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From the State Veterinary Serum Laboratory, Department for Jutland, Århus, Denmark.

# PEPTOCOCCUS (S. MICROCOCCUS) INDOLICUS

## THE DEMONSTRATION OF TWO VARIETIES OF HEMOLYSIN FORMING STRAINS

By

*Gunner Høi Sørensen*

HØI SØRENSEN, GUNNER: *Peptococcus (s. Micrococcus) indolicus. The demonstration of two varieties of hemolysin forming strains.* Acta vet. scand. 1975, *16*, 218—225. — On the basis of biochemical and serological criteria 2 hemolysin forming varieties of peptococci were identified as Peptococcus indolicus.

Of a total of 16 hemolytic strains examined 9 originated from the vagina of clinically healthy cows, 4 from mastitis secretions from dry cows, 2 from the interdigital skin of clinically healthy sows, and 1 from a subcutaneous abscess in a pig.

Two strains were designated α-hemolytic and 14 β-hemolytic.

On blood agar plates colonies of the α-hemolytic variety were surrounded by narrow zones of almost complete hemolysis, while colonies of the β-hemolytic variety were surrounded by broad zones of incomplete hemolysis. The hemolysins were termed α- and β-hemolysin, respectively.

The β-hemolysin, but not the α-hemolysin, could be demonstrated in cultures grown in liquid media. The β-hemolysin was found to be filtrable, relatively thermoresistant, and non-dermonecrotic.

By gel diffusion analyses the 2 α-hemolytic strains were referred to Serotype C. Ten of the β-hemolytic strains belonged to Serotype C, 2 to Type B, 1 to Type D, and 1 to Type E.

p e p t o c o c c u s (s. m i c r o c o c c u s) i n d o l i c u s; h e m o l y s i n f o r m a t i o n.

Micrococcus indolicus, described and named by *Christiansen* in 1934, can regularly be isolated from summermastitis secretions, and in addition it occurs associated with a variety of suppurative conditions in cattle and swine. It is most often found in a mixed infection, especially with Corynebacterium pyogenes and an unclassified, microaerophilic coccus (*Leth Jørgensen*

1937, 1966, *Stuart et al.* 1951, *Cornelisse et al.* 1970, *Høi Sørensen* 1974 a).

Recent studies have shown that Mi. indolicus is of common occurrence in the tonsils and various mucous membranes of apparently healthy cattle, and that it occurs in clinically healthy swine also (*Høi Sørensen* 1973, 1975).

In a previous paper (*Høi Sørensen* 1973) some biochemical criteria for the identification of Mi. indolicus were formulated, and it was shown that the organism can be identified serologically by gel diffusion analysis and complement fixation test. By gel diffusion analysis 6 precipitin-types, designated A, B, C, D, E, and F, were demonstrated.

According to the present taxonomy of anaerobic cocci it seems to be most correct to assign Mi. indolicus to the genus Peptococcus, and the name Peptococcus indolicus has therefore been suggested (*Høi Sørensen* 1974 b).

Until now Pc. indolicus has been considered non-hemolytic. The present report deals with 2 varieties of hemolysin forming peptococci, both identical with Pc. indolicus.

## MATERIAL AND METHODS

Sixteen strains (9 originating from the vagina of clinically healthy cows, 4 from mastitis secretions from dry cows, 2 from the interdigital skin of clinically healthy sows, and 1 from a subcutaneous abscess in a pig) were studied. Details about sources and methods employed for collection of samples will be reported in a subsequent publication (*Høi Sørensen* 1975).

The media employed, and the methods of isolation and storage of strains, investigation of growth characteristics and biochemical properties, determination of sensitivity to antibiotics, and serological identification, were the same as described by *Høi Sørensen* (1973, 1974 a) except that 0.3 % yeast extract (Bacto) was added to the blood agar.

### *Hemolysin formation in liquid media and hemolysin test*

Cultures grown in nutrient broth and in Robertson's cooked meat medium were examined. After incubation for 1, 2, 3, 4, 5, and 10 days the hemolysin content in cultures and in culture filtrates (Millipore 0.22 µm) was determined as follows:

Serial doubling dilutions were prepared in 6 tubes (from 1/5

ml culture or culture filtrate in the first tube to 1/160 ml in the sixth, the total volume in each tube being 0.5 ml). The diluent was veronal buffer, ph 7.4 (*Altan & Jones* 1963). To the tubes was added 0.5 ml of a 0.5 % suspension in veronal buffer of calf or sheep erythrocytes, washed 3 times. The tubes were incubated in a water bath at 37°C for 2 hrs., stored overnight at 5°C, and thereafter read.

The hemolysin titre was defined as the reciprocal of the smallest fraction of a ml culture or culture filtrate showing lytic activity.

*Heat resistance of β-hemolysin*

One-ml amounts of hemolysin-containing culture filtrate, distributed in tubes with a diameter of approx. 1 cm, were heated momentarily in a water bath to 50°, 60°, 70°, 80°, 90°, and 100°C, respectively, and 1 ml was autoclaved at 120°C for 15 min. After cooling in iced water the hemolytic activity was determined as described above.

*Rabbit and guinea pig skin test*

Culture filtrates of 2 β-hemolytic strains (hemolysin titres = 40—80) were injected intradermally on 1 guinea pig and 1 rabbit in doses of 0.2 ml.

The animals were observed for 6 days.

*Pathogenicity for mice and guinea pigs*

Mice and guinea pigs were inoculated i.p. with 0.2 ml doses of 24-hour nutrient broth cultures of the 2 α-hemolytic strains and 2 of the bovine β-hemolytic strains (1 originating from the vagina and 1 from a mastitis secretion, β-hemolysin titres = 40).

The animals were observed for 3 months after the inoculation.

## RESULTS

The hemolytic peptococci were isolated from surface cultures on blood agar. One of the 2 varieties was tentatively called α-hemolytic (Fig. 1), the other β-hemolytic (Fig. 2), and the respective hemolysins α- and β-hemolysin. For comparison a blood agar culture of a non-hemolytic strain of Pc. indolicus is shown in Fig. 3.

*Growth characteristics*

All the 16 strains were strictly anaerobic.

Two strains, both originating from the interdigital skin of sows, were α-hemolytic. Usually the hemolytic zones were visible around single colonies of these strains after incubation for 48 hrs.; if not, the hemolysis could be observed after removal of the colony. After incubation for 72 hrs. the colonies were surrounded by narrow zones of almost complete hemolysis. After 5—7 days incubation colonies of hemolytic strains (Fig. 1) would differ slightly from colonies of non-hemolytic strains (Fig. 3) in size and shape, the former reaching a diameter of 2.5 mm and being less convex than the latter, which never exceeded 2.0 mm in diameter. Furthermore α-hemolytic colonies regularly showed some opacity in the center, which non-hemolytic colonies did but seldom.

Fourteen strains (9 originating from the vagina of cows, 4 from mastitis secretions from dry cows, and 1 from an abscess in a pig) were β-hemolytic. The hemolysis produced by these strains was incomplete. Broad hemolytic zones with indefinite margins would appear after incubation for 24 hrs. (Fig. 2). In older cultures, or if the plates had been seeded too heavily, the β-hemolysis, owing to coalescence of the zones, would often extend over the whole plate and therefore easily be overlooked. The β-hemolytic colonies were somewhat smaller than those of the majority of non-hemolytic strains, 48-hour colonies being less than 0.5 mm in diameter, as against 0.5—0.7 mm for colonies of non-hemolytic strains. Besides, β-hemolytic colonies were less glistening and more convex than non-hemolytic colonies.

*Microscopy and biochemical reactions*

Gram stained films prepared from 48-hour blood agar cultures and from 24-hour nutrient broth cultures showed Gram-positive cocci, occurring singly, in pairs, short chains, tetrades, or small clusters.

All the strains produced gas, indole, hydrogen sulphide, and plasma coagulase (rabbit and calf plasma being coagulated), while non of them produced catalase, decomposed urea, liquefied serum gelatine of utilized citrate. None of the following carbohydrates were fermented: Glucose, Lactose, Saccharose, Trehalose, Raffinose, Mannitol, Sorbitol, Salicin, and Aesculin.

*Sensitivity to antibiotics*

All the strains were sensitive to Penicillin, Bacitracin, Terramycin, Aureomycin, Chloramphenicol, and Neomycin.

One strain was sensitive, 11 relatively resistant, and 4 resistant, to Polymyxin.

One strain was sensitive, the rest of the strains relatively resistant to Streptomycin.

*Serological investigations*

By gel diffusion analysis and complement fixation test all the strains were found to be identical with Pc. indolicus.

Gel diffusion analyses revealed that the 2 α-hemolytic strains were of Serotype C. The β-hemolytic strains were classified as follows: 8 of the 9 strains originating from the vagina of cows were referred to Serotype C and 1 to Type B; 2 of the 4 strains originating from mastitis secretions were referred to Serotype C, 1 to Type D, and 1 to Type E; the strain originating from a subcutaneous abscess in a pig was of Serotype B.

*Pathogenicity for mice and guinea pigs*

Clinical signs of disease were not noted in the observation period, and pathologic changes were not demonstrated post mortem.

*Hemolysin formation in liquid media and examination of β-hemolysin*

In 13 of the 14 β-hemolytic strains a moderate hemolysin formation was observed in nutrient broth cultures as well as in cultures in Robertson's cooked meat medium (titres varying from 5 to 80), while repeated experiments with the remaining β-hemolytic and the 2 α-hemolytic strains showed negative results.

The β-hemolysin could be detected in 24-hour cultures, and a maximum content was reached after 2—4 days. In the hemolysin test the lysis appeared after 1½—2 hrs. incubation at 37°C, and it was progressive during storage overnight at 5°C. Both calf and sheep erythrocytes were hemolyzed, and both were found equally suitable for the test. In more active lytic cultures the lysis was usually complete in the first or 2 first tubes and incomplete in the following tubes.

The β-hemolysin would pass a Millipore filter (0.22 μm), but some of its activity was often lost in passage. It was relatively resistant to heat, a slight reduction in titre being observed after momentary heating to 80°C, but autoclaving at 120°C for 15 min. being necessary to inactivate the hemolysin completely.

The culture filtrates examined were found non-dermonecrotic in rabbit and guinea pig skin test, but after 24 hrs. slight erythema was observed on the injection sites. The erythema gradually diminished during the following days.

## DISCUSSION

Apparently, hemolysin forming strains of Pc. indolicus have not been described previously. In the present study 16 strains, representing 2 varieties of hemolytic peptococci, were found to agree with Pc. indolicus in cell morphology and biochemical properties, and the identity with this organism was confirmed by gel diffusion analysis and complement fixation test. By gel diffusion analysis all of the 16 strains could be referred to one or other of the 4 commonest serotypes in the classification scheme given by *Høi Sørensen* (1973), namely Type B, C, D, or E.

The term α-hemolytic is suggested for the variety producing narrow zones of almost complete hemolysis (Fig. 1), and the term β-hemolytic for the variety producing broad zones of incomplete hemolysis (Fig. 2), the hemolysins being termed α- and β-hemolysin, respectively.

The colonies of the α-hemolytic Pc. indolicus strains were first thought to be contaminated with other hemolytic organisms (e.g., Corynebacterium pyogenes). However, repeated subculturing consistently gave monocultures of peptococci.

The colonies of the β-hemolytic strains might be mistaken for colonies of certain β-toxic staphylococci grown anaerobically, but their hemolytic zones differed from the staphylococcal β-toxin zones in having poorly defined margins.

Using cultures in nutrient broth and Robertson's cooked meat medium the β-hemolysin was shown to be filtrable and relatively resistant to heat. In rabbit and guinea pig skin tests it produced only slight erythemas on the sites of injection. The α-hemolysin was not demonstrated in cultures grown in liquid media.

The 2 α-hemolytic strains and 2 β-hemolytic strains were found apathogenic for mice and guinea pigs; this, together with

the negative results of the skin tests, would seem to indicate that the hemolysins are non-toxic. It cannot be excluded, however, that toxic activity may develop in cultures grown under more suitable conditions.

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## SAMMENDRAG

*Peptococcus (s. Micrococcus) indolicus. Påvisning af to varianter af hæmolysindannende stammer.*

To hæmolysindannende varianter af peptokokker blev på grundlag af biokemiske og serologiske kriterier fundet identiske med Peptococcus (s. Micrococcus) indolicus.

Af ialt 16 undersøgte stammer var 9 isoleret fra klinisk sunde køers vagina, 4 fra mastitis sekreter fra goldkøer, 2 fra huden i klovspalten hos klinisk sunde søer og 1 fra en subkutan absces hos en gris.

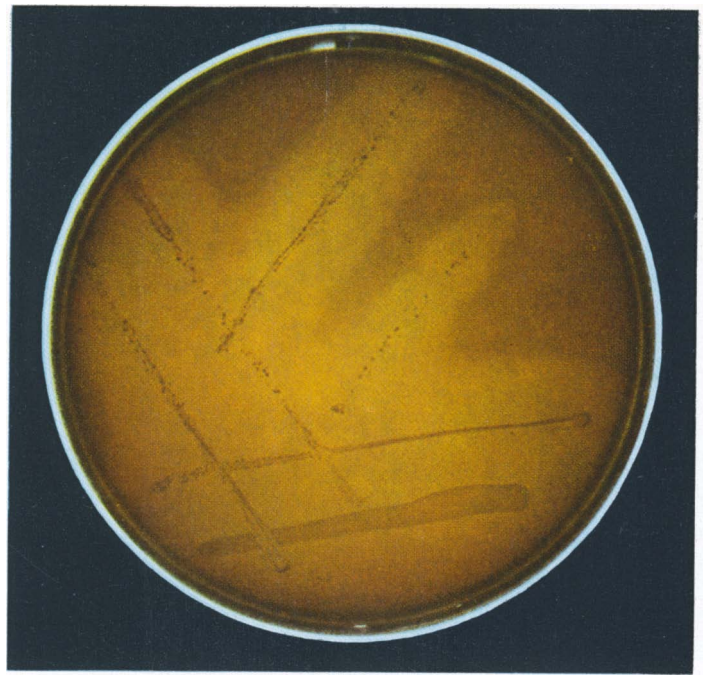

Figure 1. α-hemolytic variety of Peptococcus indolicus. Five-day surface culture on blood agar plate, showing narrow zones of nearly complete hemolysis around the colonies. (Strain SoI-K-7).

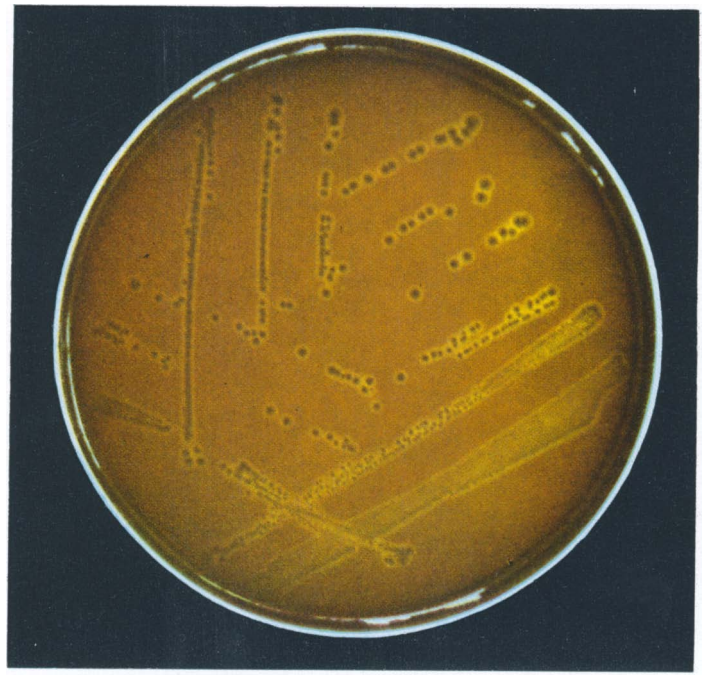

Figure 2. β-hemolytic variety of Peptococcus indolicus. Twenty-four hour surface culture on blood agar plate, showing broad zones of incomplete hemolysis around the colonies. (Strain BI-1-107).

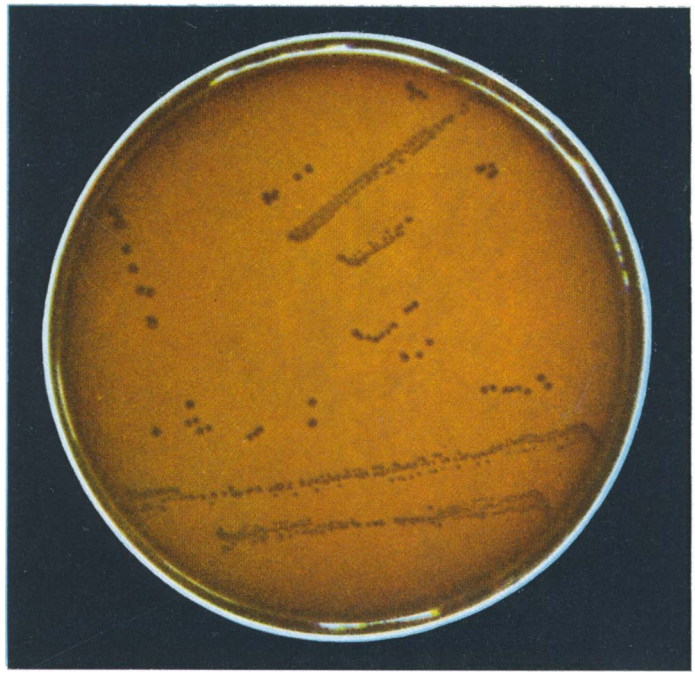

Figure 3. Non-hemolytic strain of Peptococcus indolicus.
Five-day surface culture on blood agar plate. (Strain R-8).

På blodagar plader var den ene variants kolonier omgivet af smalle, næsten klare hæmolysezoner (fig. 1). Denne variant foreslås betegnet α-hæmolytisk. Den anden variant, som blev betegnet β-hæmolytisk, dannede kolonier, omgivet af brede zoner med ufuldstændig hæmolyse (fig. 2). Hæmolysinerne blev betegnet henholdsvis α- og β-hæmolysin.

To stammer var α-hæmolytiske, 14 var β-hæmolytiske.

β-hæmolysinet kunne påvises i kulturer i kødvands pepton bouillon og i Robertson's cooked meat medium, hvorimod α-hæmolysinet ikke blev påvist i sådanne kulturer.

β-hæmolysinet fandtes at være filtrerbart, relativt termoresistent og non-dermonekrotisk.

Ved gel diffusionsanalyse fandtes de 2 α-hæmolytiske stammer at være af serotype C. Ti β-hæmolytiske stammer var af serotype C, 2 af type B, 1 af type D og 1 af type E.

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Reprints may be requested from: Gunner Høi Sørensen, The State Veterinary Serum Laboratory, Department for Jutland, Hangøvej 2 DK-8200 Århus N, Denmark.