

BOVINE COAGULASE-NEGATIVE STAPHYLOCOCCI: BIOCHEMISTRY AND POLYMERASE CHAIN REACTION

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Abstract

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Staphylococcus (*S.*) *simulans*, *S. chromogenes*, and *S. epidermidis* were the species of coagulase-negative staphylococci (CNS) most frequently involved in subclinical mastitis of dairy cattle. The same biochemical features characterized the successive isolates of a particular chronically infected udder quarter. Occurrence rate of the various species differed between the herds examined. In cases of clinical mastitis of cows *S. xylosus*, *S. simulans*, *S. haemolyticus*, and *S. chromogenes* predominated. A comparison between strains of the particular CNS species from infections in man and cattle did not show host attributable biochemical features or profiles obtained by polymerase chain reaction using arbitrary primers (AP-PCR).

Bovine mastitis, Coagulase-negative staphylococci, biochemical features, polymerase chain reaction

Coagulase-negative staphylococci (CNS) can be frequently isolated from milk samples of dairy cows, often in association with subclinical mastitis (Timms et al. 1987; Harmon and Langlois 1989; Watts et al. 1989; Davidson et al. 1992; Matthews et al. 1992; Todhunter et al. 1993). For differentiation between species of CNS several approaches have been supposed. Typing schemes primarily based on panels of biochemical tests have often been applied in tandem with other methods including composition of cell wall, isoenzyme pattern, polymorphism of whole cell protein and penicillin binding protein, pattern of rRNA gene and of endonuclease cleaved DNA, polymerase chain reaction using arbitrary primers (AP-PCR). By AP-PCR Welsh and McClelland (1990) obtained species specific patterns of AP-PCR products. Moreover, *Staphylococcus* (*S.*) *haemolyticus* of human and non-human primate origin produced different AP-PCR patterns related to their ecological origin and supposedly to host specificity of strains. The purpose of this work is to study (1) involvement of the various CNS species in udder infections of cows and (2) comparison between strains within the particular CNS species originating from man and cattle by means of AP-PCR.

Materials and Methods

Bacterial strains

The CNS examined in this study originated from cases of clinical mastitis (54 strains) and of subclinical mastitis (57 strains) in cattle. For comparison, 8 strains from infections in man were included in these studies.

Of the strains from bovine clinical mastitis 41 were isolated from udder secretions in 39 dairy herds submitted for bacteriological examination to the regional veterinary laboratory. An additional 13 strains originated from Belgium.

The 57 strains from subclinical mastitis were selected in five large herds of cattle (Table 1) with 300 to 500 cows each. An infection of the mammary gland means isolation of CNS in pure culture together with an elevated total cell count (>300 000 cells/ml milk). Sampling of udder secretions was carried out before and after milking. Repeated isolates from a particular udder quarter possessed of the same biochemical features were considered a single strain. The 57 strains represented a total of 580 isolates. All of the herds practised post milking teat dipping but only two of them (herds Np and Lu) applied a proper hygiene at milking. There was an occasional history of *Streptococcus agalactiae* in these herds and the infected cows were removed for slaughter.

In herd Np quarter milk samples from 463 cows at freshening were investigated for the presence of CNS infections. 21 cows with 29 chronically CNS infected quarters in herd Np were resampled monthly or bimonthly to the next freshening. The 29 strains represented a total of 502 isolates. In herds Mo and Zi, respectively, 7 out of 197 and 3 out of 293 cows could be found chronically infected. Three consecutive samplings were performed and a total of 42 isolates gathered. Herds Ka and Lu have been sampled only once.

Table 1
CNS infections in herds of cattle

	herd of cattle Np	Mo	Zi	Ka	Lu	MST
cows/quarters examined	463/1846	197/774	293/1150	207/822	97/394	
cows/quarters CNS infected	43/59 (9%/14%)	21/21 (4%/1%)	20/26 (11%/4%)	25/29 (7%/1%)	3/3 (3%/<1%)	
cow/quarters chronically infected	21/29	7/7	3/4	ne	ne	
SCC ^x in quarters free of CNS	4x10 ⁴	5x10 ⁴	1x10 ⁵	8x10 ⁴	2x10 ⁴	
with CNS	5x10 ⁵	6x10 ⁵	8x10 ⁵	6x10 ⁵	4x10 ⁵	
strains identified to species level						
<i>S. simulans</i>	17	2			1	9
<i>S. chromogenes</i>	9	1	2	3	2	6
<i>S. epidermidis</i>	2	4	1	9		2
<i>S. xyloso</i>	1		1			11
<i>S. warneri</i>				1		3
<i>S. haemolyticus</i>				1		8
<i>S. cohnii</i>						1
<i>S. saprophyticus</i>						1
total number	29 ^y	7 ^y	4 ^y	14 ^z	3 ^z	41

Explanatory notes:

MST, strains from cases of clinical mastitis from 39 herds of cattle; x, somatic cell count from quarter milk samples of CNS infected cows only; y, chronically infected quarters; z, quarters sampled but once; ne, not examined.

Sampling procedures

Prior to sampling, the udder of each cow was washed, dried, and disinfected thoroughly, and about 15 ml of fore-milk from each quarter were discarded. Then the two individual quarter samples, one before, one after automatic milking, were collected in separate test tubes. Samples were kept on ice until starting the laboratory examination within the next 6 to 15 hours.

Microbiology and somatic cell count

Each milk sample (0,1 ml) was streaked onto the quadrant of a 3% blood agar plate. Plates were incubated for 48 hours at 37 °C. A single colony of each primary culture of abundant growth (at least 10 colonies) yielding pure cultures with only one morphologically homogenous type of CNS was selected for further studies. Somatic cell counts (SCC) were done using a Fossomatic cell counter (A/SN, Hillerod, Denmark).

Biochemical characterization of CNS

Methods described by Kloos et al. (1974) were applied for biochemical characterization of strains. In addition, sensitivity to furazolidone was tested according to Rheinaben and Hadlock (1981); sensitivity to novobiocin according to Devriese (1979). Production of phosphatase, urease, hyaluronidase, heat stable DNase and splitting of Tween 80 was studied as described by Devriese et al. (1983). Production of glucosidase and glucuronidase was determined according to White et al. (1990). Inhibition of delta-hemolysin was detected as described by Salka (1991). Strains were identified to genus level on the basis of culture and cell morphology, pigmentation, Gram stain, catalase activity, hemolysis, clumping factor, coagulase of rabbit and human plasmas and sensitivity to furazolidone. The identification to species level was based on features contained in table 2. Production of heat stable DNase, hyaluronidase, splitting of Tween 80 and inhibition of delta-hemolysin were only determined for differentiation between *S. hyicus* and *S. chromogenes*. From the reaction patterns thus obtained CNS species were determined by using the data base of Geary et al. (1989) modified at the Institute for Experimental Epidemiology, Wernigerode.

For control, the following strains were used: *S. epidermidis* (CCM 2124), *S. hominis* (CCM 2732), *S. haemolyticus* (CCM 2737), *S. warneri* (CCM 2730), *S. saprophyticus* (CCM 883), *S. simulans* (CCM 2705), *S. xyloso* (CCM 2738), *S. sciuri* (CCM 3433), *S. cohnii* (CCM 2736), *S. hyicus* (CCM 2368), *S. chromogenes* (CCM 3387), *Micrococcus luteus* (CCM 169).

AP-PCR

A total of 50 strains of bovine and 8 of human origin were examined. Table 3 shows the origin of the strains. Genomic DNA of the strains was isolated by the method of Bialkowska-Hobrzanska et al. (1990). AP-PCR procedure was carried out according to Williams et al. (1990) using the following three 10mer primers already applied by Williams et al. (1990): namely

5'TCACGATGCA (designated primer 1), 5'ACGGTACACT (primer 2), 5'CACATGCTTC (primer 3). The primers have been synthesized by Dr. Birch-Hirschfeld, Institute for Molecular Biotechnology, Jena. Concentration of the primers used was 4 µM and 0,2 µM for both primers 1 and 2, while 1 µM was applied for primer 3. Two runs

of PCR experiments in duplicate tubes were carried out with each of the template DNA. The reagent control that should not produce any amplification product contained all components used for AP-PCR but without addition of template DNA. Amplification products were analyzed by electrophoresis in 1.4% agarose gel and detected by staining with ethidium bromid. Size markers were the 1 kb DNA ladder from GIBCO BRL.

Results

Mastitis and CNS species

The occurrence of subclinical mastitis associated with CNS in 1257 cows in the five herds of cattle, mean values of somatic cell count (SSC), and CNS species involved are presented in Table 1. On the basis of biochemical features six CNS species were identified among the 57 strains recovered from the five dairy herds. The same biochemical features characterized the successive isolates of a particular quarter, and the successive isolates were considered a single strain. Occurrence and prevalence of the various species differed between the five herds of cattle, but, *S. simulans*, *S. chromogenes*, and *S. epidermidis* predominated in chronic infections. Among the 41 CNS strains from 39 farms isolated from quarters with clinical mastitis, the four species *S. xylosum*, *S. simulans*, *S. haemolyticus*, and *S. chromogenes* were found in relatively high frequency (Table 1), while another four species (*S. warneri*, *S. epidermidis*, *S. saprophyticus*, and *S. cohnii*) were infrequent.

Characteristics of the CNS strains

The features of the CNS strains investigated by biochemical methods are shown in Table 2. All of the strains could be identified to species level with high certainty except for some of the *S. chromogenes* strains. 9 out of the 26 *S. chromogenes* strains were classified being *S. chromogenes* despite their ability to split Tween 80 and their lack in pigment production.

The various patterns of biochemical reactions within the particular CNS species were detected among the strains originating from the same herd of cattle, from different dairy herds as well as among the strains from diseases in man.

AP-PCR

The AP-PCR experiments performed with 58 strains representing four different CNS species resulted in reproducible patterns of amplified DNA.

Origin and species of the strains tested, the patterns of amplified DNA, and the number of different AP-PCR profiles observed within each of the species are listed in Table 3. Here, an AP-PCR profile includes all patterns of amplified DNA generated with a given strain by each of the three primers at the various concentrations applied.

For the species *S. chromogenes*, *S. epidermidis*, *S. simulans* and *S. xylosum* two, three, five, and six AP-PCR profiles, respectively, were observed. There was no correlation between the groups of AP-PCR profiles within a CNS species and the origin of strains neither from cattle of different dairy herds nor from man or cattle.

Between one and five amplicates resulted from each fingerprinting. The size of the amplicates increased in most of the cases as the concentration of a primer in the AP-PCR reaction decreased. Each primer at each of the concentrations used gave different patterns of DNA amplicates with a potential of detecting polymorphisms between species and between groups of strains. Some of these patterns observed within a particular species, however, were rather similar or even the same.

Discussion

The results of the study indicating the infrequent occurrence of CNS associated subclinical mastitis in dairy cows are in agreement with reports from the literature (Timms and Schultz 1987; Harmon and Langlois 1989; Rainard et al. 1990; Davidson et al.

terns indicating the existence of host adapted subspecies. For CNS of human and non other primate origin Kloos and Wolfshohl (1979; 1983) confirmed the hypothesis of a conjugate evolution of microorganisms with their host by DNA hybridization. The question rises whether the conditions in animal husbandry favour a mutual exchange of CNS strains between man and animals, thus being the reason for a close relationship between them. To answer this question, DNA homology studies with human and bovine strains of the particular CNS species are needed that can provide the most exacting data to determine the taxonomic status and relatedness of the species and the existence of subspecies.

Bovinní koaguláza-negativní stafylokoky: biochemie a polymerázová řetězová reakce

Druhy *Staphylococcus (S.) simulans*, *S. chromogenes*, a *S. epidermidis* jsou druhy koaguláza-negativních stafylokoků (CNS), nejčastěji se vyskytujících při subklinických mastitidách dojníc. Izoláty z chronicky infikovaných čtvrtí mléčné žlázy byly charakterizovány stejnými biochemickými vlastnostmi. Frekvence výskytu různých druhů se u vyšetřovaných stád lišila. V případech klinických mastitid dojníc převládaly *S. xylosus*, *S. simulans*, a *S. chromogenes*. Srovnáním kmenů CNS humánní a bovinní provenience nebyly nalezeny odlišné biochemické vlastnosti ani profily získané pomocí polymerázové řetězové reakce.

Коагуляза-негативная крупного рогатого скота стафилококками: биохимия и цепная реакция полимеризации

Виды *Staphylococcus (S.) simulans*, *S. chromogenes* и *S. epidermidis* являются видами коагуляза-негативных стафилококков (CNS), чаще всего встречающиеся в время субклинических маститов дойных коров. Изоляты хронически инфицированных четвертой молочной железы отличались одинаковыми биохимическими свойствами. Частота наличия разных видов у отдельных исследуемых стад отличалась. В случае клинического мастита дойных коров преобладающими явились *S. xylosus*, *S. simulans* и *S. chromogenes*. Сопоставлением CNS человека и крупного рогатого скота не выявили отличающихся биохимических свойств ни профилей, полученных с помощью цепной реакции полимеризации.

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