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Studies on Microaerophilic Cocci (Stuart-Schwan Cocci) Isolated from Summer Mastitis and Other Pyogenic Infections of Cattle

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Madsen M.: Studies on microaerophilic cocci (Stuart-Schwan cocci) isolated from summer mastitis and other pyogenic infections of cattle. Acta vet. scand. 1989, 30, 165–174. – Forty-nine strains of microaerophilic gram-positive cocci (Stuart-Schwan cocci) isolated from summer mastitis, "pyogenes"-mastitis, other pyogenic conditions of Danish cattle and swine, and from the sheep headfly *Hydrotaea irritans* were biochemically characterized with the API 50 CH and API ZYM test kit systems, and screened for production of a variety of extracellular enzymes by agar plate methods. For comparison 4 strains isolated from Swedish cases of heifer and dry cow mastitis were included in the study.

Similarity calculations indicated a high degree of homogeneity within the strains studied (similarity level 92 %; group mean similarity 87 %). The strains probably represent one species, although the taxonomic position of the organism remains unclear. The biochemical feature of the strains studied were very similar for strains isolated from cases of summer mastitis and strains from other sources of origin. It is suggested that the Stuart-Schwan coccus occurs as a natural cohabitant to *Actinomyces pyogenes, Peptostreptococcus indolicus* and the anaerobic organism characteristic of the bacterial complex isolated from summer mastitis and similar pyogenic conditions in ruminants and swine.

API 50 CH; API ZYM.

Introduction

On clinical grounds, summer mastitis in cattle may be defined as an acute suppurative mastitis occurring in non-lactating cows and heifers, predominantly during the summer months, although a similar syndrome occurs occasionally in calves and dry cows at other times of the year (*Marshall* 1981).

Despite the fact that summer mastitis is also known as *Corynebacterium* (s. *Actinomyces*) *pyogenes* mastitis it should be noted that the isolation of *Actinomyces pyogenes* is not a prerequisite to classification as summer mastitis. In recent years application of adequate procedures for the culture of more fastidious microorganisms have revealed that most cases yield mixed infections with at least 5 to 6, and possibly more, bacteria present. The main species concerned are *Actinomyces pyogenes, Peptostreptococcus indolicus,* a microaerophilic coccus (Stuart/Schwan coccus), *Streptococcus dysgalactiae, Bacteroides melaninogenicus* and *Fusobacterium necrophorum* (*Høi Sørensen* 1974, 1978, 1979, *Schwan & Holmberg* 1978/1979, *Tolle et al.* 1983, *Egan* 1986, *Madsen* 1985, 1987).

Of these bacterial species the Stuart-Schwan cocci still remain to be named and properly classified in the present taxonomic system.

Essential information on the habitats and biochemical properties of the organism is lacking and it is not quite clear whether all investigators reporting on an "unidentified microaerophilic coccus" refer to the same organism.

Stuart et al. (1951) examined udder secretions from dairy cattle with summer mastitis and reported for the first time the occurrence of a gram-positive organism which they designated a "microaerophilic coccus". The involvement of this organism in the bacterial aetiology of summer mastitis has been confirmed in later studies (*Høi Sørensen* 1972, 1974, 1978, *Schwan* 1979, *Madsen* 1985, *Hillerton et al.* 1987); recently, the isolations of apparently similar microaerophilic cocci from a range of pyogenic infections of cattle, sheep and goats have been reported (*Slee* 1985, *Slee & McOrist* 1985) suggesting a close association of this organism with *Actinomyces pyogenes, Peptostreptococcus indolicus* and other anaerobes commonly isolated from pyogenic conditions in ruminants.

In the present communication a number of Danish strains of Stuart-Schwan cocci isolated from mastitis and other pyogenic infections of cattle are characterized by their activity in a variety of biochemical tests.

Materials and methods

Bacterial strains

Forty-nine strains of microaerophilic gram-positive cocci isolated from summer mastitis (20), "pyogenes"-mastitis (22), other pyogenic conditions of Danish cattle and swine (6) and from the headfly *Hydrotaea irritans* (1) (Table 1) were examined. The bacteria had been isolated from blood agar plates on which they occurred in mixed culture mostly with *A. pyogenes, P. indolicus, Streptococcus dysgalactiae* and/or *Bacteroidaceae.* They were tentatively identified as Stuart-Schwan cocci on the basis of growth characteristics and bacterial morphology as described by *Stuart et al.* (1951) and *Schwan et al* (1979). In addition 4 Swedish strains isolated from cases of heifer and dry-cow mastitis (*Schwan & Holmberg* 1978/1979) were included in the study for comparison.

Culture media and chemicals

Blood agar (BA): Tryptose blood agar base (Difco) containing 5 % (vol/vol) defibrinated calf blood (National Veterinary Laboratory, Ringsted Dept.), ph 7.2–7.4.

Peptone-yeast broth (VLB): VL basal medium (*Fiévez* 1963) with an addition of 0.2 g glucose (Merck), 0.04 g 1-cystein hydrochloride (Sigma), 1.0 ml hemin-mena-

Table 1. Number of strains, and source of origin, of the investigated strains of microaerophilic cocci (Stuart-Schwan cocci).

Number of strains	Source of origin
20	Summer mastitis
22	Other mastitis, stabled cattle
2	Pulmonary abscess, heifer
1	Hock phlegmone, cow
1	Arthritis, bull
1	Umbilical abscess, calf
1	Croupous pneumonia, swine
1	Sheep headfly (*Hydrotaea irritans*)
4	Heifer and dry cow mastitis (Swedish strains)

dione solution (Sigma) (*Holdeman & Moore* 1973), 0.1 mg resazurin (Sigma), and 0.06 g Bacto agar (Difco) per 100 ml, pH 7.2–7.4.
Hyaluronidase agar: Sodium hyaluronidate (Sigma) agar prepared according to *Smith & Willett* (1968).
Tellurite agar: Tryptose blood agar base (Difco) containing 10 mg potassium tellurite (Merck) per 100 ml (1:10,000).
API 50 CH: Test kit system providing 49 different carbohydrates and carbohydrate derivatives (API System S.A., France).
API ZYM: Test kit system providing 19 different enzyme substrates (API System S.A., France).
All other media: According to *Cowan* (1974).
With the exception of BA, 10 % (vol/vol) horse serum (National Veterinary Laboratory, Ringsted Dept.) was added to all media.

Incubation and maintenance procedures
For short term storage (4–6 weeks) 48–72 h cultures on BA or in VLB were kept at 4°C. For prolonged storage 72 h BA cultures were suspended in horse serum with 7.5 % glucose (wt/vol) and freeze-dried. Prior to examination the strains were subcultured twice on BA to ensure purity.
All solid media were heavily inoculated with a streak of a 48 h culture and incubated anaerobically by the pyrogallol technique (*Fiévez* 1963) at 37°C. Plates were read after 5–7 days.
The API 50 CH and API ZYM test kit systems were, according to the manufacturer's instructions, inoculated in each cupule with two drops of a heavy suspension of bacterial cells in 0.9 % NaCl recovered from two 48 h BA plate cultures. The cupules of the API 50 CH kit were covered with liquid paraffin to obtain anaerobic conditions and then incubated at 37°C for 35 days. Readings were performed daily during the first week, and subsequently at intervals of 2–3 days. For the API ZYM kit the strips were incubated in moist chambers for 4 h at 37°C. API ZYM developing reagents were then added to the cupules, exposed to a strong (1,000 W) light source for 5 min, and the reactions read according to a scaled colour chart supplied by the manufacturer. Tests giving grade 0 and grade 1 reactions were regarded as negative whereas reactions of grades 2 to 5 were considered positive.
All Danish strains were tested once and, if doubtful reactions occurred, twice in all substrates. All Swedish strains were tested 3 times.

Similarity calculations
The homogeneity of the strains was studied in a computerized cluster analysis programme (Chr. Rovsing A/S) routinely used in identification procedures at the Institute of Hygiene and Microbiology. The programme calculates mean similarities, expressed as a similarity matrix, within and between groups (clusters) at a specified level. A comparison was performed at 80 %, 85 %, 90 %, and 95 % levels of similarity.

Results

Growth characteristics
All strains grew on BA aerobically, anaerobically and in atmospheric air containing 10 % carbon dioxide. Visible colonies appeared after 2 to 3 days of incubation anaerobically and in 10 % carbon dioxide, whereas aerobic growth was slower with visible colonies as a rule appearing after 4 to 5 days of incubation.
Satellitism was a constant feature when strains were co-cultivated with *A. pyogenes* or *Micrococcus spp.* However all strains grew without "helper" colonies as well. As compared to ordinary blood agar, growth

was enhanced on chocolate agar and lecithovitellin agar.

Colony morphology
Two days of incubation anaerobically produced pin-point colonies which on continued incubation developed into small (d = 1.0–1.5 mm), smooth, translucent dew-drop colonies. None of the strains produced haemolysis on the bovine blood agar employed but all strains produced zones of greening around the colonies when left for several days at room temperature.

Cell morphology
In gram-stained smears and India ink preparations from broth cultures the bacteria were coccoid, somewhat uneven in size (d = 1.0–1.5 μm), and were arranged singly, in pairs, clumps, or short chains of 3 to 4 cells. In fresh cultures the bacteria were gram-positive whereas older cultures were gram-labile. In older cultures large coccal forms were often observed. Capsule formation was not detected using phase-contrast microscopy or ordinary microscopy of India ink preparations.

Catalase and cytochrome oxidase
None of the strains produced catalase or cytochrome oxidase.

Motility
All strains were non-motile when examined by phase-contrast microscopy of 48 h broth cultures.

OF test
All strains attacked glucose fermentatively in Hugh & Leifson's medium. Acid production was weak and most strains had to be incubated for 5 to 7 days before a definite reading could be performed.

API 50 CH
All strains were positive in the ribose, fructose, N-acetylglucosamine, esculin, lactose, D-turanose and D-tagatose tests, and all were negative in the erythritol, adonitol, β-methylxyloside, rhamnose, dulcitol, inositol, α-methylmannoside, inulin, L-arabitol and 2-ketogluconate tests, whereas the remaining characters were variable. Late fermentation reactions were recorded in a number of substrates (Table 2).
In 40 of the 49 substrates tested in the API 50 CH system the bacterial strains gave very similar reactions, where a "typical" reaction is defined as positive or negative in 80 % or more of strains tested (see Table 2). A subdivision of strains according to source of origin (Table 3) did not reveal any significant differences between strains of different origin.

API ZYM and extracellular enzymes
All strains were positive for esterase, esterase lipase, leucine arylamidase, phosphoamidase, hyaluronidase and tellurite reduction (1:10,000), and all were negative for amylase, lecithinase and caseinase. With a typical reaction defined as above, the reactivity pattern was consistent for 20 of the 24 tests (Table 4). No significant differences were recorded between strains from different sources.

Miscellaneous tests
Five strains were subjected to indole, methyl red, Voges-Proskauer, nitrate reduction, esculin, bile solubility and optochin sensitivity tests in conventional media. All strains were negative.
The 5 strains were sensitive to chloramphenicol, tetracycline and cephalosporins, and resistant to novobiocin and polymyxin B when tested on BA by the disc sensitivity method (NeoSensitabs, Rosco).

Table 2. Biochemical reactions of 49 Danish strains of Stuart-Schwan cocci in the API 50 CH system. Positive reaction in relation to time.

	Total number of strains positive on day					Positive (%)
Substrate	1	2	5	15	35	
Glycerol	0	3	4	10	13	27
Erythritol	0	0	0	0	0	0
D-arabinose	0	0	0	38	45	92
L-arabinose	0	0	0	32	38	78
Ribose	0	0	3	46	49	100
D-xylose	0	0	0	39	43	88
L-xylose	0	0	0	38	46	94
Adonitol	0	0	0	0	0	0
Beta-methyl-xyloside	0	0	0	0	0	0
Galactose	28	34	35	44	46	94
Glucose	15	21	22	35	38	78
Fructose	27	33	34	48	49	100
Mannose	22	28	29	43	43	88
Sorbose	0	0	0	40	43	88
Rhamnose	0	0	0	0	0	0
Dulcitol	0	0	0	0	0	0
Inositol	0	0	0	0	0	0
Mannitol	0	0	0	2	2	4
Sorbitol	0	0	0	1	1	2
Alpha-methyl-mannoside	0	0	0	0	0	0
Alpha-methyl-glucoside	0	0	0	41	41	84
N-acetyl-glucosamine	36	41	42	49	49	100
Amygdalin	0	5	6	10	10	21
Arbutin	1	3	3	9	10	21
Esculin	0	4	4	47	49	100
Salicin	4	14	16	44	44	90
Cellobiose	5	21	21	43	44	90
Maltose	14	21	23	35	38	78
Lactose	28	40	42	48	49	100
Melibiose	0	1	1	2	2	4
Sucrose	2	5	6	43	44	90
Trehalose	26	33	34	46	46	94
Inulin	0	0	0	0	0	0
Melezitose	0	2	2	40	42	86
Raffinose	0	0	0	3	3	6
Amidon	17	28	29	38	38	78
Glycogen	19	26	27	35	35	71
Xylitol	0	0	0	1	1	2
Gentiobiose	0	10	11	15	16	33
D-turanose	28	34	35	47	49	100
D-lyxose	0	1	1	44	46	94
D-tagatose	5	12	14	47	49	100
D-fucose	0	0	0	1	1	2
L-fucose	0	0	0	2	3	6
D-arabitol	0	0	0	1	1	2
L-arabitol	0	0	0	0	0	0
Gluconate	0	0	0	1	1	2
2-keto-gluconate	0	0	0	0	0	0
5-keto-gluconate	1	12	18	46	47	96

Table 3. Biochemical reactions of 53 strains of Stuart-Schwan cocci in the API 50 CH system, grouped according to source of origin.

Substrate	Summer mastitis (20)	Other mastitis (22)	Other isolates (7)	Total (49)	Swedish strains (4)
Glycerol	d	d	d	d	d
Erythritol	–	–	–	–	–
D-arabinose	+	+	+	+	+
L-arabinose	d	d	d	d	+
Ribose	+	+	+	+	+
D-xylose	+	d	+	+	+
L-xylose	+	+	+	+	+
Adonitol	–	–	–	–	–
Beta-methyl-xyloside	–	–	–	–	–
Galactose	+	+	d	+	+
Glucose	d	+	d	d	+
Fructose	+	+	+	+	+
Mannose	+	+	d	+	+
Sorbose	+	d	+	+	+
Rhamnose	–	–	–	–	–
Dulcitol	–	–	–	–	–
Inositol	–	–	–	–	–
Mannitol	–	–	–	–	–
Sorbitol	–	–	–	–	d
Alpha-methyl-mannoside	–	–	–	–	–
Alpha-methyl-glucoside	d	+	+	+	d
N-acetyl-glucosamine	+	+	+	+	+
Amygdalin	d	d	–	d	d
Arbutin	d	d	–	d	d
Esculin	+	+	+	+	+
Salicin	+	+	d	+	+
Cellobiose	+	+	+	+	+
Maltose	d	+	d	d	+
Lactose	+	+	+	+	+
Melibiose	–	–	–	–	–
Sucrose	+	+	+	+	d
Trehalose	+	+	+	+	+
Inuline	–	–	–	–	–
Melezitose	d	+	+	+	d
Raffinose	–	–	–	–	–
Amidon	d	+	d	d	+
Glycogen	d	+	d	d	+
Xylitol	–	–	–	–	–
Gentiobiose	d	d	d	d	d
D-turanose	+	+	+	+	+
D-lyxose	+	+	+	+	+
D-tagatose	+	+	+	+	+
D-fucose	–	–	–	–	–
L-fucose	–	–	–	–	–
D-arabitol	–	–	–	–	–
L-arabitol	–	–	–	–	–
Gluconate	–	–	–	–	–
2-keto-gluconate	–	–	–	–	–
5-keto-gluconate	+	+	+	+	+

Symbols: – 0–20 % positive, d 21–79 % positive, + 80–100 % positive.

Table 4. Enzymatic reactions of 53 strains of Stuart-Schwan cocci in the API ZYM system, grouped according to source of origin.

Substrate	Summer mastitis (20)	Other mastitis (22)	Other isolates (7)	Total (49)	Swedish strains (4)
Alkaline phosphatase	+	+	+	+	+
Esterase (C4)	+	+	+	+	+
Esterase lipase (C8)	+	+	+	+	+
Lipase (C14)	–	–	–	–	–
Leucine arylamidase	+	+	+	+	+
Valine arylamidase	–	d	–	d	–
Cystine arylamidase	+	+	+	+	+
Trypsin	–	–	–	–	–
Chymotrypsin	–	–	d	–	–
Acid phosphatase	+	+	+	+	+
Phosphoamidase	+	+	+	+	+
Alfa-galactosidase	–	–	–	–	–
Beta-galactosidase	d	d	d	d	d
Beta-glucuronidase	–	–	–	–	–
Alfa-glucosidase	d	d	d	d	+
Beta-glucosidase	–	–	–	–	–
N-acetyl-beta-glucosaminidase	d	d	d	d	+
Alfa-mannosidase	–	–	–	–	–
Alfa-fucosidase	–	–	–	–	–
Hyaluronidase	+	+	+	+	+
Amylase	–	–	–	–	–
Lecithinase	–	–	–	–	–
Caseinase	–	–	–	–	–
Tellurite reduction (1:10,000)	+	+	+	+	+

Symbols as in Table 3.

Similarity calculations

A comparison of all strains at 80 %, 85 % and 90 % levels of similarity described all strains as one homogenous cluster, whereas 9 strains were excluded without being able to form a separate cluster when the level of similarity was increased to 95 %. The 9 strains were distributed in the 3 subgroups, viz. summer mastitis 3 (of 20), other mastitis 4 (of 22), and other isolates 2 strains (of 7). At a similarity level of 92 % no strains were excluded, and the strains displayed homogeneity with a group mean similarity of 87 %.

Discussion

On the whole the cultural and biochemical features of the strains studied showed a high degree of similarity and it thus seems likely that the strains examined represent one species. The homogeneity included the Swedish reference strains as well (cf. Table 3 and 4). According to the limited description given by *Stuart et al.* (1951), and to the more extensive examination carried out by *Schwan et al.* (1979), it seems reasonable that the strains studied herein are identical to the microaerophilic cocci isolated from English and Swedish cases of heifer and dry cow

mastitis. Also, the findings conform to the description of microaerophilic cocci recently isolated from pyogenic infections of ruminants in Australia (*Slee* 1985). Minor differences in fermentation reactions as regards arabinose, ribose, xylose, sucrose and esculin may be noted but these differences may be most easily explained by differences in incubation periods; in the present investigation all carbohydrates were incubated for 35 days, compared with 24 h (*Slee* 1985), or 48 h (*Schwan et al.* 1979) in earlier studies. As may be seen from Table 2 a substantial number of carbohydrate fermentation tests in the present study were negative after 24 h or 48 h of incubation but became positive on prolonged incubation, which seems to correlate well with the records of the slow growth of this organism.

In agreement with the results of *Stuart et al.* (1951) one of the distinct features of the strains in the present series was the satellitic growth exhibited around other bacterial colonies such as those of *A. pyogenes.* Although freshly isolated strains can be adapted by serial subculture to become independent of helper bacteria the feature strongly suggests an almost symbiotic relationship between the members of the complex of bacteria isolated from summer mastitis and other infectious conditions commonly known as "pyogenes"-infections. This view is supported by the observations of *Høi Sørensen* (1980) in which a stimulating effect of *P. indolicus* and the Stuart-Schwan coccus on the toxin production of *A. pyogenes* was demonstrated. The active participation of the Stuart-Schwan coccus in the pathogenesis of summer mastitis seems obvious as judged by the studies of *Schwan* (1980) in which high and prolonged IgG titres against the Stuart-Schwan coccus were recorded in experimentally infected animals. The specific contribution of the organism in the pathogenesis is somewhat obscure, but might be attributed to its production of hyaluronidase (*Høi Sørensen* 1972, *Schwan et al.* 1979), a potential virulence factor produced by all strains in the present study as well.

In light of the recent reports of the isolation of similar cocci from pyogenic infections other than mastitis (*Slee* 1985, *Slee & McOrist* 1985), and on the grounds of the homogeneity of isolates from various sources including swine and the sheep headfly *H. irritans* in the present investigation, it seems logical to assume that the Stuart-Schwan coccus is a natural cohabitant to *A. pyogenes, P. indolicus* and the other microorganisms involved in this complex.

The taxonomic position of the Stuart-Schwan cocci remains unsolved. Identification as micrococci, staphylococci or anaerobic cocci seems highly unlikely, and, although some characteristics such as microaerophilic growth, hyaluronidase production and a moderate resistance to tellurite suggest a relatedness to streptococci, this is not consistent with the end products of its glucose metabolism (*Schwan et al.* 1979). Furthermore, attempts to identify isolates by Lancefield grouping have not been successful (*Slee* 1985, own unpublished results). Thus a proper taxonomic classification awaits the application of more sophisticated methods such as the determination of guanin plus cytosin content and DNA/RNA homology values.

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Sammendrag

Studier over mikroaerofile kokker (Stuart-Schwan kokker) isoleret fra sommermastitis og andre pyogene infektioner hos kvæg

49 stammer af grampositive, mikroaerofile kokker (Stuart-Schwan kokker) isoleret fra tilfælde af sommermastitis, "pyogenes-mastitis", andre pyogene processer fra danske køer og svin, samt fra plantagefluen *Hydrotaea irritans,* karakteriseredes ved deres biokemiske profil i testsystemerne API 50 CH og API ZYM. Desuden undersøgtes stammernes produktion af extracellulære enzymer under anvendelse af udvalgte pladesubstrater. 4 stammer isoleret fra svenske tilfælde af sommermastitis og goldkomastitis inkluderedes i undersøgelsen som referencestammer.

Similaritetsstudier placerede alle undersøgte stammer i een gruppe på et højt similaritetsniveau (92 %), med en indbyrdes similaritet på 87 %. De undersøgte stammer udgør formentlig een species, omend den eksakte taksonomiske placering endnu er uafklaret.

Der kunne ikke påvises nogle væsentlige forskelle mellem den biokemiske profil af stammer isoleret fra mastitistilfælde og stammer af anden oprindelse. Det må således antages, at Stuart-Schwan kokken findes naturligt associeret til *Actinomyces pyogenes, Peptostreptococcus indolicus* og andre anaerobe bakterier, der indgår i det kompleks af bakterier, der ofte isoleres fra sommermastitis og lignende pyogene infektioner hos drøvtyggere og svin.

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