tongue and saliva .Transmitted from mother to child During intra oral surgical procedures (tooth extraction) Viridans group enter the blood stream causing endocarditis infection

Pathogenicity
The major agent of dental caries is Strep. Mutants .Produce sticky extracellular polysaccharide in the presence of dietary carbohydrates these help tenacious binding of the organisms to enamel and to each other Opportunistic pathogen Breakdown dental enamel from the acidic fermentation products ,carious lesion ,invasion of dentin by microorganism and pulpal infection.

Mutans streptococci are recovered almost exclusively from hard, non-shedding surfaces in the mouth, such as teeth or dentures, and they can act as opportunistic pathogens, being isolated from cases of infective endocarditis (biofilms growing on damaged heart valves.

Mutans streptococci possess cell wall carbohydrate antigens, lipoteichoic acid, lipoproteins and cell wall or cell wall-associated proteins. Streptococci antigen may be involved in the initial adherence of S. mutans to the tooth surface by interacting with components of the salivary pellicle.

Mutans streptococci make extracellular soluble and insoluble extracellular polysaccharides ( glucan, mutan and fructan) from sucrose that are associated with plaque maturation and cariogenicity.

Mutans streptococci can also synthesise intracellular polysaccharides when there is excess sugar, and these can act as carbohydrate reserves, and be converted to acid during periods when dietary carbohydrates are not available.

**Factors related to cariogenicity of S.mutans**

1-significant correlation in human between S.mutans count in saliva and plaque with the prevalence and incidence of caries .
2-S.mutans isolated from precise sits on the tooth surface before the development of caries .
3-correlation between the progression of carious lesions and S.mutans counts .
4-produces extracellular polysaccharides from sucrose help in colonization and attachment to the teeth .
5-Most effective streptococcus in experimental caries .
6-Ability to initiate and maintain growth in low pH .
7-Rapid metabolism of sugars to lactic acid .
8-can attain the critical pH for enamel demineralization more rapidly than other bacteria .
9-produces intracellular polysaccharide which can act as a food
10-Immunization of animals with S.mutans reduced the incidence of caries .

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