



口腔微生物與免疫學
Oral microbiology & immunology

**Characteristics of
Oral Gram-Positive Bacteria**

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學習目標

- 讓學生能瞭解口腔內感染疾病之疾病發生論中致病菌之致病機轉與宿主的免疫反應



參考資料

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2. Oral microbiology and immunology. Michael G Newman and Russel Nisengard. Saunders.
3. 齒學微生物學. 醫齒藥出版株式會社.
4. 相關期刊論文。

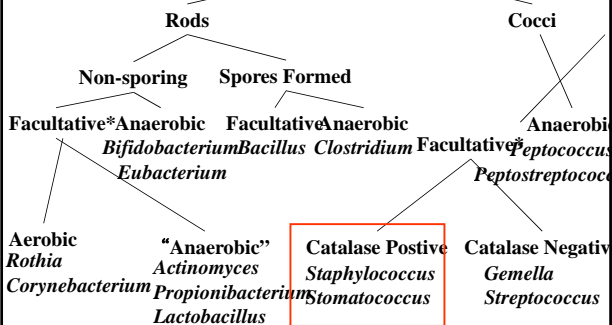


Summary

- 從口腔內微生物之感染及人體免疫發炎反應的學理觀點來介紹口腔內感染疾病 (infectious disease). 之疾病發生論 (pathogenesis)
- 從口腔內之特定感染菌種的致病因子及致病機轉開始講解至宿主的免疫反應及免疫反應所導致的口腔內之硬組織及軟組織的破壞機轉



Oral Gram-Positive Species



Gram-positive cocci

Facultative and aerotolerant cocci

Genus *Micrococcus*

Genus *Staphylococcus*

Staphylococcus aureus

Staphylococcus epidermidis

Genus *Stomatococcus*

Genus *Gemella*



Staphylococcus

- *Staphylococcus*- a bunch of grapes.
- Characteristic:
Large cocci, occurring singly, in pairs, or in irregular clumps.
Nonmotile and no endospores.
- Culture and identification:
All *staphylococcus* species grow well on trypticase soy, brain-heart infusion and blood agars.



Staphylococcus

- Metabolism and Energy:
Facultatively anaerobic, but they grow under aerobic conditions.
- Amino acids can be used as sole carbon and energy sources.



Staphylococcus

- Habitat: the skin and mucous membranes of humans and other animals.
- Also isolated from animal products, water, and soil.
- Virulence factors: hemolysins, lipases, proteases, hyaluronidase, nucleases, and enterotoxins.



Staphylococcus

- The major pathogenic species, causing serious infections and food poisoning.
- Both *S. aureus* and *S. epidermidis* associated with endodontic infections, osteomyelitis of the jaw, parotitis, and several other orally related conditions.
- *S. epidermidis* is prevalent in saliva samples from healthy individuals.



Staphylococcus

- Up to 50% of plaque samples from periodontitis, gingivitis, local juvenile periodontitis, and early onset periodontitis patients may contain low levels of *Staphylococcus* species (at about 1% of the total cultivable flora, much higher in some sites).



Staphylococcus

- Periimplantitis: In case of failing dental implants, a significant proportion (69%) of the affected sites had high proportions of *Staphylococcus* species (15% to 100% of the total flora) in the associated plaque.



Staphylococcus

- The emergence of *staphylococci* with multiple antibiotic resistances, especially in hospital infections, has been of major concern in recent years.
- The nasopharynx is a major reservoir of *S. aureus*.



Staphylococcus aureus

- Aureus- golden (the color of the colonies).
- Colonies may be gray/white to yellow or orange.
- This species requires an organic nitrogen source, B-group vitamins, uracil, and a fermentable carbon source for anaerobic growth.



Staphylococcus aureus

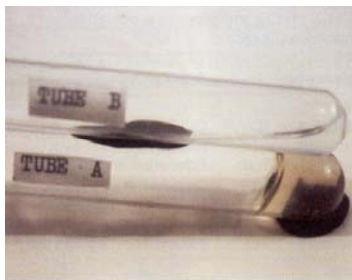


Staphylococcus aureus

- One important characteristic for identifying *S. aureus* is the production of coagulase.
- Coagulase: a factor that clots plasma and is considered an indicator of pathogenic potential.
- *S. aureus* represents about 22% of *staphylococci* isolated from



Staphylococcus aureus



A positive tube coagulase test (tube A)



Staphylococcus aureus

- *S. aureus* produces a wide range of enzymes active against biopolymers -
 - staphylococcal hyaluronidase (hyaluronic acid lysase)
 - a staphylococcal lysozyme
 - several lipases.



Staphylococcus aureus

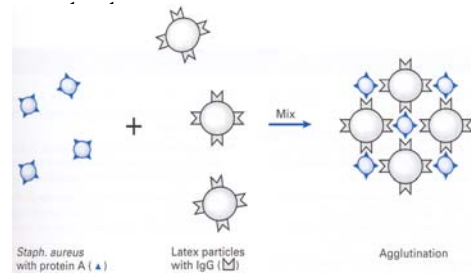
Table 11.2 Toxins and enzymes produced by Staphylococcus aureus

Toxin/enzyme	Activity
Toxins	
Cytotoxins ($\alpha, \beta, \gamma, \delta$)	Cell lysis
Leucocidin	Kills leucocytes
Epidermolytic toxin	Exfoliation and splitting of epidermis
Toxic shock syndrome toxin	Shock, rash, desquamation
Enterotoxin (A-E)	Induces vomiting and diarrhoea
Enzymes	
Coagulase	Clots plasma
Catalase	Affects bactericidal activity of polymorphs
Hyaluronidase	Connective tissue breakdown
DNAase (nuclease)	DNA hydrolysis
Lipase	Breaks lipids of cell membranes
Penicillinase	Breaks down β -lactam drugs
Protein A	Antiphagocytic



Staphylococcus aureus

• Protein A-latex agglutination



Staphylococcus aureus

- Capsule of some strains may contribute to increased virulence.
- Toxin including four different hemolysins, an epidermolytic toxin, and five types of enterotoxin.



Staphylococcus aureus

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Staphylococcus aureus

- Clinically the most serious of the antibiotic-resistant *S. aureus* that have arisen are the methicillin-resistant *S. aureus* (MRSA), which have multiple antibiotic resistances, including resistance to cephalosporin.
- Vancomycin remains an effective therapy in cases of MRSA infection.



Staphylococcus aureus

- An important pathogen, causing boils, carbuncles, impetigo, toxic epidermal necrolysis, abscesses, bacteremia, endocarditis, meningitis, osteomyelitis, pneumonia, toxic shock syndrome, urogenital infections, and food poisoning.

Staphylococcus epidermidis

- The major staphylococcal species colonizing human skin.
- An opportunistic pathogen in a wide range of infections, especially those related to indwelling medical devices, postoperative infections and infections of patients on immunosuppressive treatment.

Staphylococcus epidermidis

- *S. epidermidis* is the predominant staphylococcal species isolated from plaque associated with gingivitis or periodontitis, periapical abscesses, and endodontic infections, representing about 46% of staphylococcal isolates.

S. Aureus v.s. S. epidermidis

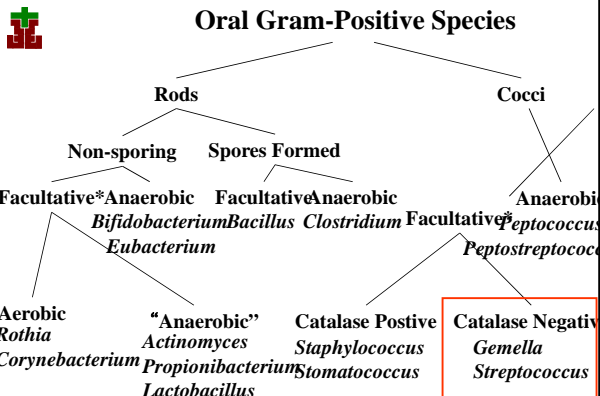
TABLE 7-2 ♦ Comparison of the Human Staphylococci

<i>S. AUREUS</i>	<i>S. EPIDERMIDIS</i>
Coagulase-positive	Coagulase-negative
Ferments mannitol	Does not ferment mannitol
DNase-positive	DNase-negative
Found in upper nasal pharynx	Found on skin
Pathogenic	Nonpathogenic

Staphylococci v.s. Streptococci

TABLE 7-1 ♦ Comparison of Staphylococci and Streptococci

STAPHYLOCOCCI	STREPTOCOCCI
Cocci arranged in bunches	Cocci arranged in chains
Catalase-positive	Catalase negative
Salt tolerant	Sensitive to high salt*
Facultative aerobe	Facultative anaerobe
Localized-type infection	Spreading-type infection
Coagulase-positive	Coagulase-negative (usually)
Antibiotic resistance	Most are sensitive to penicillin†

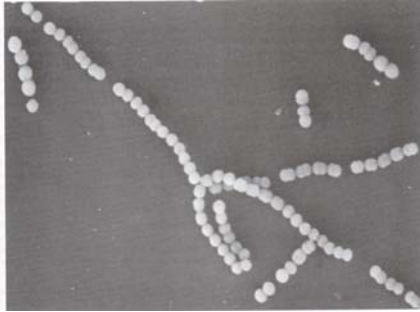


Streptococcus

- *Streptococcus*- “pliant berry”.
- The genus *Streptococcus* now consists of the pyogenic (β -hemolytic) streptococci, and the oral streptococci.



Streptococcus



Streptococcus

Table 11.1 Some recognized species of oral streptococci

Group	Species
mutans group	<i>S. mutans</i> , serotypes c, e, f <i>S. sobrinus</i> , serotypes d, g <i>S. cricetus</i> , serotype a <i>S. rattus</i> , serotype b and others
salivarius group	<i>S. salivarius</i> <i>S. vestibularis</i>
anginosus group	<i>S. constellatus</i> <i>S. intermedius</i> <i>S. anginosus</i>
mitis group	<i>S. sanguis</i> <i>S. gordonii</i> <i>S. parasanguis</i> <i>S. oralis</i> and others



Streptococcus oralis group

The *S. oralis* group:

- *S. oralis*,
- *S. sanguis*
- *S. gordonii*
- *S. mitis*
- *S. anginosus*
- *S. constellatus*
- *S. intermedius*
- *S. pneumoniae* (previously grouped with the pyogenic streptococci).



Streptococcus mutans group

The *S. mutans* group:

- *S. mutans*
- *S. rattus*
- *S. cricetus*
- *S. sobrinus*
- *S. ferus*
- *S. macacae*
- *S. downeii*

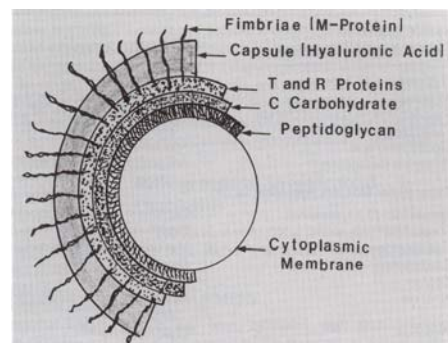


Streptococcus

- Characteristic: nonmotile and do not form endospores.
- Some species produce hyaluronic acid or polysaccharide capsules.
- Fimbriae, which mediate adherence and interspecies aggregation, occur in several species.



Streptococcus





Streptococcus

TABLE 6-2 ♦ Virulence Factors of the Group A Streptococci

STRUCTURAL COMPONENTS	BIOLOGIC ACTIVITY	ANTIGENICITY
Hyaluronic acid capsule	Antiphagocytic	No
M protein	Antiphagocytic, attachment to cells	Yes
Peptidoglycan-C polysaccharide complex	Recurrent inflammation of joints and skin	Yes
Lipoteichoic acid	Attachment to cells	Yes
Protoplast membranes	Antigenically similar to heart tissues	Yes
Extracellular Enzymes (spreading factors)		
Streptolysin O (O ₂ sensitive)	Hemolysin, cardiotoxic	Yes
Streptolysin S (O ₂ stable)	Hemolysin, leukotoxic	No
Hyaluronidase	Hyaluronic acid depolymerase	Yes
Streptokinase	Digests fibrin	Yes
DNase	Hydrolyzes DNA	Yes
NADase	Hydrolyzes nicotinamide adenine dinucleotide	Yes
Proteases	Hydrolyzes protein	Yes

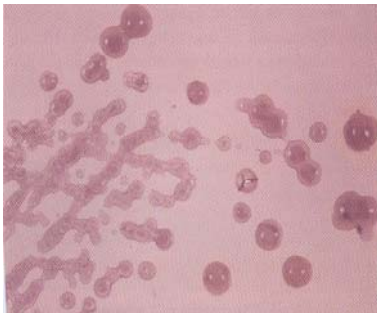


Streptococcus

- Several species produce large amounts of the extracellular polysaccharides (EPS), dextrans (polyglycans) and levans (polyfructans), when grown in the presence of sucrose.



Streptococcus mutant

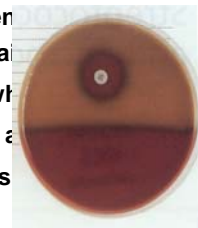


Extracellular polysaccharides



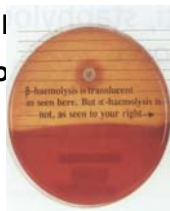
Streptococcus

- α -hemolysis is a partial hemolysis that produces a greening of the medium around a colony as a result of the production of hydrogen peroxide. The cells are incubated in a medium that contains hydrogen peroxide.
- Colonies are usually white but may appear green as a result of α -hemolysis

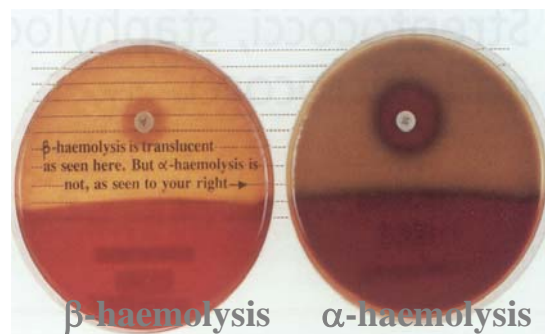


Streptococcus

- β -hemolysis is complete hemolysis, with a well-defined clear zone around a colony, produced by specific hemolysins released.
- β -hemolytic streptococci are of greatest medical importance.



Streptococcus





Streptococcus

- No hemolysis is termed γ -hemolysis.
- Most streptococcus species are facultatively anaerobic and capnophilic; aerobic growth is stimulated by the presence of 5% to 10% CO₂.



Streptococcus

- *Streptococci* are fermentative, producing major amounts of L-lactic acid from a wide range of sugars.
- Some streptococcal species produce hydrogen peroxide.



Streptococcus

- The mouth - the major habitat of the oral species.
- the oral mucosa - *S. salivarius*.
- the hard smooth surface of tooth enamel -- *S. sanguis* and *S. mutans*.



S. pyogenes

- *S. pyogenes* produces two hemolysins erythrotoxic toxin, pyogenic exotoxin, proteinases, nucleases, DNase, hyaluronidase, and streptokinase (activates the conversion of plasminogen to plasmin).



Streptococcus

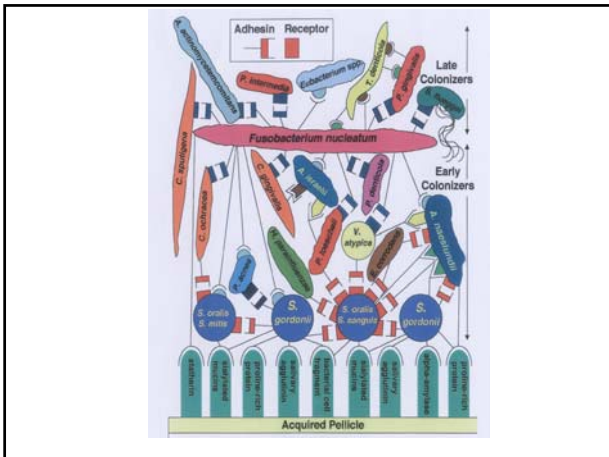
The *S. oralis* group:

- *S. oralis*,
- *S. sanguis*
- *S. gordonii*
- *S. mitis*
- *S. anginosus*
- *S. constellatus*
- *S. intermedius*
- *S. pneumoniae* (previously grouped with the pyogenic streptococci).



Streptococcus oralis

- *S. oralis* produce polysaccharide from sucrose and have neuraminidase and IgA1 protease activity but do not hydrolyze arginine or esculin or ferment insulin.
- *S. oralis* is isolated from the human mouth.
- No association with any pathological conditions has been reported.



Streptococcus sanguis

- *Sanguis*- “blood.”
- Colonies on blood agar are gray-white or green.
- The human oral cavity - the major habitat of *S. sanguis*.



Corncob



Streptococcus sanguis

S. sanguis are differentiated from the other oral species by:

- 1 hydrolyze arginine and esculin
2. produce hydrogen peroxide
3. form glucan from sucrose.
4. produces IgA1 proteases (differentiate from *S. gonorii*).



Streptococcus sanguis

- *S. sanguis* produce hydrogen peroxide, therefore may play a protective role in periodontal disease by inhibiting the colonization of gram-negative periodontal pathogens.



Lancerfield serologic group

- Heat the streptococci at 100 C for 10 min. at PH 2 extracted
Components from the *streptococci*.
- Extracts from the human β -haemolytic *streptococci* reacted with rabbit antisera prepared against the human isolates but not against the bovine isolates.
These isolates were called the Lancefield serologic groups A.
- In contrast, extracts from bovine strains reacted with homologous Antisera (antisera prepared against the bovine strain) but not with antisera prepared against the human strains. These strains called the



Lancerfield serologic group

TABLE 6-1 + Diseases Caused by Streptococci

SEROLOGIC GROUP	SPECIES	DISEASES
Group A	<i>Streptococcus pyogenes</i>	Acute Pharyngitis, pyoderma (impetigo), erysipelas, scarlet fever, pneumonia, otitis media, sinusitis, puerperal fever, septicemia
		Post-Streptococcal Rheumatic fever, glomerulonephritis
Group B	<i>S. agalactiae</i>	Neonatal sepsis, meningitis, puerperal sepsis
Group C	<i>S. equisimilis</i> , <i>S. equi</i> , <i>S. dysgalactiae</i> , <i>S. zooepidemicus</i>	Bovine mastitis, mild pharyngitis in humans
Group D	<i>Enterococcus faecalis</i> , <i>E. faecium</i> , <i>E. durans</i>	Genitourinary infections, wound infections, root canal infections, endocarditis
Group H	<i>S. sanguis</i>	Dental plaque, endocarditis



Streptococcus

TABLE 6-4 + Biochemical Identification of Oral Streptococci

TESTS	<i>S. SALIVARIUS</i>	<i>S. SANGUIS</i>	<i>S. MITIS</i>	<i>S. MUTANS</i>
Hemolysis	Nonhemolytic	Alpha	Alpha	Alpha
Catalase	Neg.	Neg.	Neg.	Neg.*
Arginine hydrolysis	Neg.	Pos.	Neg.	Pos.
Esculin hydrolysis	Neg.	Pos.	Neg./pos.	Pos.
Levan ("gumdrop" colonies)	Pos.	Neg.	Neg.	Neg.
Dextran	Neg.	Pos.†	Pos.†	Pos.†
Mannitol	Neg.	Neg.	Neg.	Pos.
Sorbitol	Neg.	Neg.	Neg.	Pos.
Inulin	Pos.	Pos.	Neg.	Pos.
Peroxide production	Neg.	Pos.	Pos.	Var.‡



Streptococcus pneumoniae

- The cells of *S. pneumoniae* are oval.
- Fresh isolates possess a thick polysaccharide capsule which is the major virulence factor and predominant antigen of *S. pneumoniae*.
- Colonies of capsulated strains are characteristically mucoid.
- Immunization against this antigen confers immunity to pneumococcal infection.



Streptococcus pneumoniae

Culture and identification:

- S. pneumoniae* is β -hemolytic when incubated anaerobically, but when grown aerobically colonies are α -hemolytic.
- Strains of *S. pneumoniae* can be distinguished from other streptococci by their sensitivity to bile and to optochin.



Streptococcus pneumoniae

- Habitat: the upper respiratory tract of humans and domestic animals.
- Pathogenicity:
 - some cases of sinusitis, conjunctivitis, pericarditis, endocarditis, and abscesses.
 - the major cause of bacterial pneumonia, a common cause of meningitis and a frequent cause of otitis media and bacteremia in children and infants.



Streptococcus mutans group

The *S. mutans* group:

- *S. mutans*
- *S. rattus*
- *S. cricetus*
- *S. sobrinus*
- *S. ferus*
- *S. macacae*
- *S. downei*

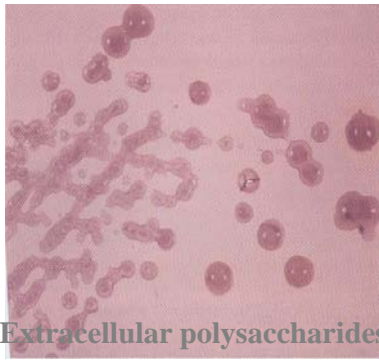


Streptococcus mutans

- Mutans- changing , refers to the appearance of colonies on sucrose agar.
- On sucrose-containing media *S. mutans* produces extracellular polysaccharide, giving characteristic opaque, rough, white colonies, usually not strongly adherent to the agar and sometimes surrounded by wet (water-soluble) glucan polymer.



Streptococcus mutant



Streptococcus mutans

- Metabolism and Energy: facultatively anaerobic, but optimal growth is often obtained anaerobically.
- Homolactic fermentation of glucose yields L-lactic acid.
- *S. mutans* forms extracellular polysaccharide from sucrose by the action of two enzymes, glucosyltransferase and fructosyltransferase.



Streptococcus mutans

- Glucosyltransferase split sucrose, joining the glucose molecules to form glucans (dextrans) with the fructose fermented.
- Fructans (Levans) are formed by the action of fructosyltransferase, with the glucose portion being fermented.



Streptococcus mutans

Habitat:

1. Primary habitat is the smooth surface of teeth.
2. *S. mutans* is absent in edentulous people, but may colonize dentures.

Pathogenicity:

1. Infective endocarditis.
2. Major causes of human caries.