MICROLAB

MICROGEN BIOPRODUCTS

Microgen Bioproducts Newsletter Nº 25 2007

The Genus *Streptococcus,* An Overview

Introduction

The genus *Streptococcus* is large, containing numerous clinically significant species that are responsible for a wide variety of infections in man and in animals. In addition to those that are considered "pathogenic," there are a number of species in this genus that are considered to be "saprophytes" which have historically not associated with any known diseases. However, over recent years, due to the increase in the number of immuosuppressed patients, and the widespread use of antibiotics in hospitals, many of the species considered as being saprophytic are now considered as being "opportunistic pathogens".

The streptococci are gram-positive cocci that are usually arranged in chains of varying lengths and in some cases pairs. Most species in this genus grow well on conventional culture media but growth is almost always enhanced on culture media containing sterile blood (usually sheep or horse) and in an aerobic environment or an environment enhanced with CO_2. Most of these species will grow both aerobically and anaerobically and are referred to as "facultative anaerobes". Some species of streptococci are true obligate anaerobes. They are catalase negative.

Classification Systems used for The Streptococci

There have been a number of classification and nomenclature systems devised for the streptococci over the years. Remnants of all of these systems are still in use today to some degree. The result is confusing terms that have been and are still being used.

Haemolysis Reactions

The identification and classification of streptococci in microbiology laboratories generally begins with their classification based on their haemolytic reactions on blood agar medium. There are 3 distinct types of reactions resulting from the breakdown of red blood cells in blood agar by enzymes classified as haemolysins.

Beta (β) Haemolysis – this is characterised by the development of a complete zone of haemolysis, generally with clearly defined edges. This reaction results from the complete haemolysis of the red blood cells in the immediate vicinity of colonies. Figure 1.

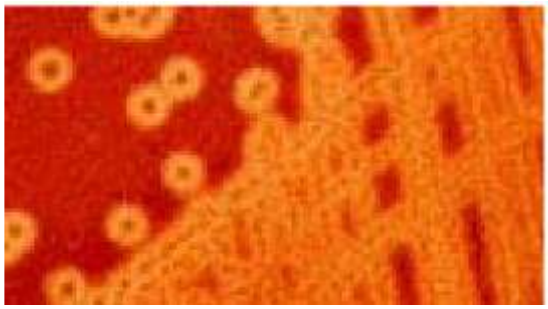

Figure 1. Typical β Haemolysis

Alpha (α) Haemolysis – is characterised by the formation of a halo of partial discolouration, usually greenish in colour around colonies. This reaction results from the partial haemolysis of red blood cells in the medium surrounding the colony in the agar medium. Streptococci

belonging to this group are commonly referred to as "Viridans" Streptococci.

Gamma (γ) Haemolysis – these strains are non-haemolytic, exhibiting no haemolysis on blood containing media. The species belonging to this group are often classified as *Enterococci*.

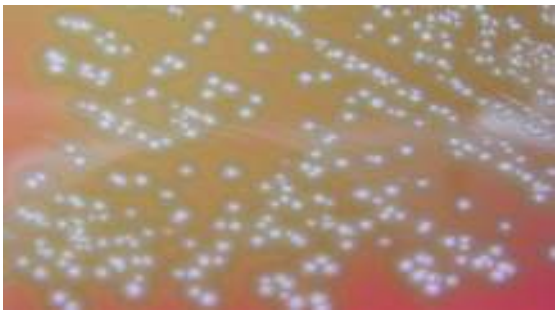

Figure 2. Typical α Haemolysis

Serological Grouping

This is the most traditional classification scheme employed for the Streptococci. There are currently 20 serological groups of Streptococci (Lancefield groups) recognised. Over the last 2 decades, the ease of performing the grouping of streptococci has been radically simplified through the introduction of latex agglutination based typing reagents (Microgen Strep Latex, M47). This typing scheme is based on specific cell wall antigens known as T proteins.

The Beta (β) Haemolytic Streptococci

The two primary streptococci exhibiting β haemolysis are *Streptococcus pyogenes* (Group A) and *Streptococcus agalactiae* (Group B). Infections due to these two groups of streptococcus can cause a range of diseases, some of which are mild and self limiting (acute pharyngitis, tonsillitis, impetigo, wound and urinary tract infections), to severe life threatening diseases (endocarditis, toxic shock, pneumonia, post partum sepsis).

The Alpha (α) Haemolytic or "Viridans" Streptococci

Although primarily comprising the α haemolytic Streptococci, a few of the species included in this category are β haemolytic or γ haemolytic. All of these species are non-pyogenic.

The taxonomy of the organisms within this classification has for many years been confusing. However recent studies using 16S rRNA gene sequencing has demonstrated clearly that most of the species can be included in one of five species groups of "Viridans" Streptococci. See Table 1. A few streptococcal species have remained ungrouped.

- Anginosus group
  - *S. anginosus*
  - *S. constellatus*
  - *S. intermedius*
- Mitis group
  - *S. sanguis*
  - *S. parasanguis*
  - *S. gordonii*
  - *S. crista*
  - *S. infantis*
  - *S. mitis*
  - *S. oralis*
  - *S. peroris*
- Mutans group
  - *S. criceti*
  - *S. downei*
  - *S. macacae*
  - *S. mutans*
  - *S. rattus*
  - *S. sobrinus*
- Salivarius group
  - *S. salivarius*
  - *S. thermophilus*
  - *S. vestibularis*
- Bovis group
  - *S. bovis*
  - *S. equinus*

Table 1. Classification of "Viridans" Streptococci

One such ungrouped member of the "Viridans" group *S. pneumoniae,* belongs to the Mitis group on the basis of phenotypic characteristics and 16S rRNA studies, but is separated from other "Viridans" streptococci on the basis of optochin sensitivity and bile solubility.

The Gamma (γ) Haemolytic Streptococci

The genus *Enterococcus* are the main members of this group. Originally classified as Group D Streptococci, the organisms belonging to this genus have now been found to be sufficiently differentiated from *Streptcooccus* on the basis of DNA-DNA and 16S rRNA studies. Based on their phenotypic characteristics, the Enterococci are divided into 5 phenotpyic groups. (Table 2) Although the majority of species included in this genus are γ haemolytic, some species may display β haemolysis when grown on rabbit, horse or human blood. The enterococci are commensal microorganisms that may be opportunistic pathogens when other predisposing factors are present (immunosuppression or prolonged antimicrobial therapy). The increasing incidence of vancomycin – resistant enterococci has become a worldwide problem and created a demand for the specific identification of these organisms.

- Enterococcus Group I
  - *E. avium*
  - *E. raffinosus*
- Enterococcus Group II
  - *E. faecalis.*
  - *E. faecium.*
  - *E. gallinarum*
  - *E. casseliflavis*
  - *E. mundtii*
- Enterococcus Group III
  - *E. dispar*
  - *E. durans*
  - *E. hirae*
- Enterococcus Group IV
  - *E. cecorum*
- Enterococcus Group V
  - *E. canis*

Table 2. Examples of Phenotypic Groups of Enterococci

Clinical Significance – Why Identify?

Streptococci belonging to the pyogenic group, *S. pyogenes*, *S. agalactiae* and *S. pneumoniae* are well established as human pathogens. However, many other species are now emerging as important pathogens. *S. dysgalactiae* subsp. *equisimilis* has been documented as an agent of pharyngitis in throat cultures and *S. anginosus* has been reported from wound specimens and abcesses. Whilst the identification of the "Viridans" streptococci can be difficult using conventional methods, the need for the identification of these species is becoming increasingly important due to their presence as sole agents in many infections, particularly those involving normally sterile sites such as bacteremic infections and brain abcesses.

In the case of Enterococci, the species specificity of emerging antimicrobial resistances amongst members of this genus (VRE) has created the need for the accurate identification of these species and the accurate determination of antimicrobial susceptibilities.

Microgen® Strep-ID (MID-62)

In response to this need for laboratories to now accurately identify members of the "Viridans" Streptococci and Enterococci, Microgen Bioproducts has introduced Microgen® Strep-ID (MID-62). This identification system is based on a total of 15 different biochemical tests and phenotypical characteristics (haemolysis reactions). Each identification comprises 12 biochemical tests provided in a standard Microgen® ID microwell strip, plus a Hippurate Hydrolysis test (separate tube test) and α and β haemolysis reactions (as determined from the primary isolation media). Figure 3

Figure 3. Interpretation of MID-Strep

The Microgen® Strep-ID microwell strip is inoculated using a suspension prepared in the special Streptococcal suspension medium provided, whilst the hippurate hydrolysis test is

performed by adding a a few drops of the Hippurate solution provided and inoculated. Both the Microgen® ID microwell strip and the hippurate hydrolysis test are incubated aerobically at 35 - 37ºC before addition of the appropriate reagents and reading. The final interpretation of the results is performed using the Microgen® Identification Systems Software (MID-60). The database provided by Microgen Bioproducts is very comprehensive and is up to date with all recent taxonomic changes.

Performace

A total of 69 cultures, comprising cultures from recognized culture collections and clinical isolates were examined. Microgen Strep-ID correctly identified 65 (93%). The API® 20 Strep product correctly identified 53 (76%) of the isolates tested. (See Table 3.)

MICROGEN BIOPRODUCTS ANNOUNCEMENT

The Microgen Bioproducts range of biochemical identification kits is further enhanced by the addition of the new Microgen® ID-Strep (MID-62).

Also Available:

Microgen® Strep Latex (M47) – Streptococcus Typing Kit.

	Number Tested	MID-62	API® 20 Strep
E. avium	1	1	1
E. durans	2	2	2
E. faecalis	10	10	10
E. faecium	5	5	5
E. gallinarum	1	1	0
E. hirae	1	1	0
G. haemolysans	1	1	1
S. acidominimus	1	1	0
S. agalactiae	5	5	5
S. anginosus	3	3	2
S. bovis	1	1	1
S. constellatus	3	2	3
S. dysgalactiae ssp. equi	1	1	1
S. equi subsp. equi	2	2	2
S. equi subsp.zooepi	1	1	1
S. gordonii	1	1	0
S. intermedius	4	3	4
S. mitis	9	9	5
S. mutans	4	3	4
S. parasanguinis	1	1	0
S. pneumoniae	5	4	2
S. pyogenes	3	3	3
S. salivarius	4	4	3
S. sanguinis	2	0	2
S. uberis	1	1	1
S. vestibularis	1	1	0
TOTAL	70	65	53

Table 3. Comparison of MID-Strep and Competitor Product

FOR MORE INFORMATION ON THE MICROGEN IDENTIFICATION PRODUCTS OR ANY OF THE OTHER MICROGEN PRODUCT, PLEASE CONTACT YOUR LOCAL MICROGEN DISTRIBUTOR

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