NOTE***Streptococcus intestinalis* Robinson et al. 1988 and *Streptococcus alactolyticus* Farrow et al. 1984 are phenotypically indistinguishable**P. Vandamme,^{1,2} L. A. Devriese,³ F. Haesebrouck³ and K. Kersters¹Author for correspondence: P. Vandamme. Tel: +32 9 264 5113. Fax: +32 9 264 5092.
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The one-dimensional whole-cell protein patterns and a variety of biochemical characteristics of the type and reference strains of *Streptococcus intestinalis* and *Streptococcus alactolyticus* were studied. All the data revealed that strains of both species were indistinguishable. Reported differences in haemolytic activity and presence of Lancefield antigens were not reproduced. All strains were α -haemolytic. The *S. intestinalis* type strain possessed the group G antigen, but a second *S. intestinalis* reference strain and all of the *S. alactolyticus* strains possessed the group D antigen. All strains produced urease activity (albeit some after prolonged incubation), a characteristic considered important for *S. intestinalis*. Given the congruence between whole-cell protein electrophoresis and percentage of DNA–DNA hybridization, these data suggest that *Streptococcus intestinalis* Robinson et al. 1988 is a junior synonym of *Streptococcus alactolyticus* Farrow et al. 1984.

Keywords: *Streptococcus intestinalis*, *Streptococcus alactolyticus*, SDS-PAGE, taxonomy

The name *Streptococcus intestinalis* was proposed in 1988 (Robinson et al., 1988) for a group of ureolytic streptococci which were predominant members of the intestinal flora of the colon of pigs (Robinson et al., 1984). To our knowledge, reports on *S. intestinalis* have not appeared since then and this organism was not included in several phylogenetic analyses of the genus *Streptococcus* (Bentley et al., 1991; Kawamura et al., 1995). In Bergey's *Manual of Systematic Bacteriology* it was classified in the species group 'other streptococci' (Hardie, 1986), and at present its real phylogenetic allocation remains unknown.

Accurate identification requires the description of differential characteristics between *S. intestinalis* and other streptococcal species. In a study on the streptococcal and enterococcal flora of pigs, strains corresponding to the description of *S. intestinalis* were identified as *Streptococcus alactolyticus* (Devriese et al., 1994). The latter species was described in 1984 by Farrow and coworkers (Farrow et al., 1984) for a group of streptococci resembling *Streptococcus equinus*, and isolated from the intestines of pigs and faeces of chickens. The present study was initiated when salient similarities were found between the whole-cell protein patterns of the *S. intestinalis* and *S. alactolyticus* type strains in the course of a general

study on the taxonomy and epidemiology of streptococcal and enterococcal species.

S. intestinalis reference strains and strains representative of the species belonging to the *Streptococcus bovis* species group (*S. alactolyticus*, *S. equinus* and *Streptococcus gallolyticus*) (Bentley et al., 1991; Sly et al., 1997) are listed in Table 1. Reference strains of other streptococcal species have been described in detail in previous studies (Vandamme et al., 1996, 1997, 1998; Devriese et al., 1997). Bacteriological purity was checked by plating and examining living and Gram-stained cells.

Whole-cell protein analysis, preparation of cellular protein extracts, PAGE, densitometric analysis, normalization and interpolation of the protein profiles, and numerical analysis were performed as described before (Vandamme et al., 1998; Pot et al., 1994) using the GelCompar 4.0 software package (Applied Maths, Kortrijk, Belgium). The profiles were recorded and stored on a PC computer. The similarity between all pairs of traces was expressed by the Pearson product-moment correlation coefficient. Growth characteristics and biochemical activities were determined using the methods described earlier (Devriese et al., 1997). All other biochemical tests were performed in a 5% CO₂ enriched atmosphere. Urease activity was tested in a

Table 1. Strains examined

Strain*	Other designation†	Depositor‡	Source
<i>Streptococcus alactolyticus</i> LMG 14588	F143		Pig, intestine (Belgium)
<i>Streptococcus alactolyticus</i> LMG 14589	F156		Pig, intestine (Belgium)
<i>Streptococcus alactolyticus</i> LMG 14590	SP62		Chicken (Belgium)
<i>Streptococcus alactolyticus</i> LMG 14591	T118		Pig (Belgium)
<i>Streptococcus alactolyticus</i> LMG 14617	94/551		Chicken (Belgium)
<i>Streptococcus alactolyticus</i> LMG 14808 ^T	NCFB 1091 ^T	NCFB	Pig, intestine
<i>Streptococcus alactolyticus</i> LMG 14810	SP 96		Chicken (Belgium)
<i>Streptococcus alactolyticus</i> LMG 14811	SP 77		Chicken (Belgium)
<i>Streptococcus alactolyticus</i> LMG 14820	STR 783		Parakeet (Belgium)
<i>Streptococcus alactolyticus</i> LMG 14872	92/2214		Dog (Belgium)
<i>Streptococcus equinus</i> LMG 14897 ^T	NCFB 1037 ^T	NCFB	Horse faeces
<i>Streptococcus equinus</i> LMG 15059	NCFB 2445	NCFB	Horse faeces
<i>Streptococcus equinus</i> LMG 14857	S232		Horse, intestine (Belgium)
<i>Streptococcus gallolyticus</i> LMG 16802 ^T	CCUG 35224 ^T	CCUG	Koala faeces (Australia)
<i>Streptococcus gallolyticus</i> LMG 15053	NCFB 2088	NCFB	Cow, mastitis lesion
<i>Streptococcus gallolyticus</i> LMG 15063	NCFB 2572	NCFB	Cow, rumen
<i>Streptococcus intestinalis</i> LMG 14685	ATCC 43493	ATCC	Pig, colon (USA)
<i>Streptococcus intestinalis</i> LMG 14906 ^T	ATCC 43492 ^T	ATCC	Pig, colon (USA)

* LMG, BCCM/LMG Culture Collection, Laboratorium voor Microbiologie Gent, Universiteit Gent, Belgium.

† ATCC, American Type Culture Collection, Manassas, VA, USA; CCUG, Culture Collection University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden; NCFB, The National Collection of Food Bacteria, Agricultural and Food Research Council, Institute of Food Research, Reading Laboratory, Reading, UK.

‡ Own isolate (L.A.D.) if not specified.

Fig. 1. Dendrogram illustrating the result of the numerical analysis of the whole-cell protein profiles of all of the strains studied. The similarity between traces was expressed by the Pearson product-moment correlation coefficient converted to a percentage similarity value for convenience. The *S. intestinalis* reference strains of the *S. alactolyticus*/*S. intestinalis* cluster are indicated in bold characters.

0.01 M sodium phosphate buffer containing 0.2 % urea and 0.05 % phenol red (pH 6.5). Results were read after 4 and 24 h incubation in air. Phenotypic characteristics covered by the Rapid ID32 and API 20 STREP (bioMérieux) microtest systems were analysed according to the recommendations of the manufacturer.

Duplicate protein extracts of several strains were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The correlation level between duplicate protein patterns was at least 94 % (data not shown). When compared with the whole-cell protein patterns of streptococcal

Fig. 2. Whole-cell protein profiles of a representative selection of strains examined. The molecular mass markers used (indicated in the bottom lane) are indicated from left to right: lysozyme (14500 Da), trypsin inhibitor (20100 Da), trypsinogen (24000 Da), carbonic anhydrase (29000 Da), glyceraldehyde-3-phosphate dehydrogenase (36000 Da), egg albumin (45000 Da) and bovine albumin (66000 Da).

reference species, the protein pattern of two *S. intestinalis* reference strains always clustered among the *S. alactolyticus* strains (Fig. 1). Fig. 2 illustrates the whole-cell protein patterns of the *S. intestinalis* type strain and a selection of strains representing the species belonging to the *S. bovis* species group. The type strains of the other streptococcal species occupy distinct positions when included in the numerical comparison (data not shown).

All strains were chain-forming, Gram-positive cocci and produced abundant sediments with clear supernatants in Brain Heart Infusion broth after 1 d incubation. Growth on Columbia blood agar at 42 °C was similar or better than at 37 °C, and definitely better than at 30 °C. No growth was seen at 25 °C. Incubation in air supplemented with 5% CO₂ considerably increased growth of three strains (*S. alactolyticus* LMG 14591 and both *S. intestinalis* reference strains); only a minor growth stimulation was noted for the other strains. All strains were α -haemolytic (this characteristic was expressed most readily at 42 °C). The strains were non-pigmented and non-motile. Colonies were small, 1 mm in diameter or less. At 42 °C, colony dissociation was seen with some strains having very small or flat colonies, as well as normal slightly heaped-up colonies.

No growth occurred in 6.5% NaCl broth. All strains grew on Edward's medium, but only *S. alactolyticus* LMG 14591, LMG 14588, LMG 14635, LMG 14808^T and LMG 14872 and *S. intestinalis* LMG 14906^T lysed aesculin. The same strains produced blackening on kanamycin aesculin azide agar and on aesculin bile agar (Difco). Growth of the other strains was poor on the former and absent on the latter medium. On Slanetz and Bartley agar, tiny slightly pink colonies appeared. Growth on Rogosa agar incubated anaerobically was absent or strongly inhibited, except for one strain (LMG 14635). All strains were positive in the Voges-Proskauer, urease and leucine arylamidase tests. Only one strain (LMG 14591) failed to react in tests for alanyl-phenylalanyl proline arylamidase. All strains produced acid from cellobiose, D-fructose, N-acetylglucosamine, D-glucose, maltose, mannose and saccharose.

All strains examined were negative in tests for arginine dihydrolase, β -glucuronidase, alkaline phosphatase, β -galactosidase, pyrrolidonyl arylamidase, glycytryptophan arylamidase, hippurate hydrolysis and N-acetyl- β -glucosaminidase. None of the strains produced acid from adonitol, D- and L-arabitol, D- and L-arabinose, cyclodextrin, dulcitol, erythritol, D- and L-fucose, methyl α -D-glucoside, gluconate, D- and L-ketogluconate, glycerol, glycogen, inositol, inulin, lactose, D-lyxose, methyl α -D-mannoside, melezitose, pullulan, rhamnose, ribose, sorbitol, L-sorbose, tagatose, xylitol, D- and L-xylose or β -methylxyloside. None of the strains clotted litmus milk. Variable reactions are listed in Table 2. One strain (LMG 14906^T) reacted with Lancefield group G antiserum, while all others were shown to possess the serogroup D antigen.

We demonstrated in several previous studies that comparative whole-organism protein electrophoresis is an excellent tool to differentiate streptococci at the species level and that there is a correlation between one-dimensional whole-organism protein pattern similarity and the level of DNA-DNA hybridization (Vandamme *et al.*, 1996, 1997, 1998; Devriese *et al.*, 1997). The whole-cell protein patterns of two reference strains of *S. intestinalis* were indistinguishable from those of *S. alactolyticus* strains (Fig. 1), suggesting that they represent the same species. Robinson *et al.* (1988) described the latter species as a β -haemolytic streptococcus and used this characteristic to differentiate it from members of the species group 'other streptococci' as listed in *Bergey's Manual of Systematic Bacteriology* (Hardie, 1986). *S. alactolyticus* was reported to be α - or non-haemolytic. In addition, strains of the former species were reported to belong to Lancefield serogroup G or to be untypable, while strains of the latter species were reported to contain the Lancefield group D antigen. Most other characteristics included in the two species descriptions are the same, including the DNA G + C ratio of 39–41 mol%. We determined the haemolysis reaction and Lancefield serogroup for the type strains of both species and a number of additional strains. All strains were α -haemolytic on Columbia agar base (LabM) supplemented with 5% sheep blood. Robinson *et al.* (1988) described *S. intestinalis* as β -

Table 2. Variable reactions of *S. alactolyticus* and *S. intestinalis*

Characteristic	Own data			Published data*	
	<i>S. alactolyticus</i> (10 strains)†	<i>S. intestinalis</i> LMG 14906 ^T	<i>S. intestinalis</i> LMG 14685	<i>S. intestinalis</i>	<i>S. alactolyticus</i>
Activity of:					
β -Glucosidase	5	+	—	NS	NS
α -Galactosidase	8	+	+	NS	+
Amylase	6 (weak)	—	+ (weak)	—	NS
Acid production from:					
Amygdalin	3	+	—	NS	V
Arbutin	3 (2 weak)	+	—	NS	V
Cellobiose	6	+	+	+	+
Aesculin	5	+	—	+	+
Galactose	7	+	+	+	+
β -Gentiobiose	4	+	—	NS	V
Methyl β -D-glucoside	3	+	—	NS	NS
N-Acetylglucosamine	5	+	—	NS	V
Mannitol	3	—	—	—	V
Melibiose	5	—	+	NS	V
D-Raffinose	9	—	+	—	+
Salicin	8	+	—	+	V
Starch	2	—	+	—	V
Trehalose	5	—	—	NS	V
D-Turanose	4	—	—	NS	V

* Data from Robinson *et al.* (1988) and Farrow *et al.* (1984). NS, Not specified; v, result is strain-dependent.

† Number of strains positive.

haemolytic on Brain Heart Infusion agar supplemented with sheep blood, a medium which is not normally used to characterize haemolysis. Only the *S. intestinalis* type strain (LMG 14906^T) reacted with Lancefield group G antiserum; all others were shown to possess the serogroup D antigen. In the description of *S. intestinalis*, strong emphasis has been given to the ureolytic nature of these bacteria but the demonstration of this characteristic was found to be medium-dependent (Robinson *et al.*, 1988). The authors advocated the use of media with not more than 0.01 % yeast extract or 0.01 % trypticase. Urease activity was not listed in the species description of *S. alactolyticus* (Farrow *et al.*, 1984), but later investigations showed that porcine strains were positive in this test (Devriese *et al.*, 1994). In the present study, urease activity was detected for two strains (LMG 14828^T and LMG 14589) after the prescribed 4 h incubation period of the microtest system. All strains were positive after 24 h incubation in the conventional test.

A characteristic which may cause difficulties in descriptions and identifications is the often strongly carboxyphilic nature of strains described as *S. intestinalis* or *S. alactolyticus*. Failure to incubate in a CO₂-supplemented atmosphere may result in supplementary variation in the outcome of biochemical tests (data not shown). All other characteristics examined in

the present study closely correspond to those originally described for *S. intestinalis* and *S. alactolyticus*. Results which were uniformly negative or positive in the strains investigated were also negative or positive in the descriptions of both species. The characteristics found to be variable in this study are listed in Table 2.

We conclude that *Streptococcus intestinalis* Robinson *et al.* 1988 and *Streptococcus alactolyticus* Farrow *et al.* 1984 are phenotypically indistinguishable as determined by whole-cell protein electrophoresis and a variety of biochemical tests. Given the congruence between whole-cell protein electrophoresis and percentage of DNA-DNA hybridization, these data suggest that *Streptococcus intestinalis* Robinson *et al.* 1988 is a junior synonym of *Streptococcus alactolyticus* Farrow *et al.* 1984.

S. alactolyticus can be differentiated from *S. gallolyticus* by its negative lactose reaction, and differs from *S. equinus* by the presence of urease activity. Positive mannitol and melibiose reactions are also indicative for *S. gallolyticus*. Another species resembling *S. alactolyticus* is *Streptococcus hyointestinalis* (Devriese *et al.*, 1988) but this species can be distinguished by its positive pullulan and alkaline phosphatase reactions, and possibly also by the absence of urease activity (Devriese *et al.*, 1994). The latter characteristic, however, needs to be reinvestigated with

appropriate media. *S. alactolyticus* can be distinguished from the enterococci by its inability to produce acid from ribose (Devriese *et al.*, 1993). Other carbohydrate reactions which are consistently positive in the enterococci and often negative in *S. alactolyticus* include amygdalin, arbutin, aesculin, *N*-acetylglucosamine, β -gentiobiose, lactose, methyl β -D-glucoside and trehalose. Inability to grow in 6.5% NaCl broth serves to differentiate *S. alactolyticus* from many, but not all, enterococci. Growth on bile aesculin agar is useful to some extent, because all enterococci produce blackening in this medium, while many *S. alactolyticus* strains do not. Difficulties may also be experienced for the differentiation of *S. alactolyticus* and *Lactococcus garvieae*. Ability to grow in 6.5% NaCl and the positive ribose and arginine reactions of the latter species are useful differentiating characteristics.

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