4 The genus *Streptococcus*

J.M. HARDIE and R.A. WHILEY

4.1 Introduction

The genus *Streptococcus* consists of Gram-positive, spherical or ovoid cells that are typically arranged in chains or pairs. These cocci are facultatively anaerobic, non-sporing, catalase-negative, homofermentative, and have complex nutritional requirements. Many of the known species are parasitic in man or other animals and some are important pathogens (Jones, 1978; Hardie, 1986; Colman, 1990). Chain-forming cocci were observed in wounds by Billroth (1874) and he applied the term 'streptococcos' to such organisms to designate their morphological arrangement (Jones, 1978). A few years later, Rosenbach (1884) first used the word *Streptococcus* in the generic sense and described the species *Streptococcus pyogenes* which is now the type species of the genus. This species was originally isolated from suppurative lesions in humans. Subsequently, in early studies by Nocard and Mollereau (1887), Schütz (1887, 1888) and Talamon (1883) – cited by Colman (1990) – several other varieties of streptococci were isolated from different sources, including *S. agalactiae* from cows with mastitis and streptococci from both equine and human cases of pneumonia.

During the period around the turn of the last century, the association between streptococci and a variety of human and animal diseases was established. Around this time the importance of morphologically similar bacteria, then classified as streptococci, in the dairy industry was also recognized. Thus, by the 1930s a large number of *Streptococcus*-like bacteria had been described and a multiplicity of names existed in the published literature. Despite numerous attempts over the years, as outlined below, it is only comparatively recently that the classification of these clinically and industrially important bacteria has been brought to a more generally acceptable level, although uncertainties remain in a few areas.

4.2 Classification

Although many distinct taxa have been recognized amongst the streptococci, their classification and nomenclature have caused considerable

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confusion over the years. One of the first characters to be recognized and used for distinguishing between isolates was the ability of certain clinically important streptococci to cause complete (β-)haemolysis around colonies grown on blood-containing culture media (Schottmüller, 1903). Other species were found to produce greening or α-haemolysis under certain conditions, whilst some caused no change to the red blood cells (Brown, 1919). Although these haemolytic changes are still useful for descriptive purposes, they have never provided a reliable basis for taxonomic subdivision of the genus.

An excellent review of the early attempts at classification of the streptococci has been provided by Jones (1978) and only a few of the major studies will be referred to in this section. Andrewes and Horder (1906) produced a classification based on a combination of biochemical, physiological and morphological characteristics which were applied to a large number of human, animal, milk and environmental isolates. They described eight groups of streptococci, designated *S. pyogenes* (identical to that of Rosenbach, 1884), *S. equinus*, *S. mitis*, *S. salivarius*, *S. anginosus*, *S. faecalis* and the pneumococci (the latter were not given a species name by these workers although they were recognized as streptococci). In a major study on the lactic acid bacteria isolated mainly from dairy products, published a few years later, Orla-Jensen (1919) extended the range of tests applied to include growth under different conditions, such as varying temperatures and salt concentrations, in addition to fermentation reactions and morphological features. He described nine groups of streptococci, some of which are now recognized as belonging to the genera (Schleifer and Kilpper-Bälz, 1987) *Lactococcus* (*S. lactis* and *S. cremoris*) or *Enterococcus* (*S. faecium*, *S. liquefaciens*).

An important development in streptococcal classification occurred with the introduction of serological methods for recognizing a series of specific cell wall antigens referred to as 'group antigens'. Lancefield (1933) first demonstrated the presence of a particular carbohydrate antigen in *S. pyogenes* which was designated Group A, and this led to the extension of the Lancefield grouping scheme to other streptococci designated B, C, G, etc. The immunochemical properties of several of these antigens were subsequently studied in great detail (e.g. Krause and McCarty, 1962), some of which, for example, Group D, were found to be teichoic acids (Krause, 1972). In some cases, including Group A (*S. pyogenes*) and Group B (*S. agalactiae*), further serological subdivisions have been made based on other antigenic components (such as M, T and R proteins in Lancefield Group A) and these have proved extremely useful for typing purposes in epidemiological investigations (Maxted, 1978).

Although detection of the Lancefield group antigens has been of

immense value for identification of some of the major human and animal pathogens, the application of serological methods to the genus as a whole has been considerably less successful. As had been shown in more recent investigations, not all streptococcal species possess a unique group antigen, whilst several of the recognized antigens are not confined to a single species. Thus, in most cases, the mere presence of a particular group antigen does not allow a streptococcal isolate to be identified to species level, unless supported by other evidence.

A significant, and to some extent prophetic, contribution to streptococcal taxonomy was made by Sherman (1937) who divided the genus into four main groups which were named 'pyogenic', 'viridans', 'lactic' and '*Enterococcus*'. These subdivisions were based on the ability to grow at 10°C and 45°C, to survive at 60°C for 30 min, to grow at pH 9.6, in 0.1% methylene blue, and at different concentrations of sodium chloride. As described below, only the first two of the groups currently remain in the genus *Streptococcus*, the 'lactic' and '*Enterococcus*' groups having been designated as separate genera.

The 'viridans' or 'oral' group of streptococci, some members of which were described at the beginning of this century by Andrewes and Horder (1906), have been a source of considerable confusion over the years (Hardie and Marsh, 1978a; Jones, 1978; Hardie & Whiley, 1992). The definition of species within this group was greatly improved during the 1960s and 1970s, thanks to the contributions of Colman and others who started to apply more modern numerical and chemotaxonomic methods to the study of these streptococci (Colman and Williams, 1965, 1972; Colman, 1968). In more recent times, the use of molecular methods such as DNA–DNA hybridization and nucleic acid sequencing, in addition to phenotypic characters, has clarified the situation still further, confirming the validity of many previously described species and enabling the description of others (Coykendall, 1989; Kilian *et al.*, 1989a; Bentley *et al.*, 1991; Hardie and Whiley, 1992).

At about the time when Vol. 2 of *Bergey's Manual of Systematic Bacteriology* (Sneath *et al.*, 1986) was under preparation, major changes to the composition of the genus *Streptococcus* were being proposed as a result of extensive molecular and chemotaxonomic studies. These led to the creation of two new genera, *Enterococcus* and *Lactococcus*, to encompass species that were formerly included in Sherman's '*Enterococcus*' and 'lactic' groups, such as *S. faecalis* (now *E. faecalis*) and *S. lactis* (now *L. lactis*) (Schleifer *et al.*, 1985; Schleifer and Kilpper-Bälz, 1984). Thus, the definition of the genus *Streptococcus sensu stricto* is now more restricted although it still includes a large number of species (currently 39), as described later in detail.

4.3 Morphology

Cells of streptococci are normally spherical or ovoid in shape, but some species may appear as short rods under certain cultural conditions. They are typically arranged in chains or pairs, chain formation being seen best in broth cultures. Individual cells are usually 0.8–1.2 μm in diameter and chains lengths vary from a few cells to over 50, depending on the strain and the growth conditions. It is not unusual for cells in older cultures to appear Gram-variable, whilst some strains may be highly pleomorphic on initial isolation.

Some species produce capsules, either of hyaluronic acid in the case of *S. pyogenes* or a variety of type-specific polysaccharides in *S. pneumoniae*, but this is not a regular feature throughout the genus as a whole. Several species produce extracellular polysaccharides when grown in the presence of sucrose, including both glucans and fructans (Hardie and Marsh, 1978a). A variety of surface structures and appendages have been described in different streptococcal species, including fimbriae and fibrils, which may be responsible for adhesion of the organisms to various surfaces (Handley, 1990; Hogg, 1992).

4.4 Cultural characteristics

Growth on solid media generally requires enrichment with blood, serum or glucose. Colonies of most species rarely exceed 1 mm in diameter after 24 h incubation at 37°C on blood agar and are usually non-pigmented, often appearing slightly translucent. On sucrose-containing media, extracellular polysaccharide producers display a variety of colonial forms which may facilitate recognition of species such as *S. mutans* and *S. salivarius* (Hardie and Marsh, 1978b). Although streptococci are facultatively anaerobic, many strains grow optimally under microaerophilic or anaerobic conditions (with CO_2) rather than in air, and some have an absolute requirement for CO_2, particularly on initial isolation. Putative obligately anaerobic streptococci such as *S. morbillorum* (now *Gemella morbillorum*), *S. parvulus* (now *Atopobium parvulus*) *S. hansenii* and *S. pleomorphus* (both more closely related to clostridia), have now been reclassified into other genera (Schleifer and Kilpper-Bälz, 1987; Kilpper-Bälz and Schleifer, 1988; Collins and Wallbanks, 1992; Hardie and Whiley, 1994).

Growth in liquid media is enhanced by addition of glucose or some other fermentable carbohydrate, but unless the medium is well buffered (as in Todd–Hewitt broth) the fall in pH will soon become inhibitory. The appearance of broth cultures varies from diffuse turbidity to granular

growth with a clear supernatant, depending on the particular species and strains.

As mentioned previously, one of the best-known characteristics of streptococci is their ability to produce different types of haemolysis on blood-containing media (Brown, 1919). Originally, these changes were described from streptococcal cultures in pour plates, but haemolysis can also be observed around surface growth on layered blood plates or in stab plates (Ruoff, 1992). The different types of haemolysis seen, namely complete (β), partial or greening (α) or none (gamma), are dependent upon the organisms concerned, the type of blood used (horse, sheep, human, etc.), the composition of the basal medium, and the atmospheric conditions. In some species, the appearance of green, α-haemolytic zones around colonies grown aerobically may be due to the production of hydrogen peroxide (Colman, 1990; Ruoff, 1992).

4.5 Biochemistry/physiology

4.5.1 Carbohydrate metabolism

Streptococci ferment glucose and other carbohydrates, yielding L-lactate as the main end product when growing rapidly under conditions of carbohydrate excess. Under glucose-limited conditions, and at low dilution rates in continuous culture, other end products are detected, such as formate, acetate and ethanol, as a result of a switch to different metabolic pathways (Ellwood, 1976). The wide and variable range of carbohydrates that can be utilized by different species of streptococci forms the basis of many of the commonly used phenotypic tests that have been employed in diagnostic identification schemes.

4.5.2 Other requirements

The nutritional requirements of streptococci are generally complex, although they have not been determined in detail for all species. For those streptococci that have been examined, they include amino acids, peptides, purines, pyrimidines and vitamins, in addition to a source of energy. In most cases such nutrients are provided by using complex culture media which often contain meat extract, peptone and blood or serum. However, some strains of 'nutritionally variant' streptococci require the addition of pyridoxal hydrochloride in order to allow growth (Bouvet *et al.*, 1981).

Some streptococcal species are able to break down arginine and this is also another energy-yielding mechanism. Under experimental conditions in the chemostat, an increased yield of glucose-limited cells can be obtained by adding arginine to the system.

4.5.3 Temperature and salt tolerance

Before the separation of enterococci and lactococci into distinct genera, determination of the range of temperatures at which isolates could grow and their ability to withstand different concentrations of sodium chloride, bile, and other chemicals, were important differential criteria. Those species which remain in *Streptococcus* generally grow within the range 20–42°C, with 37°C or thereabouts as the optimum temperature in most cases.

4.5.4 Oxygen

As mentioned previously, streptococci are facultatively anaerobic and are usually not markedly affected by the presence of oxygen. They are catalase negative, cannot synthesize haem compounds, and some species produce hydrogen peroxide when grown aerobically. For routine purposes, almost all strains will grow satisfactorily in atmospheres of air+10% CO_2, or anaerobically in a mixture of nitrogen (70–80%), hydrogen (10–20%) and CO_2 (10–20%).

4.6 Cell wall composition

As with other Gram-positive bacteria, the main structural component of the cell wall of streptococci is peptidoglycan (murein), together with various other associated polysaccharides, some of which form the basis of the Lancefield serological grouping system. Peptidoglycan consists of glucan chains that are cross-linked by short peptides and which contain alternating units of β-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid (Schleifer and Kandler, 1972). Different types of peptidoglycan structure have been described, depending on the chemical nature of the cross-linking of the adjacent stem peptides, and these types have been shown to have considerable value as taxonomic markers (Schleifer and Seidl, 1985). Cell wall polysaccharides have also been used as chemotaxonomic markers within the streptococci (Colman and Williams, 1965). Most species characteristically contain rhamnose as one of the sugar components, together with various combinations of glucose and galactose. However, rhamnose is absent from *S. oralis* and *S. pneumoniae*, both of which contain a ribitol teichoic acid. The chemical composition of the polysaccharide antigens of several species of streptococci have been determined, including Lancefield groups A, A-variant, B, C, E and G (Schleifer and Kilpper-Bälz, 1987; Colman, 1990), as well as the type-specific antigens within the *S. mutans* group (Hamada and Slade, 1980) and some other species. Some of the known chemical and serological characteristics of streptococci are summarized in Table 4.1.

Table 4.1 Serological markers and cell compositions of streptococci (species names are arranged in currently recognized species groups)

Species groups	Serological markers	Murein type	Characteristic cell wall polysaccharide components*
Oral streptococci			
S. mutans	Serotype c, e or f	Lys-Ala_{2-3}	Rha, Gluc
S. sobrinus	Serotype d, or h, g (or –)	Lys-Thr-Ala	Rha, Gluc, Gal
S. cricetus	Serotype a	Lys-Thr-Ala	Rha, Gluc, Gal
S. rattus	Serotype b	Lys-Ala_{2-3}	Rha, Gal, Glyc
S. macacae	Serotype c	ND	ND
S. downei	Serotype h	Lys-Thr-Ala	ND
S. ferus	Serotype c	Lys-Ala_{2-3}	ND
S. salivarius	Lancefield K, –	Lys-Ala_{2-3} Lys-Thr-Ala	Rha, Gluc, Gal, GalNAc
S. vestibularis	–		
S. thermophilus	–	Lys-Ala_{2-3}	ND
S. intermedius	–	Lys-Ala_{1-3}	Rha, Gluc
S. constellatus	– or Lancefield F,† A or C	Lys-Ala_{1-3}	Rha, Gluc, Gal,
S. anginosus	– or Lancefield F,† A, C, or G	Lys-Ala_{1-3}	Rha, Gluc, Gal, GalNAc
S. sanguis	Lancefield H,‡ –	Lys-Ala_{1-3}	Rha, Gluc,
S. gordonii	Lancefield H,‡ –	Lys-Ala_{1-3}	Rha, Glyc
S. parasanguis	– (or Lancefield F, G, C or B)	ND	ND
S. crista	ND	ND	ND
S. oralis	–	Lys-direct	Gluc, Gal, GalNAc, (Rha), Rtl
S. mitis	– (Lancefield K or O)	Lys-direct	(Rha), Rtl
S. pneumoniae	C-polysaccharide capsular antigens	Lys-Ala_2 (Ser)	Gluc, (Gal), GalNAc, (Rha), Rtl
S. adjacens	–	ND	ND
S. defectivus	– (or Lancefield H)	ND	ND

Table 4.1 *continued*

Species groups	Serological markers	Murein type	Characteristic cell wall polysaccharide components*
Pyogenic streptococci			
S. pyogenes	Lancefield group A§	Lys-Ala_{1-3}	Rha
S. canis	Lancefield group G	Lys-Thr-Gly	ND
S. agalactiae	Lancefield group B	Lys-Ala_{1-3} (Ser)	Rha, Gal, Glucitol
S. dysgalactiae	Lancefield group C, G, L	Lys-Ala_{1-3}	Rha, GalNAc
S. parauberis	– or Lancefield group E, P	ND	ND
S. uberis	– (Lancefield group E, P, G)	Lys-Ala_{1-3}	Rha, Gluc
S. porcinus	Lancefield groups E, P, U, V	Lys-Ala_{2-4}	ND
S. iniae	–	Lys-Ala_{1-3}	Rha, Gluc, Gal,
S. equi subsp. *equi*	Lancefield group C	Lys-Ala_{1-3}	Rha, GalNAc
S. equi subsp. *zooepidemicus*	Lancefield group C	Lys-Ala_{2-3}	ND
S. hyointestinalis	–	Lys-Ala (Ser)	ND
Other streptococci			
S.alactolyticus	Lancefield group D	ND	ND
S. bovis	Lancefield group D	Lys-Thr-Ala	Rha, Gluc, Gal
S. equinus	Lancefield group D	Lys-Thr-Ala	ND
S. suis	Lancefield group R, S, RS, T	Lys-direct	Rha, Gluc, (Gal), (GalNAc)
S. acidominimus	–	Lys-Ser-Gly	Rha, Gal
S. intestinalis	– (or Lancefield group G)	ND	ND
S. caprinus	ND	ND	ND

*ND, not determined; Gal, galactose; GalNAc, *N*-acetyl galactosamine; Gluc, glucose; Glyc, glycerol; Rha, rhamnose; Rtl, ribitol; and ( ), trace amounts.

†Further subdivision of Lancefield Group F strains has been described on the basis of type-specific carbohydrate antigens (Ottens and Winkler, 1962).

‡Reactions with Group H antiserum vary according to the immunizing strain used.

§Further subdivision of Lancefield Group A strains on the basis of M, T and R antigens.

4.7 Genetics

It is beyond the scope of this chapter to review in any detail the large body of work on streptococcal genetics that has been published since the early studies on transformation in pneumococci by Avery *et al.* (1944). Several recent books and proceedings of conferences have been devoted to this topic and others are known to be in preparation (e.g. Ferretti and Curtiss, 1987; Dunny *et al.*, 1991; Orefici, 1992). A number of streptococcal genes have been cloned and sequenced, including those coding for various surface components and virulence determinants in *S. pyogenes* and other pathogenic species (Ferretti, 1992; Fischetti *et al.*, 1992), as well as transport systems and metabolic activities in *S. mutans* (Russell *et al.*, 1991, 1992). The molecular genetics of *S. pyogenes*, *S. agalactiae*, *S. pneumoniae* and several species amongst the oral streptococci has provided the focus for many of the reported studies in recent years (Ferretti and Curtis, 1987; Fischetti, 1989; Kehoe, 1991; Boulnois, 1992; Shiroza and Kuramitsu, 1993; Russell, 1994). Such studies have helped to cast new light on the molecular mechanisms behind some of the pathogenic and metabolic activities of the streptococci and will lead, hopefully, to improved methods for prevention and treatment of streptococcal infections and their sequelae.

4.8 Phylogeny

As mentioned previously, the results of DNA–DNA and DNA–rRNA hybridization studies, together with other chemotaxonomic data, led to the separation of the former 'enterococcal (or faecal)' and 'lactic' groups of streptococci into separate genera (Schleifer and Kilpper-Bälz, 1984, 1987; Schleifer *et al.*, 1985). These proposals were supported by subsequent comparison of 16S rRNA sequences from the redefined taxa (Collins *et al.*, 1989; Williams *et al.*, 1989). Apart from *Enterococcus* and *Lactococcus*, several other genera of gram-positive cocci have been described, some quite recently, which are phylogenetically distinct from *Streptococcus*. These include *Aerococcus*, *Alloiococcus*, *Atopobium*, *Dolosigranulum*, *Gemella*, *Helcococcus*, *Leuconostoc*, *Melissococcus*, *Pediococcus*, *Tetragenococcus* and *Vagococcus*. The phylogenetic relationship of some of these genera to *Streptococcus*, as revealed by 16S rRNA sequence analysis, is illustrated in Figure 4.1.

The intrageneric relationships between species within the genus *Streptococcus*, again determined from 16S rRNA sequence data, have been reported by Bentley *et al.* (1991). From this study, in which 31 of the 39 currently known species were included, it is evident that several clusters

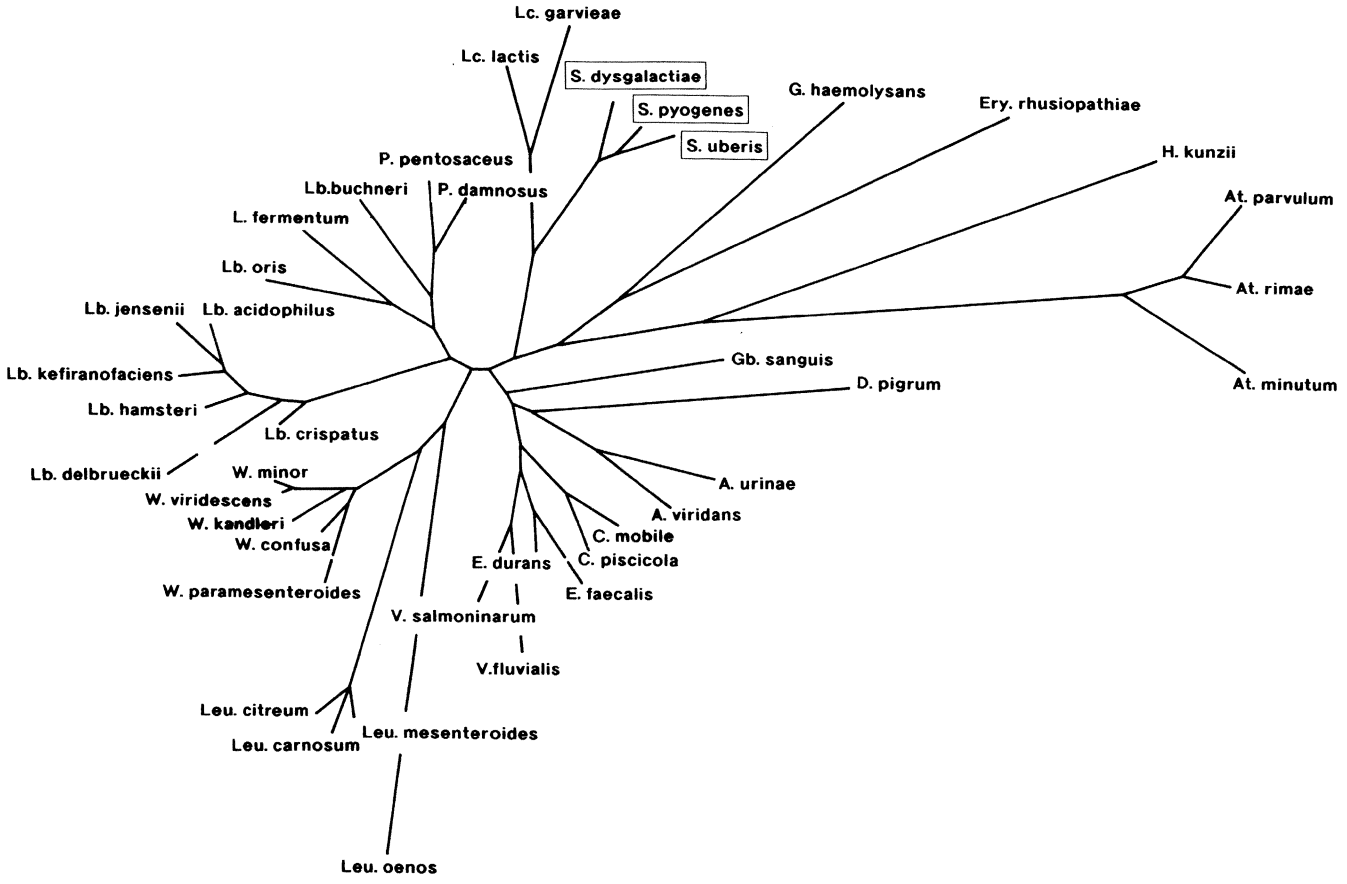

Figure 4.1 Phylogenetic network showing the relationships between *Streptococcus*, selected lactic acid bacteria and other low G+C Gram-positive taxa. *A, Aerococcus*; *At, Atopobium*; *C, Carnobacterium*; *D, Dolosigranulum*; *E, Enterococcus*; *Ery, Erysipelothrix*; *G, Gemella*; *Gb, Globicatella*; *H, Helococcus*; *Lb, Lactobacillus*; *Lc, Lactococcus*; *Leu, Leuconostoc*; *P, Pedicoccus*; *S, Streptococcus*; *V, Vagococcus*; *W, Weisella*. (Adapted, with permission, from Aguirre *et al.*, 1993; Figure 1.)

can be discerned with the genus. In the main these correspond quite closely to species groupings revealed by other techniques, although a few exceptions were reported. The pyogenic group was found to include *S. agalactiae*, *S. parauberis*, *S. porcinus* and *S. uberis*, in addition to *S. pyogenes*, *S. equi*, *S. canis*, *S. dysgalactiae*, and *S. iniae*, but the position of *S. hyointestinalis* remained uncertain. A distinct cluster was formed by *S. bovis*, *S. equinus* and *S. alactolyticus* and the close relationship previously demonstrated by other methods between *S. bovis* and *S. equinus* was confirmed. Four groups were found amongst the oral streptococci, centred around *S. mutans*, *S. salivarius*, *S. anginosus* (often referred to as the '*S. milleri* group') and *S. oralis*, the last mentioned species being closely related to *S. pneumoniae* (which has misleadingly been included in the pyogenic group in previously published descriptions of the genus (Sneath *et al.*, 1986)). The species *S. acidominimus* and *S. suis* did not fall into any of the discernible clusters.

The unrooted tree showing the phylogenetic relationships between these species of streptococci is reproduced from the paper by Bentley *et al.* (1991) in Figure 4.2. The phylogenetic groupings and order of species shown have been utilized for the construction of tables presented later in this chapter. It would be useful to add to the database in the future by inclusion of 16S rRNA sequence data from the species not hitherto included in the published reports. To take one example, it would be particularly interesting to determine the phylogenetic relationships of the nutritionally variant streptococci, *S. adjacens* and *S. defectivus*, both to each other and to other species.

4.9 Importance of the genus

4.9.1 Normal commensal flora

Streptococci comprise a significant component of the commensal flora of man and animals, colonizing mucous membranes of the mouth, respiratory tract, alimentary tract and genitourinary tract. Some species are also found on the skin, and others may be isolated from milk and dairy products (in addition to lactococci and enterococci) (Skinner and Quesnel, 1978).

Data on the distribution of streptococcal species between different animal hosts, and of their specificities for particular body sites, are incomplete, although some comparative ecological studies have been reported (Devriese, 1991). There is a need for further investigations along the lines of those recently reported on the streptococcal flora of the tonsils in cattle (Cruz Colque *et al.*, 1993), dogs and cats (Devriese *et al.*, 1992a, b), pigs (Devriese *et al.*, 1994) and the intestinal flora of poultry (Devriese *et al.*, 1991), in order to obtain a fuller picture across the animal kingdom. Several of the streptococci associated with the oral flora in humans have

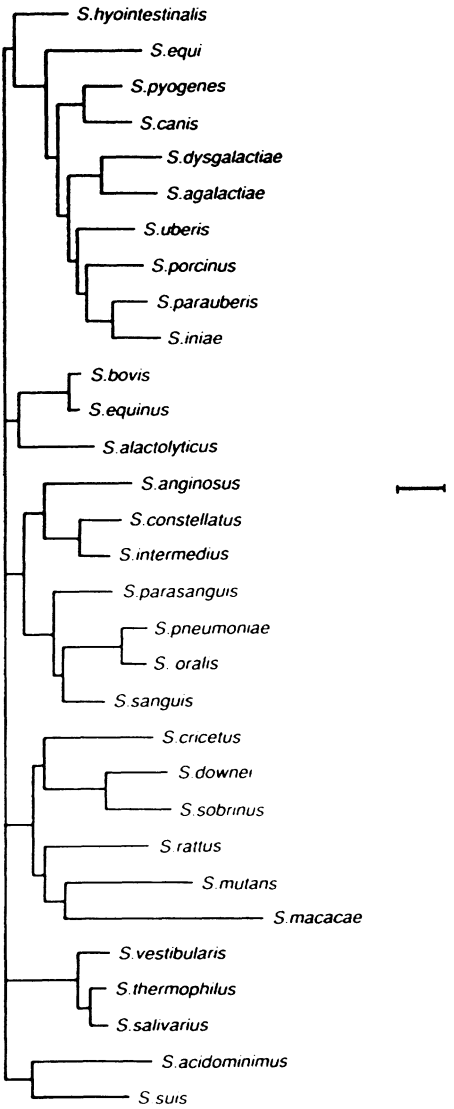

Figure 4.2 Unrooted tree showing the phylogenetic interrelationships of streptococci. The tree was based on a comparison of *c.* 1340 nucleotides (ranging from position 107 [G] to position 1431 [A] of the *E. coli* numbering system [1]). The evolutionary distance between any two species is the sum of the horizontal lines between them. Bar – K_{nuc}, 10^2. (From Bentley *et al.* (1991), reproduced by permission.)

also been isolated from the mouths of several animal species resident in a zoo (Dent *et al.*, 1978), but there is little available information about the streptococcal flora of wild animals living in their natural habitats. One recent study on feral goats in Australia led to the isolation of a new species from the rumen, *S. caprinus*, which is capable of degrading tannic acid–protein complexes (Brooker *et al.*, 1994). This unusual metabolic activity is significant because the goats browse on tannin-rich *Acacia* species. Tannin-degrading streptococci, identified as *S. bovis* biotype 1, have also been isolated from the caecum of koalas (Osawa and Mitsuoka, 1990).

A summary of the distribution of streptococcal species, albeit incomplete, is given in Table 4.2, together with their main disease association where known.

Studies on the streptococcal flora of the human oral cavity have shown that different species have a predilection for colonizing particular surfaces, such as the epithelia of the tongue, cheek and palate, or the hard tissues of the teeth (Hardie and Marsh, 1978a; Marsh and Martin, 1992). Several factors may be responsible for these variations, including surface structures (Handley, 1990), different adherence mechanisms (Gibbons and Van Houte, 1975), bacterial coaggregation (Kolenbrander and Andersen, 1986; Kolenbrander and London, 1993), and evasion of host mechanisms, such as by the production of IgA1 proteases (Kilian *et al.*, 1989b). The colonization of clean tooth surfaces to form dental plaque follows a recognized sequence of events (microbial succession), with species such as *S. mitis*, *S. oralis* and *S. sanguis* being prominent amongst the earliest bacteria to become established at these sites (Nyvad and Kilian, 1987). The ecological distribution of currently recognized streptococcal species in the mouth has been reported recently by Frandsen *et al.* (1991).

Less information is available about the streptococcal flora of the intestine in man and other animals, although several of the species normally associated with the oral cavity are known to be present, usually in low numbers (Mead, 1978). Similarly, there is limited information about the streptococci associated with the genitourinary tract.

4.9.2 Human diseases

The genus *Streptococcus* includes several species that are important pathogens in humans (Parker, 1978; Colman, 1990). In addition to the more highly virulent species, such as *S. pyogenes*, *S. pneumoniae* and *S. agalactiae*, many of the oral streptococci are capable of acting as opportunistic pathogens under appropriate circumstances. Because so many of the recognized species are found, at least on occasions, to be associated with disease, it is not possible to regard any of them as totally non-pathogenic. However, there are clearly different levels of disease-causing potential, and the more overt pathogens are known to possess a

Table 4.2 Ecological distribution and disease associations of streptococci

Species*	Main host	Main location†	Known disease associations
Oral streptococci			
S. mutans (–, E)	Man	Mouth, faeces	Caries, endocarditis
S. sobrinus (–)	Man	Mouth	Caries
S. cricetus (–)	Hamster, rats, man	Mouth	Caries
S. rattus (–)	Rats, man	Mouth	Caries
S. macacae (–)	Monkeys	Mouth	Caries (?)
S. downei (–)	Monkeys	Mouth	Caries
S. ferus (–)	Rats	Mouth	Caries
S. salivarius (–, K)	Man, animals	Mouth	Occasionally endocarditis
S. vestibularis (–)	Man	Mouth	–
S. thermophilus (–)	Milk, dairy products	?	–
S. intermedius (–, F, G)	Man	Mouth, URT	Abscesses
S. constellatus (–, A, C)	Man	Mouth, URT	Abscesses
S. anginosus (–, F, A, C, G)	Man	Mouth, URT, vagina	Abscesses
S. sanguis (–, H‡)	Man	Mouth, URT	Endocarditis
S. gordonii (–, H‡)	Man	Mouth, URT	Endocarditis
	Man	Mouth, URT	Endocarditis
S. parasanguis (–)	Man	Mouth, URT	?
S. crista (–)	Man	Mouth, URT	Endocarditis, infections in immunological compromised patients
S. oralis (–)			
S. mitis (–, K, O)	Man	Mouth, URT	Endocarditis, infections in immunological compromised patients
S. pneumoniae (–)	Man (domestic animals)	Mouth, URT	Pneumonia, meningitis, sinusitis, RTI, conjunctivitis, otitis media (occasional infectiosn in animals)
S. adjacens (–)	Man	Throat, urine	Endocarditis
S. defectivus (–)	Man	Throat, UGT, intestine	Endocarditis

Pyogenic streptococci			
S. pyogenes (A)	Man	Throat	Pharyngitis, tonsillitis, scarlet fever, pyoderma, invasive infections (rheumatic fever and acute glomerulonephritis are late complications)
S. canis (G)	Dogs, cats, cows and other animals	Skin, upper respiratory tract, udder	Mastitis
S. agalactiae (B)	Man	Genital tract, URT, faeces	Neonatal meningitis, septicaemia
S. agalactiae (B)	Cattle	Udder, milk	Mastitis
S. dysgalactiae (C)	Cattle	Udder, tonsils	Mastitis
S. dysgalactiae (C)	Man	URT. vagina, skin	?
S. dysgalactiae (C)	Pigs	Tonsils	
S. dysgalactiae (L)	Pigs		
S. dysgalactiae (G)	Man		
S. parauberis (–, E)	Cattle, milk	Lips, udder, skin	Mastitis
S. uberis (–, E)	Cattle	Udder, tonsils, lips, milk	Mastitis
S. porcinus (E, P, U, V)	Pigs	?	Cevical lymph node abscesses, pneumonia, septicaemia
S. iniae (–)	Freshwater dolphins	?	Subcutaneous abscesses
S. equi (C)	Horses, donkeys	?	Equine strangles, submaxillary gland abscess
S. hyointestinalis (–)	Pigs	Intestine	?
Other streptococci			
S. alactolyticus (D)	Pigs, chickens	Alimentary tract	?
S. bovis (D)	Cows, sheep, pigs, man, milk, dogs, pigeons	Alimentary tract, faeces, tonsils	Endocarditis in man (possibly colon cancer)
S. equinus (D)	Horses, other animals	Alimentary tract	?
S. suis (D, R, S, T)	Pigs, cattle, cats, dogs	Alimentary tract, tonsils	Bacteraemia, meningitis, respiratory disease
S. acidominimus (–)	Cattle	Vagina, skin, raw milk	?
S. intestinals (–, G)	Pigs	Alimentary tract	?
S. caprinus (–)	Feral goats	Rumen	?

*Lancefield Group antigens found.
†URT, upper respiratory tract; UGT, urogenital tract; RTI, respiratory tract infections; and ?, location or disease associations uncertain.
‡Group H varies according to immunizing strain employed.

number of important virulence determinants. As described by Parker (1978), streptococcal infections can present clinically in a number of ways. In some cases the infection is localized to a particular anatomical site, usually causing acute inflammation in the local tissues, but systemic spread, sometimes leading to the development of septicaemia, may also occur. Pus formation, as abscesses in various organs or within body cavities, is another characteristic of many streptococcal infections, hence the term pyogenic streptococci. An important feature of *S. pyogenes* infections, as described below, is the possible development of non-septic complications, such as scarlet fever, rheumatic fever and glomerulonephritis, following the initial acute condition.

4.9.2.1 Streptococcus pyogenes. The Lancefield Group A streptococcus, as *S. pyogenes* is often designated, is the most common cause of streptococcal infections in humans. It is highly communicable and can give rise to outbreaks or epidemics in susceptible populations. Patients most frequently present with either sore throat (pharyngitis, tonsillitis) or skin lesions (impetigo, pyoderma) as the primary infection, which may become invasive and lead to bacteraemia or septicaemia.

S. pyogenes produces a number of toxins and virulence determinants, including haemolysins (streptolysin O and streptolysin S), erythrogenic toxins (pyrogenic exotoxins), streptokinase (fibrinolysin), nucleases, hyaluronidases, proteinase, serum-opacity factor, nicotinamide adenine dinucleotidase and neuraminidase (Maxted, 1978; Colman, 1990). Group A streptococci also possess various surface proteins, such as the M, T and R proteins, which are utilized in serological typing schemes and may also be important virulence factors. M proteins are recognized as particularly significant because of their antiphagocytic activity and have been studied in considerable detail, several of their *emm* genes having been cloned and sequenced (Fischetti, 1989; Kehoe, 1991; Ferretti, 1992). There are over 90 known M antigens, and identification of these, together with the T antigens, forms the basis of current typing methods for investigating the epidemiology of Group A streptococcal infections (Colman, 1990; Colman *et al.*, 1993).

Some of the newer aspects of work on the pathogenicity have been discussed recently by Kehoe (1991). In this helpful review of an increasingly complex topic, the significance of some previously unrecognised factors, such as C5a peptidase is highlighted, in addition to current theories about the role of M, T and R proteins, adhesins, immunoglobulin-binding proteins, streptolysins, hyaluronidase, streptokinase and the pyrogenic exotoxins. At least one of the toxins (SPE A) is now considered to be a 'superantigen', grouped together with the staphylococcal enterotoxins, TSST-1 and exfoliative toxin (Marrack and Kappler, 1990).

One of the damaging and perplexing features of diseases caused by *S. pyogenes* is the possibility of developing later (post-streptococcal) complications following a primary infection of the upper respiratory tract or skin. Such conditions include scarlet fever (in which a skin rash is induced by the production of erythrogenic toxin in some strains), erysipelas (an erythematous skin lesion, usually on the face), rheumatic fever, acute glomerulonephritis, and toxic shock-like syndrome (Parker, 1978; Stevens *et al.*, 1989). The pathogenesis of these conditions which may follow *S. pyogenes* infections is complex and involves damaging immunological responses to the streptococci, either as a result of antigenic cross-reactivity between bacterial and host antigens (in the heart in rheumatic fever) or because of the deposition of antigen–antibody complexes in the kidney (in glomerulonephritis) (Maxted, 1978).

The prevalence of different serotypes of *S. pyogenes* in the UK over the period 1980–1990 has recently been reported, and shows that some M types (such as M1 and M49) are more often associated with epidemics. Serotypes M1 and M3 were found most commonly in invasive and fatal infections (Colman *et al.*, 1993), whilst M80 and M81 were most often isolated from patients with pyoderma. It has long been known that there is a connection between the type of *S. pyogenes* causing infection and the subsequent development of glomerulonephritis or rheumatic fever (Maxted, 1978). The occurrence of these serious, life-threatening conditions had declined in developed countries, but epidemiological studies have indicated a resurgence in recent years in several different geographical areas (Kaplan, 1992). Thus it is important to maintain surveillance on the pattern of streptococcal infections, both by *S. pyogenes* and other species, in order to detect significant shifts in their distribution. Such changes may develop as a result of alterations in the organisms themselves, or in the susceptibility of their human hosts (Barnham, 1989).

The UK is recovering from a feverish outburst of press and media interest in a small series of reports on serious cases of necrotizing fasciitis caused by *S. pyogenes*, emotively reported as 'The Killer Bug' and the 'Flesh-Eating bug (or virus!)'. Although some of these cases were from the same geographical area, no epidemiological connection between the strains isolated has so far been reported (Anon, 1994a, b). However, such episodes do serve to remind people of the potential seriousness of infections with Group A streptococci.

4.9.2.2 Streptococcus agalactiae. The streptococci of Lancefield Group B are associated with septicaemia and other infectious meningitis in man, and are a particularly important cause of infection in neonates (Jelinková, 1977; Ross, 1978; Henrichsen, 1985). Both early onset (24–36 h up to 5 days) and late onset (10 days or more after birth) forms of neonatal disease are recognized, the former having a considerably higher mortality rate due

to rapid, fulminating progression of the infection. The most likely source of Group B streptococci in neonatal infections is the genital tract of the mother, although these bacteria are also carried in the intestine and upper respiratory tract (Ross, 1978).

Four serotypes of Group B streptococococci (Ia, Ib, II, III) were originally described by Lancefield (1934, 1938), but additional types (IV, V) have subsequently been identified, based on capsular polysaccharide antigens. Further serological subdivision is possible, using the protein antigens C, R and X (Rotta, 1986; Motlova *et al.*, 1986). The capsular antigens are thought to be virulence factors although several other potential virulence determinants have been investigated (Wibawan and Lämmler, 1991; Orefici, 1992). For further differentiation between strains within serotypes, a phage typing scheme has been described (Stringer, 1980), and, more recently, molecular typing methods based on pulsed-field electrophoresis and restriction enzyme analysis of chromosomal DNA have been reported (Gordillo *et al.*, 1993).

Strains of *S. agalactiae* are usually sensitive to penicillin, although some tolerant strains have been isolated. Resistance to tetracyclines and macrolides is not uncommon and may be determined by the presence of plasmids (Colman, 1990).

4.9.2.3 Streptococcus pneumoniae. Although phylogenetically and taxonomically close to some of the oral streptococci, it is necessary to distinguish the pneumococci because of their important role as a human pathogen. In the diagnostic laboratory, the pneumococci are usually differentiated from other α-haemolytic streptococci by observing a zone of inhibition around a paper disc containing 5 μg of optochin (ethylhydrocupreine). These cocci are normally arranged in pairs and surrounded by a polysaccharide capsule. Over 80 distinct types of capsular antigens have been described which form the basis of a serological typing scheme. The cell walls of pneumococci possess a choline-containing ribitol teichoic acid, referred to as the C-substance or C-polysaccharide, which has also been detected serologically in strains of the closely related species *S. oralis* and *S. mitis* (Gillespie *et al.*, 1993). Although serological typing has usually been employed for epidemiological investigations, DNA fingerprinting by means of pulsed-field gel electrophoresis can be considered as an alternative approach (Lefevre *et al.*, 1993). DNA probes and PCR-based methods for diagnosing *S. pneumoniae* infections have also been described (Denys and Carey, 1992; Rudolph *et al.*, 1993). *Streptococcus pneumoniae* infections are particularly important in the very young and the very old, and in patients who are debilitated in some way. It is the major cause of community-acquired pneumonia, especially lobar pneumonia, and is commonly involved in meningitis, sinusitis, and otitis media. Less frequently, pneumococci are found as aetiological agents in peritonitis,

infective endocarditis and suppurative arthritis (Roberts, 1985; Colman, 1990).

Notwithstanding the role of the pneumococcus as a major human pathogen, it is also found as part of the normal commensal flora of the nasopharynx. How the organism reaches other sites, such as the lung, in order to initiate disease is not clearly understood at present (Brusse, 1991; Johnston, 1991).

A number of potential virulence factors from pneumococci have been described and the extensive published literature on them has been reviewed recently by Boulois (1992). The importance of the capsule has been recognized for many years and it is known to protect the pneumococci from phagocytosis by host cells. Vaccines based on selected capsular antigens have been effective in some population groups, although these polysaccharides are often only weakly immunogenic and may elicit poor antibody responses. Other virulence factors include the pneumococcal surface protein A, neuraminidase, the toxin pneumolysin, and autolysin. The latter cell-wall-associated enzyme may be particularly important because it facilitates the release of some of the other factors, such as pneumolysin and neuraminidase, which are located in the cytoplasm (Boulnois, 1992).

Since 1967 there has been an increasing number of pneumococci that are resistant to penicillin and other antibiotics (Hansman and Bullen, 1967; Spika *et al.*, 1991). Because of difficulties with detection of penicillin resistance by conventional disc-sensitivity tests, particular care must be taken in selecting the appropriate methodology (Marshall *et al.*, 1993).

4.9.2.4 The oral streptococci. Although the various species that have been grouped together within the oral streptococci are generally found as part of the commensal flora of the mouth and upper respiratory tract, almost all of them have been implicated as opportunistic pathogens. The types of infections associated with these streptococci include local conditions, such as dental caries and a variety of inflammatory conditions in the mouth, as well as more distant effects exemplified by infective endocarditis and abscesses in various organs (Hardie and Whiley, 1992). Some species, including *S. mitis*, *S. sanguis* and *S. oralis*, are also increasingly being recognized as aetiological agents in infections of immunologically compromised patients (Hardie and Whiley, 1994). Unlike the situation with some pathogens, it is generally not possible to link each individual species in this group with one specific disease for which it is the sole aetiological agent. In the following section, some examples of infections associated with oral streptococci are briefly discussed.

4.9.2.5 Infective endocarditis. Infective endocarditis, a serious and life-threatening infection of the endocardium of the heart, may be caused by a

large variety of microoganisms. The condition can occur in an acute form in patients with previously undamaged heart valves, in which case it is usually associated with highly virulent pathogens such as *Staphylococcus aureus* or *Streptococcus pyogenes*, but it is more commonly found as a subacute disease in patients with pre-existing valvular abnormalities, which may be either congenital or acquired. In the latter situation, streptococci are the most frequently isolated aetiological agents, although many other genera and species have been reported on occasions, including enterococci, staphylococci and several Gram-negative genera.

It has been suggested that the proportion of cases of infective endocarditis due to oral streptococci may have fallen over the last 30 years or so (Bouvet and Acar, 1984), although analysis of several published retrospective surveys still suggests that they account for 60% or more of the total (Hardie and Whiley, 1992). Organisms which appear to have increased in frequency over this period include staphylococci and Lancefield group D cocci (encompassing both *S. bovis* and *Enterococcus* species). Such alterations in proportions of taxa isolated from infective endocarditis may be a reflection of changes in the age distribution of the disease, with increasing numbers of older individuals, and the introduction of new methods of treatment, such as valve replacements and other kinds of cardiac surgery.

It is generally assumed that when oral streptococci are implicated in endocarditis, their most likely portal of entry into the bloodstream is the mouth. It is certainly true that many forms of dental treatment, notably tooth extraction, periodontal surgery and deep scaling, will induce a transient bacteraemia which would put at risk patients with previously damaged and susceptible heart valves. However, even toothbrushing or chewing may carry some risk in people with unhealthy and inflamed gums. Unfortunately, however, it is extremely difficult in most cases to establish a definite cause-and-effect link between any particular treatment episode and the subsequent development of endocarditis in individual patients, and because of the inevitable time delay between these events the evidence is, at best, circumstantial. Nevertheless, the potential danger is recognized and all 'at risk' patients should be protected by appropriate antibiotic prophylaxis when undergoing dental treatment.

Other potential portals of entry for endocarditis-inducing bacteria include the skin, gastrointestinal tract and genitourinary tract, and these are more likely to be the source of infection for organisms such as staphylococci, enterococci, *S. bovis*, and coliforms.

Although there have been many reports on the species of streptococci associated with endocarditis, recent changes in the taxonomy and nomenclature of the oral group make it necessary to re-evaluate such data. From many of these studies it would appear that *S. sanguis* (which may include *S. gordonii*), *S. oralis* (under various names) and *S. mutans* were

prominent amongst the streptococci identified, although other species were recorded on occasions. In one recent study of 47 endocarditis isolates, using current terminology, 31.9% were identified as *S. sanguis*, 29.8% as *S. oralis*, and 12.7% as *S. gordonii*, together with smaller numbers of *S. bovis* (6.4%), *S. parasanguis* (4.2%), *S. mutans* (4.2%), *S. mitis* (4.2%) and *S. salivarius* (4.2%) (Douglas *et al.*, 1993).

The nutritionally variant streptococci (NVS), now known as *S. defectivus* and *S. adjacens*, are estimated to account for 5% or more of cases of streptococcal endocarditis (Ruoff, 1991). Because of their requirement for growth media supplemented with pyridoxal, it is quite likely that these species have been underestimated or missed altogether in some studies.

Considerable interest has been shown in the possible virulence determinants of streptococci associated with infective endocarditis (Hardie and Whiley, 1994). Several features have been considered, including the production of extracellular polysaccharides, aggregation of platelets, and attachment to cellular components such as fibronectin and laminin (Herzberg *et al.*, 1990; Tart and Van de Rijn, 1991; Sommer *et al.*, 1992; Douglas *et al.*, 1993; Manning *et al.*, 1994). Further studies on such mechanisms, coupled with more extensive epidemiological surveys using the currently accepted classification schemes, should help in the search for more effective ways of preventing and treating this devastating disease.

Diagnosis of infective endocarditis depends to a large extent on isolation and identification of the causative agent from repeated blood cultures. However, the clinician needs a highly developed 'index of suspicion' in order to recognize the often insidious onset of the condition. Once the diagnosis has been made, appropriate antibiotic therapy should be instituted immediately. Early surgical intervention to replace severely damaged heart valves is increasingly recommended as part of the clinical management in many hospitals.

4.9.2.6 'Streptococcus milleri-*group*'. The species *S. anginosus*, *S. constellatus* and *S. intermedius*, which comprise the '*S. milleri*-group' (SMG), are found in the mouth, gastrointestinal and genitourinary tracts as part of the commensal flora, but have increasingly been recognized as significant pathogens (Gossling, 1988; Hardie and Whiley, 1992; Piscitelli *et al.*, 1992). They are mainly associated with purulent conditions, such as abscesses, from which they may be isolated as pure cultures or as part of a polymicrobial infection. The SMG have been reported from various oral infections, including dental abscesses, pericoronitis and Ludwig's angina; brain abscesses; ear, nose and throat infections; thoracic infections; abdominal infections (including liver abscesses); obstetric and neonatal infections; skin and subcutaneous infections; osteomyelitis and septic arthritis; and infections involving muscle (Gossling, 1988).

Because of confusion in the past about the nomenclature of these streptococci, it is not always possible to discern from published reports which of the three species has been isolated from the various clinical conditions. However, more recent studies have indicated that there is some specificity in their distribution (Whiley *et al.*, 1990b, 1992). The high level of association of *S. intermedius* with brain abscesses (23 out of 27 cases, 82%), and the relative frequency of occurrence of *S. anginosus* amongst genitourinary and intestinal isolates were particularly worthy of note. *Streptococcus anginosus* was also the species found most frequently in oral samples when examined on a nalidixic acid-sulphamethazine-containing selective medium (Whiley *et al.*, 1993).

A number of potential tissue-destroying enzymes, like hyaluronidase and chondroitin sulphatase, have been found in streptococci from this group, as well as surface-binding properties which may be of relevance to their pathogenicity (Beighton *et al.*, 1990; Homer *et al.*, 1993; Willcox *et al.*, 1993). There is clearly a need for further studies on these interesting and widespread opportunistic pathogens.

4.9.2.7 Infections in immunocompromised patients. Opportunistic infections are common in immunocompromised patients, whatever the underlying cause of their predisposition, and streptococci feature among the long list of microorganisms which may be involved. In some reports the exact identity of the streptococcal species is unclear, although it is apparent that they often belong to the 'oral' or 'viridans' group. However, from some of the more recent studies, it appears that members of the *S. oralis*-group, especially *S. oralis*, *S. mitis* and *S. sanguis*, are significant isolates from neutropenic patients (Classen *et al.*, 1990; McWhinney *et al.*, 1993; Hardie and Whiley, 1994). It will be interesting to determine from future studies whether there is any specificity in the streptococci associated with septicaemia and the adult respiratory distress syndrome (ARDS) in subjects who are immunocompromised.

4.9.2.8 Dental caries and the Streptococcus mutans *group.* It has been recognized since the last century that dental caries occurs as a result of the fermentation of carbohydrates by oral lactic acid bacteria, the acids they produce causing demineralization of the tooth surface (Miller, 1890). It is also well known that the mixed microbial community which colonizes both surfaces, in the form of dental plaque, is highly complex and contains a wide variety of bacteria with different metabolic activities (Hardie and Bowden, 1974; Marsh and Martin, 1992). A more detailed account of the lactic microflora of the oral cavity, and of their role in dental caries, is given in Volume 1 of this series (Hogg, 1992).

Many of the bacteria present in dental plaque, including several species of streptococci and lactobacilli, are capable of producing sufficient acid to

decalcify dental enamel, and a number of these are also able to induce dental caries in experimental animals (Hardie, 1992). The search for a specific aetiological agent in human dental caries has largely been concentrated on the *S. mutans*-group, especially *S. mutans* and *S. sobrinus*, which are the species most commonly found in man. There is a considerable body of published work implicating these species in the disease, and many aspects of their biochemistry, physiology, antigenic structure, epidemiology and pathogenicity have been investigated (Hamada and Slade, 1980; Loesche, 1986; de Soet *et al.*, 1992). Increasingly such studies have used molecular approaches and several putative virulence determinants have been examined by genetic methods (Russell, 1994). Molecular typing methods have been applied successfully to the study of transmission of mutans streptococci, confirming earlier observations that these organisms are usually acquired by infants from their mothers (Caufield *et al.*, 1993).

Prevention of dental caries can be achieved in most cases by measures such as restriction of dietary sugar intake, use of systemic and topically applied fluorides, toothbrushing with fluoride toothpastes, and application of fissure sealants to susceptible teeth. The search for a caries vaccine, based on *S. mutans* antigens, has produced a large amount of valuable and interesting experimental data which have helped to develop understanding of the caries process and of immune responses in the mouth (Krasse *et al.*, 1987; Russell and Johnson, 1987; Klein and Scholler, 1988). However, despite successful results in experimental animals, no human trials of active immunization with such vaccines have so far been reported. Concerns about potential safety problems, and the availability of other effective caries preventive measures, have probably contributed to this lack of progress. However, the possibility of using preformed antibodies for the purposes of passive immunization against cariogenic streptococci is still under investigation in some laboratories.

Estimation of salivary levels of mutans streptococci and lactobacilli has been used as a method of assessing caries risk in patients, although this approach has not been universally recommended or adopted (Krasse, 1988; Johnson, 1991; Hardie, 1992). Commercial kits for this purpose have been developed and can be used by the dental practitioner, requiring only facilities for incubation of cultures (Davenport *et al.*, 1992). Levels of mutans streptococci of 2.5×10^5 cfu/ml of saliva, or greater, are often taken as being indicative of 'high risk' of caries, and may well be correlated to a high frequency of sucrose intake (Hardie, 1992).

4.9.3 Animal diseases

As indicated in Table 4.2, many species of streptococci are found in animals and some are responsible for important diseases. A more detailed

account of streptococcal infections in animals can be found elsewhere (Buxton and Fraser, 1977; Wilson and Salt, 1978).

4.9.3.1 Cattle. Bovine mastitis is the most common and economically important condition in cattle which is frequently caused by streptococci. Species most often implicated in mastitis are *S. agalactiae*, *S. uberis*, *S. parauberis*, *S. dysgalactiae*, *S. canis*, although others are occasionally reported. Other types of streptococcal infections in cattle are less common, but may give rise to endocarditis, abortion, genitourinary infections or arthritis (Wilson and Salt, 1978).

4.9.3.2 Pigs. Streptococcal infections in pigs can take various forms, such as meningoencephalitis, arthritis, cervical lymphadenitis, endocarditis, abscesses, pneumonia and septicaemia. Several of the streptococci that have been reported in older studies on pig isolates as belonging to various Lancefield groups (e.g. E, P, U, V, R, S, T) have more recently been classified as either *S. porcinus* or *S. suis* (Devriese, 1991).

4.9.3.3 Horses. Equine strangles and other manifestations of streptococcal infection in horses are caused mainly by the Lancefield group C streptococci which belong to the species *S. equi*. The disease is characterized by the production of pharyngeal and submaxillary abscesses (Wilson and Salt, 1978).

4.9.3.4 Sheep. Suppurative arthritis, sometimes followed by bacteraemia and the development of endocarditis due to *S. dysgalactiae* (Lancefield group C), has been reported in lambs, but does not appear to be a common problem (Wilson and Salt, 1978). Ewes may develop mastitis, which can be caused by *S. agalactiae*.

4.9.3.5 Poultry and birds. Streptococci are not normally regarded as a major problem in poultry, although infections with *S. equi* subsp. *zooepidemicus* have been recorded. Recently, it has been shown that *S. bovis* is an important cause of septicaemia in pigeons (Devriese *et al.*, 1990).

4.10 Identification

The genus *Streptococcus* has rapidly undergone major taxonomic revision within recent years and currently consists of 39 recognized species. Undoubtedly the application of nucleic acid analyses to these studies has greatly advanced our understanding of the genetic relationships of these species at both intra- and intergeneric levels, as discussed earlier in this

chapter. However, the rapid improvements in the classification of the genus have not been equalled in pace by the development of comprehensive identification schemes using phenotypic tests nor by the construction and application of species specific DNA probes. That this situation will undoubtedly improve has already been indicated by recent advances on both phenotypic and genotypic fronts; the shift in emphasis away from traditional tests such as Lancefield grouping reactions, production of haemolysis on blood agar and physiological tests for differentiating streptococci and the use, for example, of fluorogenic and chromogenic substrates for the rapid detection of preformed enzyme activities, has characterized several identification schemes aimed at particular species groups (Kilian *et al.*, 1989a; Whiley *et al.*, 1990b; Beighton *et al.*, 1991) as well as the complete genus (Freney *et al.*, 1992). In the latter investigation, a 32 test commercial, rapid identification kit was used, consisting of chromogenic substrates for detecting glycosidases together with carbohydrate fermentation tests, arylamidase reactions, alkaline phosphatase, arginine hydrolase, acetoin production, hippurate hydrolysis and urease production. This approach has the advantage of combining a sufficiently large number of tests to enable the identification of most of the recognized species of streptococci, together with a standardized test formulation to give increased confidence when comparing results between laboratories. The performance of the test kit was commendable with 413/433 (95.4%) of strains correctly identified, including 109 stains which required further tests for complete identification (16 strains remaining unidentified and four strains misidentified). Alternative approaches that have been applied to the problem of streptococcal identification include whole cell derived polypeptide patterns by SDS-PAGE (Whiley *et al.*, 1982), pyrolysis–mass spectrometry (Winstanley *et al.*, 1992) and monoclonal antibodies (de Soet *et al.*, 1990), although, as yet, none of these has been evaluated for the complete genus.

Some progress has also been made using DNA based approaches: restriction fragment polymorphisms of whole chromosomal digests stained with ethidium bromide (Rudney *et al.*, 1992) as well as RFLPs of rRNA genes (ribotyping) (Rudney and Larson, 1993), have been attempted but the ease of use of these techniques, especially in studies involving large numbers of strains, remains untested. The extensive application of ribosomal RNA sequencing in phylogenetic investigations of the streptococci and related genera in recent years has carried with it the implicit promise of allowing construction of species specific probes to facilitate identification. The study by Bentley *et al.* (1991) represents the most thorough application of this technology to date with most, but not all, streptococcal species sequenced. The extension of this strategy to the provision of 'working' rDNA probes has begun with the recent description of oligonucleotide probes for the differentiation of *Streptococcus uberis*

and *S. parauberis* using PCR and dot blot formats (Bentley *et al.*, 1993; Harland *et al.*, 1993). However, the full impact of nucleic acid based techniques on routine laboratory identification of presumptive streptococci is still awaited.

4.11 Isolation and enumeration

Growth on non-enriched nutritient agar is usually poor, most species growing best on media supplemented with blood, serum, or with carbohydrates such as glucose or sucrose (Colman, 1990). A number of selective agents have been used in some isolation media, including crystal violet, thallous acetate, sodium azide (Hardie, 1986). More specific media, for isolation of particular groups or species of streptococci, have also been described (e.g. Barnes *et al.*, 1978; Whiley *et al.*, 1993), incorporating a variety of different antimicrobial agents as selective agents. For routine purposes, a good quality, non-selective blood agar will support the growth of most, if not all, species and also allows the recognition of haemolysis around the colonies. For selective isolation of extracellular polysaccharide producing streptococci, such as *S. bovis* and several of the oral streptococci, a sucrose-containing agar is useful (Hardie and Marsh, 1978b). Todd–Hewitt broth is commonly used as a liquid growth medium for streptococci.

4.12 Maintenance and preservation

Strains can usually be maintained by regular subculture on appropriate media. Many strains will survive storage for several days or even weeks on plates, either at room temperature or at 4°C. Streptococci can also be kept in agar stabs or in litmus milk + 1% chalk + 0.3% yeast extract + 1% glucose (Garvie *et al.*, 1981).

Long-term preservation can be achieved by freezing at −70°C or in liquid nitrogen, conveniently on beads, or by freeze-drying using standard methods (Hardie, 1986).

4.13 Species of the genus *Streptococcus*

All species of the genus *Streptococcus* are Gram-positive cocci, which may be spherical or ovoid in shape and are usually arranged in chains or pairs. They are non-motile and do not form endospores. Most are facultatively anaerobic, but some strains require CO_2 for growth, particularly on initial isolation. They are chemo-organotrophs, ferment carbohydrates with the

production of lactic and other acids, and have complex nutritional requirements. They are catalase negative. The mol% G+C of the DNA is in the range 34–46%, and the type species is *Streptococcus pyogenes* (Rosenbach, 1884). Most streptococcal species occur as commensals or parasites on man and other animals, and several are highly pathogenic. Further descriptive details may be found in other reference works, such as *Bergey's Manual of Systematic Bacteriology* (Sneath *et al.*, 1986) and *The Prokaryotes* (Balows *et al.*, 1992).

Each of the 39 currently recognized species is listed alphabetically and described briefly in the following sections. Detailed phenotypic characteristics of these species are given for the oral, pyogenic, and 'other' groups of streptococci in Tables 4.3, 4.4 and 4.5, respectively.

4.13.1 Streptococcus acidominimus

First described by Ayers and Mudge (1922) from bovine udders, the taxonomic position of *S. acidominimus* has remained uncertain. Jones (1978) included this species within the 'other streptococci', a term used for a small group of mainly α-haemolytic streptococci not included within the pyogenic, oral, faecal, lactic or anaerobic streptococcal groups recognized at that time. Wilson and Miles (1975) considered *S. acidominimus* to be a variant of *S. uberis* but by 16S rRNA analysis this has been shown not be the case. Cells are cocci occurring in short chains. α-Haemolysis is produced on blood agar. Strains are weakly fermentative with most failing to decrease the pH of the growth medium below 6.0. The biochemical reactions of this species are shown in Table 4.5. DNA G+C content is 40 mol%. No group specific antigen has been demonstrated. 16S rRNA sequence analysis (Bentley *et al.*, 1991) has shown that *S. acidominimus* does not group with any other species with the possible exception of *S. suis*. Source/habitat: bovine vagina, skin of calves and raw milk. Type strain NCDO 2025.

4.13.2 Streptococcus adjacens

Streptococcus adjacens (Bouvet *et al.*, 1989) is one of two currently recognized species of nutritionally variant (pyridoxal dependant) streptococci (NVS). These clinically important streptococci were originally assumed to be variants of some already recognized α-haemolytic streptococci but taxonomic studies have shown them to be distinct species in their own right. Cells are 0.4–0.6 μm in diameter, small ovoid cocci occurring in chains of variable length, in pairs or singly in CDMT semi-synthetic medium. Stationary phase cells may tend to be rod shaped. However, strains may produce cocci, coccobacilli and rods within chains during growth on pyridoxal or cysteine-supplemented broth. Strains are

Table 4.3 Biochemical characteristics of the oral streptococci and closely related species*

	Streptococcus mutans group†						
	S. mutans	*S. sobrinus*	*S. cricetus*	*S. rattus*	*S. macacae*	*S. downei*	*S. ferus*
Acid from							
N-Acetyl-glucosamine	+	−	+	+	+	NT	+
Aesculin	+	−	+	+	NT	NT	+
Amygdalin	+(−)‡	−	+	+	+	NT	+
Arbutin	+	−	+	+	NT	NT	+
Cellobiose	+	+(−)‡	+	+	+	−	+
Erythritol	−	−	−	−	NT	NT	−
Fructose	NT	NT	NT	NT	+	+	NT
Galactose	+	−(+)	+	+	+	+	+
Glycerol	−	−	−	−	−	−	−
Glycogen	−	−	−	−	NT	−	−
Inulin	+	+(−)	+	+	−	+	−
Lactose	+	+(−)	+(−)	+(−)	NT	+	+
Maltose	+	+	+	+	+	+	+
Mannitol	+	+(−)	+	+	+	+	+
Melibiose	+(−)	−	+	+	−	+	−
Methyl-D-glucoside	+(−)‡	−	−	−	NT	NT	−
Pullulan	−	−	NT	NT	NT	−	NT
Raffinose	+	−	+	+	+	−	−
Ribose	−	−	−	−	−	NT	−
Salicin	+	−	+	+	NT	+	+
Sorbitol	+	−(+)‡	+	+	+	−	+
Starch	−	−	−	−	−	−	+
Tagatose	+(−)	+	NT	NT	NT	NT	NT
Trehalose	+	+	+(−)	+	+	+	+
Hydrolysis of							
Aesculin	+	+(−)	+(−)	+	+	−	+
Starch	+	NT	−	−	+§	−	NT
Arginine	−	−	−	+	−	−	−

Production of							
α-Arabinosidase	−	−	NT	NT	NT	NT	NT
Acid phosphatase	−(+)§	NT	NT	NT	NT	NT	NT
Alkaline phosphatase	−	−	NT	NT	NT	−	NT
α-Fucosidase	−	−	NT	NT	NT	NT	NT
β-Fucosidase	−	−	NT	NT	NT	NT	NT
α-Galactosidase¶	+	−	NT	NT	NT	NT	NT
β-Galactosidase¶	−	−	NT	NT	NT	NT	NT
Glycyl-tryptophan arylamidase	−	−	NT	NT	NT	NT	NT
α-Glucosidase¶	+	+	NT	NT	NT	NT	NT
β-Glucosidase¶	+	−	NT	NT	NT	NT	NT
β-Glucuronidase	−	−	NT	NT	NT	NT	NT
Neuraminidase	−	−	NT	NT	NT	NT	NT
N-Acetyl-β-glucosaminidase	−	−	NT	NT	NT	NT	NT
N-Acetyl-β-galactosaminidase	−	−	NT	NT	NT	NT	NT
Leucine arylamidase	NT	NT	NT	NT	NT	NT	NT
Pyrolidonyl arylamidase	−	−	NT	NT	NT	NT	NT
Valine arylamidase	NT	NT	NT	NT	NT	NT	NT
Urease	−	−	−	−	NT	NT	−
Hydrogen peroxide	−	+	−	−	−	−	−
Extracellular polysaccharide	+	+	+	+	+	−	+
IgA protease	−	NT	NT	NT	NT	NT	NT
Hyaluronidase	−	−	NT	NT	NT	NT	NT
Acetoin (VP)	+	+	+	+	+	+	+(−)
Amylase binding	−	−	NT	NT	NT	NT	NT

*Species are ordered in the table according to data from Bentley *et al.* (1991). No species reported produces acid from adonitol, arabinose, arabitol, cyclodextrin, dulcitol, gluconate, rhamnose, sorbose or xylose. All species produce acid from glucose, mannose and sucrose. No species hydrolyses hippurate.

Data taken from Anon (1991); Beighton *et al.* (1984, 1991); Bouvet *et al.* (1989); Colman and Ball (1984); Colman and Williams (1972); Coykendall (1983); Handley *et al.* (1991); Hardie (1986); Hardie and Whiley (1992); Kilian *et al.* (1989a); Kilpper-Bälz *et al.* (1985); Kral and Daneo-Moore (1981); Whiley (1987); Whiley, R.A., unpublished data; Whiley and Beighton (1991); Whiley and Hardie (1988, 1989); Whiley *et al.* (1990a, b).

†+, >90% of strains give a positive result; +(−), 50–89% of strains give a positive result; −(+), 11–49% of strains give a positive result; −, <10% of strains give a positive result; V, reported as 'variable'; and NT, not tested.

‡Proportion of strains reported as giving a positive result for this test differs between studies.

§Weak or slow reaction given by some strains.

¶Variation in results obtained depending on method of testing.

Table 4.3 *continued*

	S. salivarius group†			*S. milleri* group†		
	S. salivarius	*S. vestibularis*	*S. thermophilus*	*S. intermedius*	*S. constellatus*	*S. anginosus*
Acid from						
N-Acetyl-glucosamine	+	+(−)	−	+	−(+)	−(+)
Aesculin	+	NT	+	NT	NT	NT
Amygdalin	+	+(−)	−	+(−)	−(+)	+
Arbutin	+	−(+)	V	+	+	+
Cellobiose	+	V	−	+(−)	−(+)	+(−)
Erythritol	NT	NT	−	NT	NT	NT
Fructose	+	+	+	NT	NT	NT
Galactose	−(+)	+	V	NT	NT	NT
Glycerol	−	−	−	−	−	−
Glycogen	−	−	−	−	−	−
Inulin	+(−)	−	−	−	−	−
Lactose	+(−)	+(−)	+	+	+(−)	+
Maltose	+	+	−	+	+	+
Mannitol	−	−	−	−	−	−(+)
Melibiose	−	−	V	−	−	−
Methyl-D-glucoside	+(−)	−(+)	−	−(+)	+(−)	+
Pullulan	+	−	−	+	−	+(−)
Raffinose	−	−	V	−	−	−(+)
Ribose	−	−	V	−	−	−
Salicin	+	+	−	+(−)	+(−)	+§
Sorbitol	−	−	−	−	−	−
Starch	−	−	NT	NT	NT	NT
Tagatose	−	−	−	−	−	−
Trehalose	−(+)	V	−	+	+(−)	+
Hydrolysis of						
Aesculin	+	+(−)	−	+	+§	+
Starch	+	NT	V	NT	NT	NT
Arginine	−	−	−	+	+	+

Production of						
α-Arabinosidase	+	+	NT	−	−	−
Acid phosphatase	+	NT	−	NT	NT	NT
Alkaline phosphatase	+	−	−	+	+	+
α-Fucosidase	−	−	−	+	−	−
β-Fucosidase	+(−)	−	NT	+	−	−
α-Galactosidase¶	−	−	−	−	−	−(+)
β-Galactosidase¶	+(−)	+	+	+	−	−(+)
Glycyl-tryptophan arylamidase	NT	NT	NT	+(−)	−	−
α-Glucosidase¶	+	+(−)	−	+	+	−(+)
β-Glucosidase¶	+	−	−	−(+)	−	+
β-Glucuronidase	−	−	−	−	−	−
Neuraminidase	−	−	NT	+	−	−
N-Acetyl-β-glucosaminidase	−	−	−	+	−	−
N-Acetyl-β-galactosaminidase	−	−	NT	+	−	−
Leucine arylamidase	+	NT	+	NT	NT	NT
Pyrolidonyl arylamidase	−	−	−	−	−	−
Valine arylamidase	+	NT	−	NT	NT	NT
Urease	−(+)	+	NT	−	−	−
Hydrogen peroxide	−	+	NT	−	−	−(+)
Extracellular polysaccharide	+	−	NT	−	−	−
IgA protease	−	NT	NT	NT	NT	NT
Hyaluronidase	−	−	NT	+	+(−)	−
Acetoin (VP)	+(−)	−	+(−)	+	+	+
Amylase binding	V	−	NT	−	−	−

*Species are ordered in the table according to data from Bentley *et al.* (1991). No species reported produces acid from adonitol, arabinose, arabitol, cyclodextrin, dulcitol, gluconate, rhamnose, sorbose or xylose. All species produce acid from glucose, mannose and sucrose. No species hydrolyses hippurate.

Data taken from Anon (1991); Beighton *et al.* (1984, 1991); Bouvet *et al.* (1989); Colman and Ball (1984); Colman and Williams (1972); Coykendall (1983); Handley *et al.* (1991); Hardie (1986); Hardie and Whiley (1992); Kilian *et al.* (1989a); Kilpper-Bälz *et al.* (1985); Kral and Daneo-Moore (1981); Whiley (1987); Whiley, R.A., unpublished data; Whiley and Beighton (1991); Whiley and Hardie (1988, 1989); Whiley *et al.* (1990a, b).

†+, >90% of strains give a positive result; +(−), 50–89% of strains give a positive result; −(+), 11–49% of strains give a positive result; −, <10% of strains give a positive result; V, reported as 'variable'; and NT, not tested.

‡Proportion of strains reported as giving a positive result for this test differs between studies.

§Weak or slow reaction given by some strains.

¶Variation in results obtained depending on method of testing.

Table 4.3 *continued*

	S. sanguis/oralis group†							Nutritionally variant streptococci†	
	S. sanguis	*S. gordonii*	*S. parasanguis*	*S. crista*	*S. oralis*	*S. mitis*	*S. pneumoniae*	*S. adjacens*	*S. defectivus*
Acid from									
N-Acetyl-glucosamine	+	+	+	+	+	+	+(−)	NT	NT
Aesculin	+(−)	+	NT	NT	−	−(+)	NT	NT	NT
Amygdalin	−	+	−(+)	−	−	−	−	NT	NT
Arbutin	+	+	−(+)	+	−	−	−	NT	NT
Cellobiose	+(−)	NT	V	NT	−	+(−)	NT	NT	NT
Erythritol	NT	NT	NT	NT	−(+)	NT	−	NT	NT
Fructose	+	+	+	NT	+	+	+	NT	NT
Galactose	+	+	+	NT	+	+	+	NT	NT
Glycerol	−	−	−	NT	−‡	NT	+§	NT	NT
Glycogen	NT	NT	−	NT	V	−	+‡	−	−
Inulin	+	+(−)	−	−	−	−(+)‡	+	V	−
Lactose	+	+	+	+(−)	+	+	+	−	V
Maltose	+	+	+	+	+	+	+	+	+
Mannitol	−	−	−	−	−	−	−‡	−	−
Melibiose	+(−)	−(+)	+(−)	−	+‡	+(−)‡	−‡	−	−
Methyl-D-glucoside	−(+)	+(−)	NT	NT	−	−	−(+)	−	−
Pullulan	+(−)	−	NT	V	+	+(−)	NT	−	+
Raffinose	+(−)	−(+)	+(−)	−	+(−)	+(−)‡	+	−	V
Ribose	−	−	NT	−	+(−)‡	−(+)	−	−	−
Salicin	+	+	V	NT	−‡	−(+)	+(−)	NT	NT
Sorbitol	−(+)	−	−	−	−	−	−	−	−
Starch	−(+)	−	−	NT	−‡	−	−(+)	−	+
Tagatose	NT	NT	NT	−	−(+)	−	−	+(−)	−
Trehalose	+	+	V	+	−(+)	−	+	−	+
Hydrolysis of									
Aesculin	+(−)	+	−(+)	−	−	−	−(+)‡	NT	NT
Starch	+(−)	+(−)	+	NT	+	+(−)	+(−)	NT	NT
Arginine	+	+	+	+(−)	−	+(−)	−‡	−	−

Production of									
α-Arabinosidase	−	−	−(+)	−	−	−	NT	NT	NT
Acid phosphatase	+(−)	+	NT	NT	+	+(−)	NT	NT	NT
Alkaline phosphatase	−	+	+(−)	−	+	V	−	−	−
α-Fucosidase	−	+	−(+)	+	−	−	−(+)	NT	NT
β-Fucosidase	−(+)‡	−	−(+)	−	+§	+	−(+)	NT	NT
α-Galactosidase¶	+(−)	−(+)	+	−	NT	+(−)	+	−	+
β-Galactosidase¶	−(+)	+(−)‡,§	NT	−(+)	+‡	+(−)‡,§	+	−	+‡
Glycyl-tryptophan arylamidase	+(−)	+	NT	NT	+	+(−)	+	NT	NT
α-Glucosidase¶	−	+(−)‡,§	+	−	+	+‡	+	NT	NT
β-Glucosidase¶	−(+)‡,§	+	−(+)	−	−	−	−(+)	−‡	−
β-Glucuronidase	−	−	−	NT	−	−	−	−(+)	−
Neuraminidase	−	−	−	−	+	−(+)	+	NT	NT
N-Acetyl-β-glucosaminidase	+(−)‡,§	+	+	+	+	+	+(−)	−(+)	−
N-Acetyl-β-galactosaminidase	−	+(−)	+	+	+	−	+	NT	NT
Leucine arylamidase	+	+	+	NT	+	+	−(+)	+	+
Pyrolidonyl arylamidase	−	−	−	NT	−	−	−(+)	+(−)‡	+(−)‡
Valine arylamidase	NT	NT	NT	NT	+	+	NT	NT	NT
Urease	−	−	−	−	−	−	−	−	−
Hydrogen peroxide	+	+	+	+	+	+	+	NT	NT
Extracellular polysaccharide	+	+	−	NT	+(−)	−	−	−	−
IgA protease	+	−	NT	NT	+	−	+	NT	NT
Hyaluronidase	−	−	−	−	−	−	+	NT	NT
Acetoin (VP)	−	−	−	−	−	−	−	V	V
Amylase binding	−	+	+(−)	+	−	+	NT	NT	NT

*Species are ordered in the table according to data from Bentley *et al.* (1991). No species reported produces acid from adonitol, arabinose, arabitol, cyclodextrin, dulcitol, gluconate, rhamnose, sorbose or xylose. All species produce acid from glucose, mannose and sucrose. No species hydrolyses hippurate.

Data taken from Anon (1991); Beighton *et al.* (1984, 1991); Bouvet *et al.* (1989); Colman and Ball (1984); Colman and Williams (1972); Coykendall (1983); Handley *et al.* (1991); Hardie (1986); Hardie and Whiley (1992); Kilian *et al.* (1989a); Kilpper-Bälz *et al.* (1985); Kral and Daneo-Moore (1981); Whiley (1987); Whiley, R.A., unpublished data; Whiley and Beighton (1991); Whiley and Hardie (1988, 1989); Whiley *et al.* (1990a, b).

†+, >90% of strains give a positive result; +(−), 50–89% of strains give a positive result; −(+), 11–49% of strains give a positive result; −, <10% of strains give a positive result; V, reported as 'variable'; and NT, not tested.

‡Proportion of strains reported as giving a positive result for this test differs between studies.

§Weak or slow reaction given by some strains.

¶Variation in results obtained depending on method of testing.

Table 4.4 Biochemical characteristics of the pyogenic streptococci*

	S. pyogenes†	*S. canis*†	*S. agalactiae*†	*S. dysgalactiae*†	*S. parauberis*†
Acid from					
Amygadalin	−	NT	−(+)	NT	+
Arbutin	−	NT	−(+)	NT	+
Cyclodextrin	−(+)	−	−	NT	NT
Dulcitol	−	NT	−	NT	V
Cellobiose	+(−)	NT	−(+)	NT	+
Glycerol	−(+)	NT	−(+)	+(−)	−
Glycogen	−(+)	−	−	V	−
Inulin	−	NT	−	−	V
Lactose	+	+(−)	−(+)	+(−)	+
Maltose	+	+	+	+	+
Mannitol	−	−	−	−	+
Mannose	NT	NT	NT	NT	+
Melezitose	−	−	−	NT	V
Methyl-D-glucoside	+	+	+	NT	−
Methyl-D-xyloside	−	NT	−(+)	NT	−
Pullulan	+(−)	+	+	NT	NT
Raffinose	−	−	−	−	V
Rhamnose	NT	NT	NT	NT	−
Ribose	−	+	+(−)	+	+
Salicin	+	NT	V	V	+
Sorbitol	−	−	−	−(+)	+
Starch	NT	NT	NT	+	NT
Sucrose	+	+	+	+	+
Tagatose	−	−	−(+)	NT	V
Trehalose	+	−(+)	+	+	+

Hydrolysis of					
Aesculin	V	NT	−	−(+)	+
Gelatin	NT	NT	NT	NT	NT
Hippurate	−	−	+	−(+)	V
Starch	NT	NT	NT	NT	NT
Arginine	+	+	+	+	+
Production of					
Alkaline phosphatase	+	+	+	+	+
α-Galactosidase	−	+(−)	V	−	V
β-Galactosidase	−	−	−	−	NT
Glycyl-tryptophan arylamidase	−	−	−	NT	NT
β-Glucosidase	−	−	−	NT	NT
β-Glucuronidase	−(+)	−(+)	V	+	−
N-Acetyl-β-glucosaminidase	−	−	−	NT	NT
Leucine arylamidase	NT	NT	NT	+	+
Pyrolidonly arylamidase	+	−	−	−	+
Urease	−	−	−	NT	NT
Acetoin (VP)	−	−	+	−	+

*Species are ordered in the table according to data from Bentley *et al* (1991). No species reported produces acid from adonitol, arabinose, arabitol, erythritol, gluconate, melibiose, methyl-D-mannoside, sorbose or xylose. All species produce acid from *N*-acetyl-glucosamine, fructose, glucose and galactose.

Data taken from Anon (1991), Collins *et al.* (1984); Devriese *et al.* (1986, 1988); Farrow and Collins (1984b); Pier and Madin (1976).

†+, >90% of strains give a positive result; +(−), 50–89% of strains give a poisitive result; −(+), 11–49% of strains give a positive result; −, <10% of strains give a positive result; V, reported as 'variable'; and NT, not tested.

‡*S. equi* subsp. *zooepidemicus* strains give positive results in these tests.

§Reported results may vary between studies.

Table 4.4 *continued*

	S. uberis†	*S. porcinus*†	*S. iniae*†	*S. equi*†	*S. hyointestinalis*†
Acid from					
Amygadalin	+	+(−)	NT	NT	V
Arbutin	+	+(−)	NT	NT	+
Cyclodextrin	NT	−	NT	+	NT
Dulcitol	−	−	−	NT	−
Cellobiose	+	+(−)	NT	NT	V
Glycerol	−	+(−)	−	−	−
Glycogen	−(+)	−	NT	+	−
Inulin	+	−	−	−	NT
Lactose	+	+(−)	−	−‡	+
Maltose	+	+(−)	NT	+	+
Mannitol	+	+(−)	+	−	−
Mannose	+	+	+	NT	+
Melezitose	−	−	NT	−	NT
Methyl-D-glucoside	−(+)	+(−)	NT	+	−
Methyl-D-xyloside	−	−	NT	NT	NT
Pullulan	NT	+(−)	NT	+	−
Raffinose	−	−	−	−	V
Rhamnose	−	−	−	NT	−
Ribose	+	+(−)	NT	−‡	−
Salicin	+	+(−)	+	+	+
Sorbitol	+	+	−	−‡	−
Starch	NT	V	NT	+	+
Sucrose	+	+(−)	+	+	+
Tagatose	+(−)	−	NT	−	−
Trehalose	+	+	+	−	+

Hydrolysis of					
Aesculin	+	+(−)	+	+(−)	+
Gelatin	NT	V	−	−	−
Hippurate	+	−	−	−(+)§	−
Starch	NT	NT	+	NT	+
Arginine	+	+	NT	+	−
Production of					
Alkaline phosphatase	−(+)	+	NT	+	+
α-Galactosidase	−	+(−)	NT	−	V
β-Galactosidase	−	−	NT	−§	−
Glycyl-tryptophan arylamidase	NT	−	NT	NT	NT
β-Glucosidase	NT	+(−)	NT	−	NT
β-Glucuronidase	+	+	NT	+	−
N-Acetyl-β-glucosaminidase	NT	−	NT	−	NT
Leucine arylamidase	+	+	NT	+	+
Pyrolidonly arylamidase	+	−	NT	−	−
Urease	NT	NT	NT	−	NT
Acetoin (VP)	+	+(−)	NT		+

*Species are ordered in the table according to data from Bentley *et al* (1991). No species reported produces acid from adonitol, arabinose, arabitol, erythritol, gluconate, melibiose, methyl-D-mannoside, sorbose or xylose. All species produce acid from *N*-acetyl-glucosamine, fructose, glucose and galactose.

Data taken from Anon (1991); Collins *et al.* (1984); Debriese *et al.* (1986, 1988); Farrow and Collins (1984b); Pier and Madin (1976).

†+, >90% of strains give a positive result; +(−), 50–89% of strains give a positive result; −(+), 11–49% of strains give a positive result; −, <10% of strains give a positive result; V, reported as 'variable'; and NT, not tested.

‡*S. equi* subsp. *zooepidemicus* strains give positive results in these tests.

§Reported results may vary between studies.

Table 4.5 Biochemical characteristics of species referred to as 'other streptococci'*

	S. alactolyticus†	*S. bovis*†,‡			*S. equinus*†	*S. suis*†	*S. acidominimus*§	*S. intestinalis*†	*S. caprinus*†
Acid from									
N-Acetyl-glucosamine									
Aesculin	+	+	+(−)	+	+	NT	NT	NT	NT
Amygdalin	NT	NT	NT	NT	NT	NT	NT	NT	NT
Arbutin	+(−)	+(−)	−(+)	+(−)	+	NT	NT	NT	NT
Cellobiose	+(−)	+(−)	−(+)	+	+	NT	NT	NT	NT
Gluconate	+	+	−(+)	+(−)	+	NT	NT	+	+
Glycerol	−	−	−	−	−	NT	NT	NT	NT
Glycogen	−	−	−	−	−	−	−(+)	−	−
Inulin	−	+	+	+(−)	+(−)	+	−	NT	NT
Lactose	−	+(−)	−(+)	−(+)	+(−)	+	NT	NT	+
Maltose	−¶	+	+	+(−)	+(−)	+	+(−)	−	+
Mannitol	+	+	+	+	+	+	−(+)	+	+
Melibiose	+(−)	+(−)	−(+)	−	−	−	−(+)	−	+
Melezitose	+(−)¶	+(−)	−	+(−)	+(−)	V	−	NT	NT
Methyl-D-glucoside	−(+)	−(+)	−	−	−	−	−	NT	NT
Methyl-D-mannoside	−(+)	+(−)	−	−	NT	NT	−	NT	−
Pullulan	−	−(+)	−	−	−	NT	NT	NT	NT
Raffinose	−	NT	NT	NT	NT	+	−	NT	NT
Rhamnose	+	+(−)	+(−)	+	+(−)	V	−	−	+
Salicin	−	−(+)	−	−	−	NT	NT	NT	−
Sucrose	(+)−	+(−)	−(+)	+	+	+	NT	+	NT
Tagatose	+¶	+	+	+	+	+	+(−)	+	+
Trehalose	−	+(−)	−(+)	−	−	−	−	NT	NT
	+(−)¶	+(−)	+	−	−(+)	+	+(−)	−	+

Hydrolysis of									
Aesculin									
Hippurate									
Starch	+¶	+	+(−)	+	+	+	NT	+	NT
Arginine	−	−	+(−)	−	−	−	NT	−	NT
	NT	NT	NT	NT	NT	+	NT	−	NT
Production of	−	−	−	−	−	+	−	−	NT
α-Galactosidase									
β-Galactosidase									
β-Glucosidase									
β-Glucuronidase	+	+(−)	−	+(−)	+(−)	+	−	NT	NT
N-Acetyl-β-glucosaminidase	−	−(+)	−	−	−	V	−	NT	+
Pyrolidonyl arylamidase	+(−)	NT	NT	NT	NT	+(−)	−	NT	NT
Urease	−	−	−(+)	−	−	+	−(+)	NT	NT
Acetoin (VP)	−	NT	NT	NT	NT	+	−	NT	+
	−	−	−(+)	−	−	−(+)	−	NT	NT
	+(−)	NT	NT	NT	NT	−	−	+	NT
	+	+	−	+	+	−	−	NT	NT

*Species are ordered in the table according to data from Bentley *et al.* (1991). No species reported produces acid from adonitol, arabinose, arabitol, cyclodextrin, dulcitol, erythritol, sorbitol, starch or xylose. All species produce acid from fructose, galactose, glucose and mannose. No species produces alkaline phosphatase.

Data taken from Anon (1991); Brooker *et al.* (1994); Devriese *et al.* (1994); Farrow *et al.* (1984); Kilpper-Bälz and Schleifer (1987).

†+, >90% of strains give a positive result; +(−), 50–89% of strains give a positive result; −(+), 11–49% of strains give a positive result; −, <10% of strains give a positive result; V, reported as 'variable'; and NT, not tested.

‡Biochemical characteristics of the three DNA homology groups reported by Farrow *et al.* (1984).

§High final pH often results in difficulty when reading tests.

¶Number of strains giving a positive result varies between reports.

α-haemolytic on sheep blood agar forming tiny (0.2 mm diameter) colonies. No extracellular polysaccharide is produced on sucrose-containing medium. A red chromophore is produced, visualized by boiling the bacteria at pH 2.0 for 5 min. The biochemical reactions of this species are shown in Table 4.3. This species has complex growth requirements including the addition of one of the active forms of vitamin B_6 such as pyridoxal hydrochloride or pyridoxamine dihydrochloride. Also, satellitism can be observed around colonies of *S. epidermidis* on horseblood agar. Strains are ungroupable with Lancefield antisera. Cell walls are characterized by the absence of rhamnose and presence of ribitol teichoic acid. DNA G+C content is 36–37 mol%. DNA–DNA hybridization studies demonstrated *S. adjacens* to be a separate species and not to be variant strains of *S. mitis* or *S. sanguis II* as previously suggested. Unfortunately this species was not included in the 16S rRNA sequence study by Bentley *et al.* (1991). Source/habitat: human throat, urine and blood of patients with endocarditis. Type strain ATCC 49175.

4.13.3 Streptococcus agalactiae

Streptococcus agalactiae (Lehmann and Neumann, 1896) is synonymous with Lancefield Group B streptococcus. *Streptococcus agalactiae* is an important cause of mastitis in cattle and in the past few decades has also become recognized as an important pathogen of man, causing neonatal meningitis and septicaemia. Cells are 0.6–1.2 μm diameter, spherical or ovoid occurring frequently in very long chains. On blood agar most strains produce β-haemolysis although some strains are α- or non-haemolytic. Addition of starch to the medium, or anaerobic incubation, may enhance the production of yellow, orange or red pigments. Most strains grow in the presence of 40% bile and all strains hydrolyse hippurate. The other biochemical reactions of this species are shown in Table 4.4. Almost all strains give a positive CAMP (named after the initials of the authors who first described the test) reaction (Christie *et al.*, 1944). Cell wall peptidoglycan type is Lys-Ala_2(Ser). DNA G+C content is 34 mol%. Strains possess the Lancefield Group B specific carbohydrate antigen in the cell walls. Further serological division is possible on the basis of both capsular polysaccharide antigens and protein antigens of which the former are virulence factors. 16S rRNA sequence analysis places this species within the pyogenic group of streptococci and DNA hybridization has also demonstrated that Lancefield Group M streptococci are included within this species. Source/habitat: vaginal mucosa, upper respiratory tract, urine, faeces of man and in the milk and udder tissues of animals. Type strain NCTC 8181.

4.13.4 Streptococcus alactolyticus

This species was first described by Farrow *et al.* (1984) in a study of strains of *S. bovis* and *S. equinus*. DNA–DNA hybridization studies by these authors resulted in the recognition of six DNA homology groups of which one, comprising strains of *S. equinus* from pigs and chickens, was given the name *S. alactolyticus*. Cells are coccoid and form short chains or pairs. Colonies on blood agar are α- or non-haemolytic, circular, smooth and entire. Growth occurs at 45°C but not 50°C or in the presence of 6.5% NaCl. The biochemical characteristics are shown in Table 4.5. DNA G+C content is 40–41 mol%. Strains contain the Lancefield Group D antigen. 16S rRNA comparative sequencing has shown a relatively close phylogenetic relationship between *S. alactolyticus*, *S. bovis* and *S. equinus*. Strains of *S. alactolyticus* have previously been designated as *S. equinus*. Source/habitat: intestines of pigs and chickens. Type strain NCDO 1091.

4.13.5 Streptococcus anginosus

Although originally described at the beginning of the century by Andewes and Horder (1906), the taxonomic position of streptococci named *S. anginosus*, together with similar 'species', remained confused for many years (Jones, 1978). DNA–DNA hybridization studies (Kilpper-Bälz *et al.*, 1984; Whiley and Hardie, 1989) finally clarified the situation with the recognition of three closely related species, including *S. anginosus* present amongst these biochemically and serologically heterogeneous streptococci (Whiley and Beighton, 1991). Cells are 0.5–1 μm in diameter, forming short chains. On blood agar most strains produce α-haemolysis or are non-haemolytic, with some strains producing β-haemolysis. No extracellular polysaccharide is produced on sucrose containing media. The biochemical reactions of this species are shown in Table 4.3. Growth is enhanced in the presence of CO_2, reduced under aerobic conditions and some strains require anaerobic conditions. Most strains are serologically ungroupable with the majority of groupable isolates belonging to Lancefield Group F. Reactions with Lancefield Groups A, C and G antiserum are also occasionally found. The cell wall peptidoglycan type is Lys-Ala_{1-3}. DNA G+C content is 38–40 mol%. DNA homology studies (Whiley and Hardie, 1989) and 16S rRNA sequence analysis (Bentley *et al.*, 1991) in particular have shown that *S. anginosus* together with *S. constellatus* and *S. intermedius* form a group of closely related species sometimes referred to as the '*Streptococcus milleri*-group'. Strains resembling *S. anginosus* have previously been referred to as *Streptococcus milleri*, *Streptococcus* MG, haemolytic and non-haemolytic streptococci of Lancefield Group F, the

minute colony-forming streptococci of Lancefield Groups F and G, *Streptococcus* MG-*intermedius* and *Streptococcus anginosus-constellatus*. Source/habitat: human oral cavity, upper respiratory tract and vagina. Frequently isolated from purulent infections of man. Type strain NCTC 10713 (ATCC 33397).

4.13.6 Streptococcus bovis

Originally described as a bovine bacterium that fermented arabinose, raffinose and starch but not mannitol (Orla-Jensen, 1919), *S. bovis* was later discovered to be of clinical importance to man as an aetiological agent in some cases of endocarditis and possible association with colon cancer (Facklam, 1972; Klein *et al.*, 1977). The biochemical heterogeneity presented by strains for a long time hindered any resolution of the taxonomy of these streptococci. The application of genetic approaches however confirmed that *S. bovis* was indeed made up of several distinct 'species' (Farrow *et al.*, 1984; Coykendall and Gustafson, 1985) although full descriptions of some of these are still awaited. Cells are spherical or ovoid and are 0.8–1.0 μm in diameter, occurring in moderate or long chains and also in pairs. Most strains give α-haemolysis on blood agar and produce large amounts of polysaccharide on sucrose containing media. This species comprises strains with heterogeneous properties that include anaerobic strains capable of growth in broth containing 6.5% NaCl and at pH 9.6, the production of urease by some strains, failure to grow at 45°C and sharing high DNA homology with *S. mutans*, fermentation of arabinose, xylose, mannitol, sorbitol, trehalose and inulin. Phenotypic similarity between *S. bovis* and *S. salivarius* has been noted by some authors. Strains from human sources have previously been designated as biotype I and II, the former being characterized by their ability to ferment mannitol and inulin and produce extracellular glucan on sucrose agar in contrast to the biotype II strains which are negative in these tests. The biochemical properties of this species are shown in Table 4.5. Peptidoglycan types Lys-Thr-Ala, Lys-Thr-Gly and Lys-Thr-Ala (Ser) occur in strains of *S. bovis*. Strains possess the Lancefield Group D antigen. DNA–DNA hybridization studies have revealed extensive genetic heterogeneity within strains classified as *S. bovis*. In the study of *S. bovis* and *S. equinus* strains by Farrow *et al.* (1984) six DNA homology groups were demonstrated: one group contained both the type strains of *S. bovis* and *S. equinus* leading these authors to propose that due to the priority of the name *S. equinus* the name '*S. bovis*' be reduced to synonymity. Another DNA homology group consisted of strains from cases of bovine mastitis capable of fermenting mannitol. Strains designated *S. bovis* were also grouped in another three DNA homology groups, one of which contained bovine strains and was proposed as a new species named *S. saccharolyticus*. *Streptococcus bovis*

strains from human sources were included in unnamed DNA homology Group 4 of Farrow *et al.* (1984). 16S rRNA sequence data together with information from DNA–DNA hybridization have demonstrated the close relationship between *S. bovis*, *S. equinus* and *S. alactolyticus*. Source/habitat: alimentary tract of cow, sheep and other ruminants, faeces of pigs. Occasionally isolated from human faeces in large numbers, from raw and pasteurized milk and cheese and from some cases of endocarditis in humans. Type strain NCDO 597.

4.13.7 Streptococcus canis

Streptococci of Lancefield Group G include the so-called large colony, β-haemoloytic strains isolated from animals which differ from human Group G isolates within *S. dysgalactiae* on the basis of α- and β-galactosidase activities, lack of fibrinolysin, hyaluronidase, or β-glucuronidase and an inability to ferment trehalose. On the basis of DNA homology and phenotypic characterization Devriese *et al.* (1986) named these streptococci *S. canis*. Cells form chains or occur in pairs. β-Haemolysis is produced on blood agar. The strain is CAMP factor negative. Strains are facultatively anaerobic and good growth occurs at 37°C. No growth occurs in the presence of 6.5% w/v NaCl or 40% w/v bile. The biochemical reactions of this species are shown in Table 4.4. The cell wall peptidoglycan type is Lys-Thr-Gly. DNA G+C content is 39–40 mol%. Strains belong to Lancefield Group G. DNA–DNA hybridization studies and comparative 16S rRNA sequence analysis have shown *S. canis* to be within the pyogenic group of streptococci. Source/habitat: dogs (skin, upper respiratory tract and genitals) cows (udders) and probably cats. Isolated from dogs (neonates with septicaemia and from a wound exudate) and from cows suffering from mastitis. Not isolated from humans. Type strain DSM 20715.

4.13.8 Streptococcus caprinus

From studies on the bacteria inhabiting the digestive tracts of animals with tannin-rich diets has emerged the species description of *S. caprinus* (Brooker *et al.*, 1994) isolated from wild goats grazing tannin-rich shrubs in Australia. Cells occur mainly in short chains. Grows on nutrient agar plates containing 0.5% w/v tannic acid, forming large mucoid colonies surrounded by clear zones in the tannic acid agar. Able to grow in complex growth medium with at least 2.5% w/v condensed tannins from the acacia tree (*Acacia aneura*). The biochemical reactions of this species are shown in Table 4.5. The DNA G+C content and presence of a Lancefield group antigen have not been reported from this recently described species. Source/habitat: rumen of feral goats. Type strain ACM 2969.

4.13.9 Streptococcus constellatus

Streptococcus constellatus was the name given by Holdeman and Moore (1974) to strains isolated from clinical specimens and vaginal swabs, that closely resembled a species first described by Prevot (1924) as *Diplococcus constellatus*. These streptococci produced major amounts of lactic acid, fermented glucose, maltose, and sucrose but not lactose and hydrolysed aesculin. Subsequently a close resemblance was reported between these streptococci, and several other 'species' already described that included '*Streptococcus* MG', *Streptococcus intermedius*, and *S. anginosus* (Facklam, 1977). These were divided into two species on the basis of lactose fermentation: *S. anginosus-constellatus* (lac−) and *S.* MG-*intermedius* (lac+). Further taxonomic studies have revealed three distinct species within these biochemically and serologically heterogeneous streptococci which includes *S. constellatus*, *S. anginosus* and *S. intermedius* (Whiley and Beighton, 1991). Cells are 0.5–1 μm in diameter forming short chains. On blood agar strains can produce α-, β- or no (γ-)haemolysis. Extracellular polysaccharide is not produced on sucrose-containing medium. The biochemical reactions of this species are shown in Table 4.3. Growth is enhanced in the presence of CO_2, reduced under aerobic conditions and some strains require anaerobic conditions. Some strains react with Lancefield Groups A and C antisera with the majority of strains remaining ungroupable in this system. The cell wall peptidoglycan type is Lys-Ala_{1-3}. DNA G+C content is 37–38 mol%. DNA homology studies and 16S rRNA sequence analysis in particular have shown that *S. constellatus* together with *S. anginosus* and *S. intermedius* form a group of closely related species sometimes referred to as the '*Streptococcus milleri*-group'. Strains resembling *S. constellatus* have also been previously referred to as *Streptococcus milleri*, *Streptococcus* MG, haemolytic and non-haemolytic streptococci of Lancefield Group F, the minute colony-forming streptococci of Lancefield Groups F and G, *Streptococcus* MG-*intermedius* and *Streptococcus anginosus-constellatus*. Source/habitat: human oral cavity and upper respiratory tract. Frequently isolated from purulent infections in man. Type strain ATCC 27823 (NCDO 2226).

4.13.10 Streptococcus cricetus

Originally described as *S. mutans* serotype a from hamster and human dental plaque (Bratthall, 1970) these streptococci were shown to be genetically distinct from other mutans-like strains. Proposed initially as *S. mutans* subsp. *cricetus* (Coykendall, 1974) these streptococci were subsequently elevated to species status as *S. cricetus* (Coykendall, 1977). Cells are approximately 0.5 μm in diameter forming chains or occurring in pairs. Colonies formed on sucrose-containing agar are rough and heaped, with

liquid glucan sometimes present. On blood agar most strains are non-haemolytic, whilst some are α-haemolytic. The biochemical characteristics of this species are shown in Table 4.3. Optimum growth is obtained with added CO_2 or under reduced O_2. Most strains possess the serotype a polysaccharide antigen (Bratthall, 1970). Cell wall peptidoglycan type is Lys-Thr-Ala. DNA G+C content is 42–44 mol%. *Streptococcus cricetus* is a species belonging to the mutans group of streptococci as demonstrated by DNA–DNA hybridization and rRNA studies. Strains of this species were previously designated as *Streptococcus mutans* serotype a. Source/habitat: oral cavities of wild rats, hamsters and man (occasionally). Type strain ATCC 19642.

4.13.11 Streptococcus crista

These streptococci isolated from the human oral cavity and throat were initially regarded as unusual strains of *S. sanguis* before DNA homology studies demonstrated that they constituted a new species named *S. crista* (Handley *et al.*, 1991). Cells are approximately 1 μm in diameter, spherical and form chains. By electron microscopy cells have fibrils arranged equatorially in lateral tufts. α-haemolysis is produced on blood agar and some strains produce glucan on sucrose-containing medium. The biochemical reactions of *S. crista* are given in Table 4.3. The peptidoglycan type of this species has not been determined. DNA G+C content is 42.6–43 mol%. Strains of this species have previously been referred to as the 'tufted fibril group', the 'CR group' and '*S. sanguis* I'. The phylogenetic position of *S. crista* has not been determined. Source/habitat: human throats and mouths. Type strain NCTC 12479.

4.13.12 Streptococcus defectivus

Streptococcus defectivus (Bouvet *et al.*, 1989) together with *S. adjacens* comprised the nutritionally variant (pyridoxal dependent) streptococci (NVS). Originally considered to be variant of already established α-haemolytic streptococci, both *S. defectivus* and *S. adjacens* have been shown to be distinct species in their own right. Cells are 0.4–0.55 μm in diameter, small ovoid cocci occurring in chains of varying length, in pairs or even singly in CDMT semi-synthetic medium. Stationary phase cells may tend to be rod shaped. However, strains may produce cocci, coccobacilli and rods within chains during growth on pyridoxal or cysteine-supplemented broth. Strains are haemolytic on sheet blood agar, forming tiny (0.2 mm diameter) colonies. No extracellular polysaccharide is produced on sucrose containing medium. A red chromophore is produced, visualized by boiling the bacteria at pH 2 for 5 min. The biochemical reactions of this species are shown in Table 4.3. Complex growth

requirements including the addition of one of the active forms of vitamin B_6 such as pyridoxal hydrochloride or pyridoxamine dihydrochloride. Also, satellitism can be observed around colonies of *Staphylococcus epidermidis* on horse blood agar. Strains are serologically ungroupable against Lancefield antisera, with an occasional weak reaction against Group H antiserum. Cell walls are characterized by the absence of rhamnose and presence of ribitol teichoic acid. DNA G+C content is 46.0–46.6 mol%. DNA–DNA hybridization studies demonstrated *S. defectivus* to be a separate species and not to be variant strains of *S. mitis* or *S. sanguis* II as had been previously thought. Unfortunately this species was not included in the 16S rRNA sequence study by Bentley *et al.* (1991). Source/habitat: human throat, urogenital tract and intestine. Most frequently isolated from the blood of patients with bacteraemia or endocarditis. Type strain ATCC 49176.

4.13.13 Streptococcus downei

Following the initial division of streptococci resembling *S. mutans* into several distinct species (*S. mutans*, *S. sobrinus*, *S. cricetus* and *S. rattus*) (Coykendall, 1977) further studies revealed the existence of additional species within the 'mutans-group' of streptococci. *Streptococcus downei* (Whiley *et al.*, 1988) were isolated from monkey dental plaque and were characterized as mutans streptococci carrying a serologically distinct carbohydrate antigen designated h (Beighton *et al.*, 1981) before being recognized as a distinct species. On sucrose-containing agar colonies are large (2–3 mm diameter), conical and are usually surrounded by a white halo within the agar. Cells adhere to glass surfaces when grown in sucrose broth, indicating the production of extracellular polysaccharide, although no cell-free, ethanol precipitable polysaccharide has been demonstrated. The biochemical reactions of this species are shown in Table 4.3. No growth occurs at pH 9.6, at 45°C or in the presence of 6.5% w/v NaCl. Variable growth occurs on 10 and 40% w/v bile agar. The cellular long-chain fatty acid composition consists of major amounts of hexadecanoic (16:0 palmitic), octadecanoic (18:0 stearic), octadecenoic (18:1 vaccenic) and eicosenoic (20:1) acids. Minor amounts (<10% of total fatty acids present) of tetradecanoic (14:0 myristic), hexadecenoic (16:1 palmitoleic), octadecenoic (18:1 oleic), eicosenoic (20:0 arachidic) and cyclopropane (*cis*-9, 10-methyleneoctadecanoic acid). Cell wall peptidoglycan type is Lys-Thr-Ala. DNA G+C content is 41–42 mol%. A distinct polysaccharide antigen, designated h, is present in strains of this species. Monoinfected 'germ-free' rats develop dental caries. DNA–DNA hybridization and 16S rRNA comparative sequence analysis have demonstrated that *S. downei* is most closely related to *S. sobrinus* within the mutans group of streptococci. Previously strains were designated as *Streptococcus mutans* serotype h.

Source/habitat: dental plaque of monkey (*Macaca fascicularis*). Type strain NCTC 11391.

4.13.14 Streptococcus dysgalactiae *(including* 'S. equisimilis')

Streptococcus dysgalactiae (Diernhofer, 1932) is a well-known cause of bovine mastitis but for reasons that remain unclear was not included on the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and was later revived by Garvie *et al.* (1983) following demonstration of its species status by DNA–DNA hybridization and studies of its lactate dehydrogenase. Cells are ovoid or coccal occurring in pairs or chains. A wide zone of β-haemolysis is produced on blood agar by some strains with others giving α-haemolysis. The biochemical reactions of this species are shown in Table 4.4. Strains will grow optimally at 37°C but not at 10°C or 45°C, at pH 9.6 or in the presence of either 6.5% NaCl or 0.1% methylene blue milk. This species does not survive heating at 60°C for 30 min. Cellular long-chain fatty acids composition consists of major amounts of hexadecanoic (C16:0) and octadecenoic (C18:1) acids. Cyclopropane-ring fatty acids and menaquinones are absent. Cell wall peptidoglycan type is Lys-Ala_{1-3}. DNA G+C content is 38.1–40.2 mol%. Strains may react with Lancefield Groups C, G or L antisera. DNA–DNA hybridization studies have demonstrated that *S. dysgalactiae* also includes streptococci designated as '*S. equisimilis*' as well as streptococci of Lancefield Groups C, G (large colony type) and L (Farrow and Collins, 1984b). 16S rRNA sequence analysis has confirmed *S. dysgalactiae* as a member of the pyogenic group of streptococci. Source/habitat: human respiratory tract, vagina and skin, throats and genital tracts of domestic animals. Isolated from mastitic bovine udders. Type strain NCDO 2023.

4.13.15 Streptococcus equi

This species is recognized as an important equine respiratory pathogen. The demonstration of the close relationship between the type strain of *S. equi* and '*S. zooepidemicus*' (Farrow and Collins, 1984b) but at the same time recognition of their respective phenotypes has resulted in the creation of the two subspecies *zooepidemicus* and *equi*.

4.13.15.1 S. equi *subsp.* equi. Cells are 0.6–1.0 μm in diameter, ovoid or spherical, sometimes resembling streptobacilli. Capsules can be demonstrated in young cultures. Wide zones of β-haemolysis are formed on blood agar. Growth is poor in media without serum. The biochemical properties of *S. equi* subsp. *equi* are shown in Table 4.4. Cell wall peptidoglycan type is Lys-Ala_{2-3}. Stains possess the Lancefield Group C antigen. DNA G+C content = 40–41 mol%. Streptococci previously assigned to '*S. zooepidemicus*' have been shown, by DNA homology studies, to belong to *S.*

equi but, because of their phenotypic differences (fermentation of lactose, ribose and sorbitol by subsp. *zooepidemicus*), were not reduced to synonymity. Source/habitat: isolated from equine strangles, abscesses in the submaxillary glands and in mucopurulent discharges of the lower respiratory system of horses and from their immediate environment. Type strain NCTC 9682.

4.13.15.2 S. equi *subsp.* zooepidemicus. The following description is based on that of Farrow and Collins (1984b). Cells are spherical or ovoid and can occur in chains or in pairs. Capsules may be present. Wide zone of ß-haemolysis is produced on blood agar. Growth is optimal at 37°C and does not occur at 10°C or 45°C, after heating at 60°C for 30 min, in the presence of 6.5% NaCl, 10% bile or 0.1% methylene blue milk or pH 9.6. The biochemical properties of subsp. *zooepidemicus* are shown in Table 4.4. Major long-chain fatty acids are hexadecanoic (C18:0) and octadecenoic (C18:1) acids. Strains react with Lancefield Group C antiserum. Cell wall peptidoglycan type is Lys-Ala_{2-3}. DNA G+C content = 41.3–42.7 mol%. Source/habitat: isolated from the blood stream, inflammatory exudates and lesions of diseased animals. Type strain: NCDO 1358.

4.13.16 Streptococcus equinus

Streptococcus equinus was originally described by Andrewes and Horder (1906) as a saprophytic streptococcus chiefly from air, dust and horse dung. Although considered by Sherman (1937) to be a distinct species there was no general agreement between bacteriologists as to the species status of both *S. equinus* and *S. bovis* which shared many common phenotypic characteristics (Jones, 1978). This confusion has been clarified somewhat by the recent application of genetic approaches to the classification of *S. equinus* and related species (Farrow *et al.*, 1984). Cells occur in medium length chains especially in broth cultures. Weak α-haemolysis is produced on blood agar. No growth occurs in the presence of 4% w/v NaCl or 0.04% w/v potassium tellurite. Does not survive 60°C for 30 min. Strains contain the Lancefield Group D antigen and possess peptidoglycan type Lys-Thr-Ala. This species has been redefined on the basis of results from extensive DNA–DNA hybridization experiments and phenotypic characterization (Farrow *et al.*, 1984). These data demonstrated that the type strains of *S. bovis* and *S. equinus* belong in the same DNA homology group and, due to the priority that the same *S. equinus* has over '*S. bovis*', that the latter should be reduced to synonymity. Consequently *S. equinus* is defined according to Farrow *et al.* (1984) as follows: cells are spherical or ovoid in moderately long chains and producing α-haemolysis of varying intensity on blood agar. Growth occurs at 45°C but not at 50°C or at 10°C. Some strains

survive heating at 60°C for 30 min. Growth occurs in 40% w/v bile but not at pH 9.6–0.1% methylene blue milk or in the presence of 6.5% w/v NaCl. The biochemical reactions of *S. equinus* as redefined are also shown in Table 4.5. Cells contain the Lancefield Group D antigen. The DNA G+C content is 36.2–38.6 mol%. Occurs in the alimentary tract of cows, horses, sheep and other ruminants, isolated occasionaly in large numbers from human faeces, and occasionally isolated from cases of human endocarditis. 16S rRNA comparative sequence analysis and DNA–DNA hybridization have shown a close relationship between strains of *S. equinus* and *S. bovis* which together with *S. alactolyticus* formed a distinct cluster within the 16S rRNA derived phylogenetic tree. Source/habitat: alimentary tract of horses. Type strain ATCC 9812 (NCDO 1037).

4.13.17 Streptococcus ferus

Following the recognition of four subspecies (*mutans*, *rattus*, *sobrinus* and *cricetus*) within *S. mutans* by Coykendall (1974) a new mutans-like *Streptococcus* was isolated from wild sucrose-eating rats living in sugar-cane fields (Coykendall *et al.*, 1974). These strains contained the serotype c antigen first described by Bratthall (1970), were found to have a relatively high DNA G+C content (43–45%) and to be genetically distinct by DNA–DNA hybridization. They were initially given the subspecific epithet *ferus* (Coykendall *et al.*, 1976) and later proposed as a separate species *S. ferus* (Coykendall, 1983). Cells are approximately 0.5 μm in diameter and occur in pairs or in chains. On sucrose-containing agar colonies are adherent and raised but without the presence of liquid glucan. Both extra- and intercellular polysaccharides are produced from sucrose. The biochemical reactions of this species are shown in Table 4.3. Strains do not grow at 45°C or in 6.5% NaCl. Serological studies have shown that strains react with *S. mutans* serotype c antiserum. Cell wall peptidoglycan type is Lys-Ala_{2-3}. DNA G+C content is 43–45 mol%. DNA–DNA and DNA–rRNA hybridization studies indicate that *S. ferus* is a species within the mutans group of streptococci although data from multilocus enzyme electrophoresis place it closer to *Streptococcus sanguis*. Source/habitat: oral cavity of wild rats. Type strain ATCC 33477.

4.13.18 Streptococcus gordonii

The classification of streptococci resembling *S. sanguis* has remained confused and unresolved until the recent application of genotypic analyses and extensive phenotypic characterization. This has resulted in the recognition of several new species and amended descriptions of these streptococci that include *S. gordonii* (Kilian *et al.*, 1989a). Cells are observed to form short chains in serum broth, to give α-haemolysis on

horse blood agar plates and pronounced greening on chocolate agar. Most strains produce extracellular polysaccharide on sucrose containing medium. The biochemical reactions of this species and its biotypes are shown in Table 4.3. Cell wall peptidoglycan type is Lys-Ala_{1-3} and the cell wall contains rhamnose and glcyerol teichoic acid. DNA G+C content is 38–43 mol%. Strains react with Lancefield Group H antiserum raised against strain Blackburn or F90A. Strains of *S. gordonii* have previously been designated as *S. sanguis*, *S. sanguis* I, *Streptococcus* sbe, Group H *Streptococcus* and *S. mitis* (strain NCTC 3165). 16S rRNA comparative sequence analysis has not been reported for this species although DNA–DNA hybridization studies previously carried out using strains now known to belong to *S. gordonii* have demonstrated that this species is grouped within the '*S. oralis* group' of Schleifer and Kilpper-Bälz, (1987) that includes *S. sanguis*, *S. oralis*, *S. parasanguis*, *S. pneumoniae*, as well as *S. intermedius*, *S. constellatus* and *S. anginosus*. Strain NCTC 3165, previously designated as the type strain of *Streptococcus mitis* has also been shown to be a phenotypically atypical strain of *S. gordonii*. Source/habitat: human oral cavity and pharynx. Type strain NCTC 7865 (ATCC 10558).

4.13.19 Streptococcus hyointestinalis

Strains of this species were originally described as *S. salivarius* before recognition of their separate species status (Devriese *et al.*, 1988). *Streptococcus hyointestinalis* strains are also phenotypically distinct from another recently described *Streptococcus* isolated from pig intestines, *S. intestinalis*, the latter being β-haemolytic, having a lower G+C content and a different fermentation pattern. Cells form chains and produce sediment with clear supernatant when grown in broth. α-Haemolytic on blood agar. No growth occurs in the presence of 6.5% NaCl or 40% bile. Optimum growth at 37°C and under anaerobic conditions. The biochemical reactions of this species are shown in Table 4.4. No reaction with Lancefield grouping sera (A–G). Cell wall peptidoglycan type is Lys-Ala(Ser). DNA G+C content is 42–43 mol%. Source/habitat: pig intestines. Type strain DSM 20770.

4.13.20 Streptococcus iniae

This member of the pyogenic streptococci was isolated from abscesses on the thorax and abdomen of Amazon river-living freshwater dolphins (Pier and Madin, 1976). It has not however been shown to be pathogenic for other animals. Cells are 0.6–1 μm diameter spherical of ovoid forming medium to long chains. On blood agar colonies are 1 mm diameter with opaque centres and translucent borders and are β-haemolytic or α-haemolytic. The biochemical characteristics of this species are shown in

Table 4.4. No growth occurs at 45°C or in bile–esculin media. Good growth is obtained in Todd–Hewitt broth with overnight incubation at 37°C. DNA G+C content is 33 mol%. Contains a specific antigen extractable by HCl or formamide that does not react that Lancefield grouping sera A–V. 16S rRNA sequence analysis has demonstrated this species to belong to the pyogenic group of streptococci. Source/habitat: freshwater dolphin (*Inia geoffrensis*); isolates from subcultaneous abscesses on thorax and abdomen. Type strain ATCC 29178.

4.13.21 Streptococcus intermedius

The taxonomy and nomenclature of this species is, as with the other members of the '*S. milleri*-group', somewhat confused. *Streptococcus intermedius* (Holdeman and Moore, 1974) was reported as an amended description of the original published description (Prévot, 1925). The source of the original Prévot strain remains unknown and *S. intermedius* was described by Holdeman and Moore as being isolated from human clinical specimens and faeces. As described previously (see description of *S. constellatus*) the close resemblance between *S. intermedius* and several other biochemically and serologically heterogeneous 'species', resulted in the division of all such strains on the basis of lactose fermentation into *S.* MG-*intermedius* (lac+) and *S. anginosus-constellatus* (lac−) (Facklam, 1977). More recent taxonomic studies have shown that three distinct species exist within this group of streptococci and these have retained the names *S. anginosus*, *S. constellatus* and *S. intermedius* (Whiley and Beighton, 1991). An association between *S. intermedius* and abscesses of the brain has also been noted (Whiley *et al.*, 1992). Cells are 0.5–1 μm in diameter, forming short chains. Most strains are α- or non-haemolytic on blood agar. No extracellular polysaccharide is formed on sucrose-containing medium. The biochemical reactions of this species are shown in Table 4.3. Growth is enhanced in the presence of CO_2, reduced under aerobic conditions and some strains require an anaerobic environment for growth. Almost all strains are serologically ungroupable using Lancefield grouping antisera. The cell wall peptidoglycan type is Lys-Ala_{1-3}. DNA G+C content is 37–38 mol%. DNA homology studies and 16S rRNA sequence analysis in particular have shown that *S. intermedius* together with *S. anginosus* and *S. constellatus* form a group of closely related species sometimes referred to as the '*Streptococcus milleri*-group'. Strains resembling *S. intermedius* have also been previously referred to as *Streptococcus milleri*, *Streptococcus* MG, haemolytic and non-haemolytic streptococci of Lancefield Group F, the minute colony-forming streptococci of Lancefield Groups F and G, *Streptococcus* MG-*intermedius* and *Streptococcus anginosus-constellatus*. Source/habitat: human oral cavity and upper respiratory tact. Reported to be present in human faeces.

Isolated from purulent infections in man. Type strain ATCC 27335 (NCDO 2227).

4.13.22 Streptococcus intestinalis

This relatively recently described species of *Streptococcus* (Robinson *et al.*, 1988) comprises approximately 50% or more of the bacteria present in the colon of pigs. Of particular interest is the ability of strains to hydrolyse urea, an important aspect of nitrogen metabolism in animals. Cells form long chains, are often elongated and can occur in pairs of unequal cell size. On blood agar colonies are tiny (1 mm in diameter or less), white, flat to convex, circular, entire and β-haemolytic. The biochemical reactions of this species are shown in Table 4.5. Strains are characterized by the ability to hydrolyse urea. Growth occurs optimally at 37°C, can occur at 45°C and strains can survive 60°C for 30 min. However, no growth occurs at pH 9.6 or in the presence of 6.5% NaCl or 40% bile. DNA G+C content is 39–40%. Some strains react with Lancefield Group G antiserum. Source/habitat: intestines and faeces of pigs. Type strain ATCC 43492.

4.13.23 Streptococcus macacae

This species, first described by Beighton *et al.* (1984) from the dental plaque of monkeys, is one of the more recent additions to the 'mutans-streptococci' species group. However, the true taxonomic position of *S. macacae* within the genus *Streptococcus* remains to be determined. A chain-forming coccus that produces greening on horse blood agar when grown anaerobically or in candle jars. Dextran is produced from sucrose; on sucrose-containing agar 1–2 mm diameter colonies are formed that are easily removed but remain intact. Vivid white, crumbly colony variants can also arise. The biochemical reactions of *S. macacae* are shown in Table 4.3. Strains grow poorly in air and CO_2 stimulates growth. Growth does not occur in the presence of 6.5% w/v NaCl, at 45°C or at pH 9.6 but occurs in media containing 10% and 40% bile. This species is serologically ungroupable against Lancefield antisera. DNA G+C content is 35–36 mol%. Source/habitat: dental plaque of monkeys (*Macaca fascicularis*). Type strain NCTC 11558.

4.13.24 Streptococcus mitis

The name *S. mitis* was first used by Andrewes and Horder (1906) to describe a saprophytic *Streptococcus* present mainly in human saliva and faeces that was short chained, grew well at 20°C on gelatin, did not clot milk, often reduced neutral red and nearly always fermented lactose and saccharose but not the glucosides salicin and coniferin. Subsequent

descriptions of *S. mitis* tended to be poor and ill-defined, with strains characterized mainly on negative criteria. Despite the lack of a clear description of this species, the name *S. mitis* was included in the Approved List of Bacterial Names (Skerman *et al.*, 1980) and has persisted in the literature. More recently Kilian *et al.* (1989a) published on amended description of *S. mitis* giving the name to a group of streptococci whose integrity as a species is better supported by phenotypic and genotypic data. Cells form short or long chains in serum broth, and give α-haemolysis on horse blood agar and pronounced greening on chocolate agar. Extracellular polysaccharide is not produced on sucrose-containing medium. The biochemical reactions of *S. mitis* and its biovars are shown in Table 4.3. Cell wall peptidoglycan type is Lys-direct and cell walls contain ribitol teichoic acid but lack rhamnose in significant amounts. DNA G+C content is 40–41 mol%. Strains may be serologically ungroupable using Lancefield grouping antisera, or may react with Group K or O antisera. *Streptococcus mitis* has not been compared with other streptococcal species using 16S rRNA sequence analyses but, from previous DNA–DNA hybridization studies that included strains now designated as *S. mitis*, it appears that this species belongs within the '*S. oralis* group' of Schleifer and Kilpper-Bälz (1987). Strains of *S. mitis* have been previously designated as '*Streptococcus viridans*', *Streptococcus* Groups O and K and '*Streptococcus mitior*'. Source/habitat: human oral cavity and pharynx. Type strain NCTC 12261.

4.13.25 Streptococcus mutans

Originally described by Clarke (1924) from carious teeth, *S. mutans* was reported as a significant factor in the aetiology of dental caries. Nevertheless, this species was virtually ignored until interest picked up again in the 1960s when experiments into the induction and transmission of dental caries in animals were initiated. Since that time there has been an enormous body of literature focused on *S. mutans*, and subsequent taxonomic studies have revealed that mutans-like cariogenic streptococci comprise a group that currently includes seven species (Coykendall, 1977). Cells are 0.5–0.76 μm in diameter cocci forming short or medium length chains and sometimes forming short rods on some solid media or under acid conditions in broths. Colonies on blood agar are sometimes hard with a tendency to adhere to the agar and are usually α- or non-haemolytic. Some strains produce β-haemolysis. On sucrose-containing agar strains produce extracellular polysaccharides to give colonies that are rough, heaped, and detachable, 1 mm in diameter, frequently with droplets of water-soluble polysaccharide. On TYC agar may yield yellow or white colonies. Strains produce both water-soluble and water-insoluble glucans as well as fructans when on sucrose-containing agar. Intracellular,

glycogen-like glucan is also produced. The biochemical characteristics of this species are shown in Table 4.3. Optimum growth occurs under anaerobic conditions, at 37°C with some strains able to grow at 45°C but no strain growing at 10°C. There are three demonstrable polysaccharide antigens, designated c, e and f. Cell wall peptidoglycan type is Lys-Ala_{2-3}. DNA G+C content is 36–38 mol%. This species gives its name to a group of seven closely related species collectively referred to as the mutans streptococci. Many strains are thought to be cariogenic in man and also induce caries in experimental animals. This species is also isolated from blood cultures in some cases of infective endocarditis. Source/habitat: surfaces of teeth in man and can also be isolated from faeces. Type strain NCTC 10449 (ATCC 25175).

4.13.26 Streptococcus oralis

Bridge and Sneath (1982) originally gave the name *S. oralis* to a cluster of oral streptococci included in a numerical taxonomy study (Bridge and Sneath, 1983) some of which resembled *S. mitis*. However, the phenotypic heterogeneity apparent in the species description was confirmed by genetic analysis which revealed several centres of variation at the species level (Kilpper-Bälz *et al.*, 1985). Kilian *et al.* (1989a) amended the species description further by phenotypic and serological approaches in a taxonomic study of 151 viridans streptococci, many of which had been included in previous taxonomic studies, so that currently the name *S. oralis* is given to a well-defined species. Cells form long chains in serum broth, give α-haemolysis on horse blood agar and pronounced greening on chocolate agar. Extracellular polysaccharide production on sucrose containing medium is a variable characteristic of this species. The biochemical characteristics of this species are shown in Table 4.3. Cell wall peptidoglycan type is Lys-direct and cell walls contain ribitol teichoic acid but little or no rhamnose. DNA G+C content is 38–42 mol%. Streptococci corresponding to *S. oralis* have previously been referred to as '*S. mitior*', *S. mitis*, '*Streptococcus* sbe', '*S. sanguis* I' or '*S. sanguis* II'. 16S rRNA comparative sequencing and DNA–DNA hybridization studies have demonstrated that this species belongs to the so-called '*S. oralis*-group' of species that also include *S. sanguis*, *S. parasanguis*, *S. mitis*, *S. pneumoniae*, *S. intermedius*, *S. constellatus* and *S. anginosus*. Source/habitat: human oral cavity. Type strain NCTC 11427.

4.13.27 Streptococcus parasanguis

The application of DNA–DNA hybridization to atypical viridans streptococci revealed the existence of *S. parasanguis* within the species group that also includes *S. sanguis* and *S. oralis* (Whiley *et al.*, 1990a). Many of the

strains that fell into this species had been included in unnamed DNA homology groups by previous authors. Cells are approximately 0.8–1 μm in diameter, coccoid and chain forming. α-Haemolysis is produced on blood agar. Extracellular polysaccharide is not produced on sucrose-containing medium. The biochemical reactions of this species are shown in Table 4.3. No growth is obtained in the presence of 4% w/v NaCl although most strains grow in the presence of 40% w/v bile and at 45°C. The cell wall peptidoglycan type of *S. parasanguis* has not been determined. DNA G+C content is 40.6–42.7 mol%. *Streptococcus parasanguis* has been shown to be most closely related to *S. sanguis* by DNA homology and 16S rRNA comparative sequence analysis. Source/habitat: human throat and clinical specimens (blood and urine). Type strain ATCC 15912.

4.13.28 Streptococcus parauberis

Streptococcus parauberis (Williams and Collins, 1990) was proposed following the demonstration by DNA–DNA hybridization and 16S rRNA sequencing that the important pathogenic species commonly responsible for bovine mastitis, *S. uberis* consisted of two phylogenetically distinct lines of descent. These had previously been designated *S. uberis* types I and II (Garvie and Bramley, 1979) and the latter were renamed *S. parauberis*. Cells are coccoid and form moderate length chains or occur in pairs. On blood agar strains are weakly α-haemoloytic or non-haemolytic. Growth occurs in the presence of 4% NaCl but not 6.5% NaCl or at pH 9.6. Some strains survive heating at 60°C for 30 min. The optimum temperature for growth is 35–37°C. The biochemical reactions of this species are shown in Table 4.4. DNA G+C content is 35–37 mol%. Some strains of *S. parauberis* have been shown to react against Lancefield E and P antisera (Garvie and Bramley, 1979). 16S rRNA comparative sequence analysis has demonstrated that *S. parauberis* falls into the pyogenic group of streptococci. Strains of this species have previously been designated *S. uberis* type II. Source/habitat: lips, skin and udder tissue of cattle, and in raw milk. Type strain NCDO 2020.

4.13.29 Streptococcus pneumoniae

This extremely important pathogenic species of *Streptococcus* causes pneumonia, meningitis, otitis media as well as being isolated from abscesses, pericarditis, conjunctivitis and other clinical conditions. Currently, this species is the focus of attention due to the emergence of penicillin resistant strains with increasing frequency worldwide (Klugman, 1990). Cells are spherical or ovoid, 0.5–1.25 μm in diameter and are usually seen in pairs or occasionally either as single cells or as short chains. Cells in pairs may be elongated of the distal ends. Strong α-haemolysis is

produced on blood agar. Colonies can be mucoid due to production of a polysaccharide capsule particularly with fresh isolates, smooth due to decreased capsule production or occasionally rough. The temperature range for growth is 25–42°C and incubation under increased CO_2 tension prevents autolysis. In defined media this species requires choline for growth. Bile soluble. The biochemical reactions of this species are shown in Table 4.3. Cell wall peptidoglycan type is Lys-Ala_2(Ser). Variation of the stem peptide has been reported within penicillin resistant strains which carry branched-stem peptides with Ala-Ser or Ala-Ala on the epsilon-amino groups of the stem peptide lysine residue. DNA G+C content is 36–37 mol%. Capsular polysaccharide is an important virulence factor of this species and forms the basis of the antigenic division of strains into types and subtypes, antibody to a particular capsule conferring type specific immunity. 16S rRNA comparative sequencing shows *S. pneumoniae* to be closely related to *S. oralis*. Source/habitat: upper respiratory tract of normal humans and domestic animals and from the upper respiratory tract, inflammatory exudates and various body fluids of diseased humans. Type strain NCTC 7465 (ATCC 33400).

4.13.30 Streptococcus porcinus

ß-Haemolytic streptococci of Lancefield Group E are important pathogens of pigs and have many biochemical characteristics in common with Lancefield Groups P, U and V strains. The somewhat controversial interrelationships of these streptococci were resolved with the demonstration that they should be included within a single species named *S. porcinus* (Collins *et al.*, 1984). Cells are ovoid and form small to medium length chains. On blood agar isolates produce ß-haemolysis. No growth occurs at 10°C and at 45°C or after heating at 60°C for 30 min. The biochemical characteristics of *S. porcinus* are shown in Table 4.4. Cell wall peptidoglycan type is Lys-Ala_{2-4}. Major long-chain fatty acids are hexadecanoic (C16:0) and octadecenoic (*cis*-vaccenic). Menaquinones are absent. DNA G+C content is 37–38 mol%. Strains may react against Lancefield Group E, P, U or V antisera. 16s rRNA comparative sequencing has shown *S. porcinus* to belong within the pyogenic group of streptococci. Strains of *S. porcinus* have previously been referred to as Lancefield Group E, P, U or V streptococci, '*Streptococcus infrequens*', '*S. lentus*' or '*S. subacidus*'. Source/habitat: associated with diseases of pigs (abscesses of the cervical lymph nodes, pneumonia and septicaemia) and from milk. Type strain NCTC 10999.

4.13.31 Streptococcus pyogenes

Streptococcus pyogenes (Rosenbach, 1884), the type species of the genus *Streptococcus* is one of the most important human pathogens within the

genus, giving rise to a number of pyogenic and septicaemic infections and is the only species of *Streptococcus* regularly causing epidemics in man (Maxted, 1978). Cells are 0.5–1 μm in diameter, spherical or ovoid, occurring in short to medium length chains or frequently as pairs in clinical samples. Broth cultures yield long chains. β-Haemolysis is produced on blood agar with three colonial types occurring: glossy, mucoid or matt (dehydrated mucoid). Growth is enhanced by the addition of blood or serum to broths and is optimum at 37°C. Strains do not grow at 10°C, 45°C or in the presence of 6.5% NaCl, 40% bile or at pH 9.6. The biochemical characteristics of this species are shown in Table 4.4. Cell wall peptidoglycan type is Lys-Ala_{2-3}. DNA G+C content is 35–39 (T_m). *Streptococcus pyogenes* possess the Lancefield Group A carbohydrate antigen and strains are also divided on the basis of M, T and R surface protein antigens. Extracellular products that are important biologically and diagnostically include streptolysins O (oxygen labile) and S (oxygen stable and responsible for the zone of β-haemolysis seen around colonies growing on blood agar), erythrogenic toxin (elicits the rash in scarlet fever), streptokinase, DNase, NADase, hyaluronidase and proteinase. 16S rRNA comparative sequencing shows that this species is grouped within the pyogenic streptococci. Source/habitat: upper respiratory tract in man, inflammatory exudates, skin lesions, blood and contaminated environmental dust. Type strain ATCC 12344.

4.13.32 Streptococcus rattus

This species within the mutans-like streptococci was first proposed by Coykendall (1977) for unusual strains of '*S. mutans*' that possessed the antigen b of Bratthall (1970) and produced ammonia from arginine. It should be noted that studies involving this species have invariably been limited to the same few strains. Cells are approximately 0.5 μm in diameter, occurring in chains or pairs. On sucrose-containing agar some strains form rubbery colonies or rough and heaped colonies, with liquid glucan present in beads or puddles. The biochemical characteristics of this species are shown in Table 4.3. Growth is improved under conditions of reduced O_2 or by the addition of CO_2. Strains of this species contain a polysaccharide antigen designated type b. Cell wall peptidoglycan type is Lys-Ala_{2-3}. DNA G+C content is 41–43 mol%. DNA–DNA hybridization studies and 16S rRNA comparative sequence analysis have shown *S. rattus* to be most closely related to *S. mutans* within the mutans streptococci. Strains of this species were previously designated *Streptococcus mutans* serotype b. Source/habitat: oral cavities of rat and man (occasionally). Type strain ATCC 19645.

4.13.33 Streptococcus salivarius

Streptococcus salivarius was first described by Andrewes and Horder (1906) from human saliva. Although not considered an important pathogenic species, *S. salivarius* has occasionally been isolated from infective endocarditis and some strains have been shown to be cariogenic in gnotobiotic animals. Cells are approximately 0.8–1 μm in diameter, spherical or ovoid and form chains of varying length. On blood agar strains are usually non-haemolytic with a few giving α- or β-haemolysis. On sucrose-containing media large mucoid colonies are formed due to extracellular polysaccharide production (soluble fructan:levan). In addition isolates can occasionally produce an insoluble glucan (dextran) with the relative proportions of these extracellular polysaccharides determining the resulting degree of roughness or smoothness of the colonial texture. The biochemical characteristics of this species are shown in Table 4.3. Growth can occur on complex media at 45°C but not at 10°C and ammonia and urea can serve as a source of nitrogen in media that include biotin, cysteine, glucose, nicotinic acid, riboflavin, thiamin, panthothenic acid and inorganic salts. Long-chain fatty acid analysis by capillary gas-liquid chromatography has demonstrated the presence of eicosenoic (C20:1) acids. Cell wall peptidoglycan type is Lys-Thr-Gly. DNA G+C content is 39–42 mol%. Some strains react with Lancefield Group K antiserum. A close relationship has been demonstrated between this species and *S. vestibularis* and *S. thermophilus* by DNA–DNA hybridization. These three species form a distinct cluster (species group) by comparative sequence analysis of 16S rRNA (Bentley *et al.*, 1991). Source/habitat: the oral cavities of man and animals, in particular the tongue and saliva. Type strain NCTC 8618 (ATCC 7073).

4.13.34 Streptococcus sanguis

Originally described by White and Niven (1946) from the blood of patients with endocarditis *S. sanguis* was shown to be biochemically, serologically and, more significantly, genetically heterogeneous before being redefined according to Kilian *et al.* (1989a). Cells usually grow as short chains in serum broth and produce alpha-haemolysis on blood agar and greening on chocolate agar. Extracellular polysaccharide (dextran) is produced on sucrose-containing agar, giving smooth, entire, hard and adherent colonies. The biochemical characteristics of this species and its biotypes are shown in Table 4.3. Cell wall peptidoglycan type is Lys-Ala_{1-3} and rhamnose and glycerol teichoic acid are present in the cell wall. DNA G+C content is 46 mol%. The majority of the strains react against Lancefield Group H antiserum raised against strain Blackburn but not with Group H antiserum raised against strain F90A. Strains of *S. sanguis* have also previously been

designated *S. sanguis* I, '*S. sbe*' and group H *streptococcus*. 16S rRNA sequence analysis has revealed a relatively close phylogenetic relationship between *S. sanguis*, *S. oralis*, *S. pneumoniae*, *S. parasanguis* and the '*S. milleri*-group' (*S. anginosus*, *S. intermedius* and *S. constellatus*). Source/habitat: human oral cavity. Type strain NCTC 7863 (ATCC 10556).

4.13.35 Streptococcus sobrinus

This species was first described by Coykendall (1983) for mutans-like streptococci possessing the groups d or g antigens (Perch *et al.*, 1974). It is thought to be an aetiological agent of dental caries in man, together with *S. mutans*. Cells are 0.5 μm in diameter and form long chains or occur in pairs. Strains are mostly non-haemolytic on blood agar with some strains producing α-haemolysis. On sucrose-containing agar colonies are rough, heaped, approximately 1 mm in diameter and are surrounded by liquid containing glucan. The biochemical characteristics of this species are shown in Table 4.3. Strains of *S. sobrinus* belong to serotypes d or g on the basis of polysaccharide antigens. However, the type strain does not react with either type d or g antisera. Cell wall peptidoglycan type is Lys-Thr-Gly. DNA G+C content is 44–46 mol%. Within the mutans streptococci *S. sobrinus* is most closely related to *S. downei* as shown by DNA–DNA hybridization and 16S rRNA sequence comparisons. This species is associated with dental caries in man and is cariogenic in experimental animals. Previously *S. sobrinus* strains were designated *S. mutans* serotypes d or g. Source/habitat: tooth surface in human oral cavity. Type strain ATCC 33478.

4.13.36 Streptococcus suis

The group of streptococci brought together by Kilpper-Bälz and Schleifer (1987) into a single species named *Streptococcus suis* resolved the taxonomic position of a serologically heterogeneous collection of strains that constitute an important pathogen of pigs. Cells are ovoid, less than 2 μm in diameter, occurring mainly singly or in pairs and occasionally in short chains. Cells can sometimes also tend to form rods. β-haemolysis is produced on horse blood agar, whereas α-haemolysis is produced on sheep blood agar. The biochemical characteristics of this species are shown in Table 4.5. Some strains are resistant to 40% bile although no growth occurs in the presence of 6.5% NaCl, 0.04% tellurite or at 10°C or 45°C. Cell wall peptidoglycan type is usually lysine-direct with occasional strains possessing Lys-Ala_{1-2}. Glucose, galactose, glucosamine and rhamnose are present in the cell wall. Strains contain a lipid-bound teichoic acid cell wall antigen that is closely related to the Lancefield Group D antigen and results in a reaction with Lancefield Group D antiserum. Strains are

groupable into Lancefield Groups R, RS, S and T or are non-groupable. Cross-reaction with Groups E and N antisera and between Group B and Group R antisera occur. In addition, strains can also be subdivided into one to eight capsular polysaccharide serotypes (serovars). Strains of *Streptococcus suis* have also been designated as streptococci of serological groups R, S or T. 16S rRNA sequence analysis has shown *S. suis* to be genetically distinct from other streptococcal species and species groups with the possible exception of *S. acidominimus*. Source/habitat: isolated from pigs with bacteraemia, meningitis or respiratory disease. Type strain NCTC 10234.

4.13.37 Streptococcus thermophilus

The taxonomic status of *S. thermophilus* (Orla-Jensen, 1919) has fluctuated in recent years due to the close relationship demonstrated between these streptococci and the species *S. salivarius*. This discovery resulted in the temporary inclusion of both in a single species *S. salivarius* as subsp. *salivarius* and subsp. *thermophilus* (Farrow and Collins, 1984a) until separate species status was reproposed by Schleifer *et al.* (1991) on the basis of both genetic and phenetic criteria. Cells are 0.7–1 μm in diameter, spherical or ovoid, forming chains or occurring in pairs. Growth at 45°C can give rise to irregular cells and segments. Strains are either α-haemolytic or non-haemolytic on blood agar. The biochemical characteristics of *S. thermophilus* are given in Table 4.3. No growth occurs at 15°C but all strains grow at 45°C and most are able to grow at 50°C. Survives heating for 30 min at 60°C. No growth occurs in 0.1% w/v methylene blue or at pH 9.6. Requires B-vitamins and some amino acids. A group antigen has not been demonstrated. Cell wall peptidoglycan type is Lys-Ala_{2-3}. DNA G+C content is 37–40 mol%. This species is closely related to *S. salivarius* and *S. vestibularis* and, as mentioned above, previously has been proposed as a subspecies of *S. salivarius* (*S. salivarius* subsp. *thermophilus*). 16S rRNA sequence data have demonstrated that *S. thermophilus* is one of a three-member species group that also includes *S. salivarius* and *S. vestibularis*. Source/habitat: milk (heated and pasteurized) – natural habitat unknown. Type strain ATCC 19258 (NCDO 573).

4.13.38 Streptococcus uberis

An important species occurring in bovine mastitis, *Streptococcus uberis* (Diernhofer, 1932) was later shown to include two distinct genetic groups called *S. uberis* type I and II. *Streptococcus uberis* II strains have now been proposed as a distinct species called *S. parauberis* (Williams and Collins, 1990). Cells form moderate length chains or pairs. Weak α-haemolysis or non-haemolysis is produced on blood agar. No growth occurs at 10°C or

45°C. May or may not survive heating at 60°C for 30 mins. Growth occurs in the presence of 4% NaCl but not 6.5% NaCl. The biochemical characteristics of *S. uberis* are shown in Table 4.4. Cell wall peptidoglycan type is Lys-Ala_{1-3}. DNA G+C content is 36–37.5 mol%. Some strains may react with Lancefield Groups E, P or G antisera (Garvie and Bramley, 1979). 16S rRNA comparative sequence analysis has demonstrated that *S. uberis* is a species within the pyogenic group of streptococci. Source/habitat: lips, skin and udder tissue of cows and raw milk. Type strain NCTC 3858 (ATCC 19436).

4.13.39 Streptococcus vestibularis

Streptococcus vestibularis (Whiley and Hardie, 1988) is a relatively new species of oral streptococcus, closely related to *S. salivarius* and *S. thermophilus*. Clinical significance of the species, if any, remains to be established. Cells are approximately 1 μm in diameter, chain-forming cocci. α-Haemolysis is produced on blood agar. Strains do not produce extra- or intracellular polysaccharide. The biochemical characteristics of *S. vestibularis* are shown in Table 4.3. Growth does not occur at 10°C or at 45°C, in the presence of 4% w/v NaCl, 0.0004% w/v crystal violet or in 40% w/v bile but most strains grow in the presence of 10% w/v bile. Long-chain fatty acid analyses have demonstrated major amounts of hexadecanoic (C16:0; palmitic) and octadecenoic (C18:1w7; *cis*-vaccenic) acids in addition to tetradecanoic (C14:0; myristic), hexadecenoic (C16:1), octadecanoic (C18:0; stearic), octadecenoic (C18:1W9; oleic) and eicosenoic (C20:1) acids. Cell wall peptidoglycan type is Lys-Ala_{1-3}. DNA G+C content is 38–40 mol%. Whole cell derived polypeptide patterns by SDS-PAGE, DNA–DNA hybridization and 16S rRNA studies have shown the close relationship between *S. vestibularis*, *S. salivarius* and *S. thermophilus*. Source/habitat: the human oral cavity, especially the vestibular mucosa. Type strain NCTC 12166.

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