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Studies on the properties of *Clostridium sordellii* and *Clostridium novyi* (A, B) with special reference to their fatty acids

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C'est le même dessin d'acide gras que *Clostridium novyi* (A) et *Clostridium novyi* (B) ont donné en chromatographie en phase gazeuse. *Clostridium novyi* (A) a été trouvé plus hémolytique pour les globules rouges de mouton, de cheval et de dromadaire que *C. novyi* (B). Les souches de *C. sordellii* testées ont montré des différences très faibles dans leurs propriétés biochimiques. *C. sordellii* (bovine) a été plus hémolytique que les souches camelines et ovines. Les spores de *C. sordellii*, souches bovines, ont été plus résistantes à la chaleur que les souches camelines et ovines. Les études en immunodiffusion ont montré que les trois souches de *C. sordellii* sont antigéniquement reliées. Les chromatogrammes des souches camelines et ovines ont révélé de l'acide acétique, de l'acide propionique et de l'acide iso-caproïque. *C. sordellii* (bovine) a donné un pourcentage élevé d'acide acétique et d'acide butyrique, mais une faible quantité d'acide propionique et d'acide iso-caproique. *Mots clés* : Bovin - Ovin - Dromadaire - *Clostridium sordelli* - *Clostridium novyi* - Acide gras.

INTRODUCTION

Clostridium sordellii was first isolated in Argentina from cases of gas gangrene in man by SORDELLII in 1922 (11). It has since been recovered from infected wounds of man and necrotic hepatitis and infected muscles in cattle. The organism was ultimately named *C. sordellii.*

CLARK and HALL (6) and STEWART (13) produced strong evidence suggesting that *C. sordellii* and *C. bifermentans* were simply pathogenic and non-pathogenic strains of the same organism, but in 1953 these organisms were demonstrated to be two distinct species by TATAKI and HUET (14). *C. sordellii* could be distinguished from *C. bifermentans* by its ability to produce urease and by certain serological characteristics.

C. sordellii liquefies gelatin, produces lecithinase serologically related to the alpha toxin of *C. perfringens* (9) and an oxygen labile related to the theta toxin of *C. perfringens.*

MATERIAL AND METHODS

Strains

Clostridium sordellii (Sheep) and *Clostridium novyi* type B were isolated from a case of black disease in sheep (1). *Clostridium sordellii* (Camel) was isolated from a camel suffering from haemorrhagic enteritis (7). *Clostridium sordellii* (Cattle) and *Clostridium novyi* type A were isolated from a cow suffering from gangrenous myositis.

Biochemical properties

The biochemical properties were conducted according to STERNE and BATTY (12).

Haemolytic activity

The erythrocytes of sheep, horse and camel were used.

Blood was collected aseptically in an equal volume of Alsever's solution, the suspensions were centrifuged at 4,000 rpm for 10 minutes at 4 °C. The sedimented erythrocytes were washed twice with physiological saline and resuspended in the same diluent to a final concentration of 2.5 suspension.

Clostridium sordellii (Sheep), *C. sordellii* (Cattle), *C. sordellii* (Camel), *C. novyi* type A, *C. novyi* type B, were grown in RCM medium, incubated at 37 °C for 24 hours. Cultures were shaken gently and centrifuged at 4,000 rpm for 20 minutes. The clear supernatants (designated haemolysins) were collected and stored at 4 °C. For each strain eight tubes were prepared ; each tube containing 1 ml of double dilution of haemolysin in normal saline. To every tube, one ml of sheep erythrocytes was added. The whole procedure was

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repeated using camel and horse erythrocytes, the racks were shaken incubated at 37 °C for one hour and then incubated at 20 °C for an overnight before they were finally read.

Resistance to various temperatures

Spores of tested strains were prepared as follows :

RCM medium was seeded with the strain under test, incubated at 37 °C for 24 hours. Then the cultures were stored at room temperature for ten days. Cultures were tested for sporulation and it was considered as maximum when 70-80 p. 100 of cells sporulated. Cultures were centrifuged, the spores suspensions were washed twice with NS.

The spore suspension was heated at 80 °C with frequent subculturing every 5 minutes for 120 minutes. The survival of the organisms was tested at 90 °C and 100 °C.

Agglutination test

Antisera was raised against the different *C. sordellii* strains using rabbits.

Two rabbits were used for each strain for the production of hyperimmune sera.

Agglutination test

The test was carried in standard plastic plates provided with small wells ; each well is capable of holding 1.5 ml. Sera, antigens as well as positive and negative controls were incorporated in every test. The plates were shaken gently and incubated at 37 °C for 60 minutes, then stored at 4 °C for an overnight.

Immunodiffusion test

1.5 g agarose was added to 50 ml of distilled water

TABLE I *Biochemical properties of* **C. novyi** *type A,* **C. novyi** *type B,* **C. sordellii** *sheep, cattle and camel strains.*

Strains	Glucose	Gas	Lactose	Salicin	Sucrose	Arabinose	Raffinose	Mannitol	Maltose	Sorbitol	Rhaminose	Xylose	Trehalose	Inositol	Indole	Gelatin	Nitrate	Milk	Protein	Urease	H_2S	L.V.	L.V. inhibition	Pearly layer
C. novyi B	A	G	–	–	W	–	–	–	W	–	–	W	A	W	–	+	+	A	–	–	ND	+	–	–
C. novyi A	Tra	Tra	–	W	W	W	Tra	W	W	–	Tra	W	W	W	–	+	–	ND	–	ND	–	+	+	+
C. sordellii (Sheep)	Tra	Tra	–	–	–	W	W	–	–	–	–	–	–	–	+	+	+	Black	–	–	+	+	+	–
C. sordellii (cattle)	Tra	Tra	W	–	–	–	–	–	–	–	W	W	–	W	–	+	ND	Black	+	ND	+	+	+	–
C. sordellii (camel)	W	Tra	–	W	W	W	–	–	W	–	W	W	–	W	–	+	ND	ND	+	ND	+	+	+	–

TABLE II *The haemolytic activity of* **Clostridium sordellii, C. novyi** *and* **C. chauvoei** *against erythrocytes of different domestic animals.*

Strains	Sheep haemolytic titre								Horse RBC haemolytic titre								Camel RBC haemolytic titre							
	N	1/2	1/4	1/8	1/16	1/32	1/64	1/128	N	1/2	1/4	1/8	1/16	1/32	1/64	1/128	N	1/2	1/4	1/8	1/16	1/32	1/64	1/128
C. sordellii (sheep)	4	3	3	2	1	0	0	0	3	2	2	1	0	0	0	0	2	1	0	0	0	0	0	0
C. sordellii (cattle)	4	4	4	3	2	1	0	0	4	4	4	4	3	2	1	0	4	3	3	2	1	0	0	0
C. sordellii (camel)	4	3	3	2	1	0	0	0	3	2	2	1	0	0	0	0	2	1	0	0	0	0	0	0
C. novyi type A	4	4	3	3	2	2	1	0	4	4	3	2	1	0	0	0	3	2	2	1	0	0	0	0
C. novyi type B	2	2	2	1	0	0	0	0	2	2	1	0	0	0	0	0	2	2	1	1	0	0	0	0

4 = + + + + 100 p. 100 haemolytic.
3 = + + + 75 p. 100 haemolytic.
2 = + + 50 p. 100 haemolytic.
1 = + 25 p. 100 haemolytic.
0 = No haemolysis.

boiled to dissolve. Fifty ml of PBS were added, mixed and poured onto sterile petri-dishes to make a layer of 3 mm thickness. When the agar was set, wells of 12.5 mm in diameter were cut using cork-borers.

Preparation of cell-free antigen

The strains were grown for 24 hours at 37 °C anaerobically in RCM. Cells were harvested by centrifugation, washed twice with phosphate buffered saline without $Mg^{2}+$ or $Ca^{2}+$ and resuspended in 10 ml of ice-cold acetone (analytical grade) allowed to stand on ice for 5 minutes. Residual acetone was removed and the proteins were extracted by incubation with 1.0 ml of 1 p. 100 sodium dodecyl sulphate (SDS) for 2 minutes.

The antiserum to be tested was added to the central well. Antigens were added to the wells at the prephery. Dishes were stored in a moist chamber for 1-4 days and examined daily for precipitation.

Fatty acids analysis

The fatty acids analysis was conducted according to SEIFERT (10) using Apye unicam 104 series gas chromatograph (England).

RESULTS

Strains

Reproducible results were obtained.

Biochemical properties

The biochemical properties are shown in table I.

Haemolytic activity

The result of the haemolytic activity of *C. sordellii* (Sheep, Camel and Cattle) strains and *C. novyi* type A and B against RBCs of different domestic animals are shown in table II.

Resistance to various temperatures

Clostridium novyi type A and *C. novyi* type B resisted temperatures up to 100 °C for 75 minutes. *Clostridium sordellii* (Sheep and Camel) strains were killed at 100 °C when boiled for 80 minutes. *Clostridium sordellii* cattle resisted temperatures of 100 °C for 80 minutes.

Agglutination test

Clostridium sordellii (Sheep) strain agglutinated its homologous antisera at a titre of 1/256. *C. sordellii* cattle strain agglutinated its homologous antisera at a titre of 1/256. *C. sordellii* camel strain agglutinated its homologous antisera at a titre of 1/16.

Immunodiffusion test

Clostridium sordellii (sheep, cattle and camel) strains were found to be identical according to the precipitation lines obtained.

The percentages of fatty acids produced by *C. sordellii* and *C. novyi* strains are shown in table III.

TABLE III Percentage of fatty acids produced by **Clostridium sordellii** *and* **Clostridium novyi** *strains.*

Strain	Fatty acids						
	Acetic acid	Propionic acid	Butyric acid	Isovaleric acid	Valeric acid	Isocaproic acid	Caproic acid
C. sordellii (sheep)	60.00	13.39	4.28	6.25	—	16.67	—
C. sordellii (cattle)	41.20	1.63	50.50	—	—	6.9	—
C. sordellii (camel)	76.40	4.96	2.23	—	—	16.40	—
C. novyi A	27.48	0.61	68.86	—	—	1.40	—
C. novyi B	16.93	1.31	76.64	—	—	5.10	—

— : not present.

DISCUSSION

Regarding the fatty acids pattern produced by the strains *C. novyi* type A and *C. novyi* type B, we found that the two strains produced acetic acid, small amount of propionic acid and isocaproic acid. *C. novyi* strains were found to produce the same acids by BROOKS and MOORE (4) and HOLDMAN and MOORE (8), but they differ in their production of valeric acid instead of isocaproic acid. Since the two strains produce the same fatty acid pattern, it is impossible to differentiate between the two organisms using the GLC fatty acid pattern. The only method to differentiate between them is by using the Nagler's reaction, the two strains were found to produce lecithinase that can be inhibited by *C. novyi* type A antisera in case of *C. novyi* type A, but was not inhibited by the same antisera in case of *C. novyi* type B. Regarding the other biochemical properties *C. novyi* type B was found to be nitrate negative and does not produce pearly layer. Comparing the haemolytic activity of the two strains, *C. novyi* type A was found to be more haemolytic than *C. novyi* type B. On sheep RBC, *C. novyi* type A showed a minimum haemolytic dose of 32, compared with 4 for *C. novyi* type B. On horse RBC *C. novyi* type A showed a minimum haemolytic dose of 4 compared with 2 for *C. novyi* type B. Both strains were found to resist a temperature up to 100 °C for 75 minutes.

Immunodiffusion test conducted in different antisera prepared in rabbits using the three strains, against the three antigens, showed that there was a cross reaction between the three strains and that they were identical.

Regarding their GLC fatty acid pattern, the three strains were found to produce different patterns ; that of *C. sordellii* (Sheep) consisted of a large number of acids ; it includes a high percentage of acetic acid and slightly low propionic acid, butyric acid, isovaleric acid, isocaproic acid. BROOKS *et al.* (3), HOLDMAN and MOORE (8) extracted acetic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid and isocaproic acid from *C. sordellii* culture. In our results, isobutyric acid was not detected because the peak of isobutyric acid coincides with that of propionic acid.

The GCL fatty acid pattern produced by the *C. sordellii* (Camel) strain resembles that of *C. sordellii* (Sheep) strain. In the major peaks produced, it produces acetic acid, propionic acid, isovaleric acid and isocaproic acid. Those findings agree with those of SEIFERT *et al.* (10), who found that *C. sordellii* strains to produce acetic acid, isobutyric acid, isovaleric acid and isocaproic acid (the peak in our chromatogram, tentatively identified as propionic acid, may be isobutyric acid because they have the same elution time).

The GCL fatty acid pattern of *C. sordellii* (Cattle) strain was different from those of *C. sordellii* (Sheep) and *C. sordellii* (Camel). In the ratio of the peaks, here we found high percentage of acetic acid and butyric acid and only small amount of propionic acid and isocaproic acid ; this chromatogram resembles that of *C. novyi* strains although biochemically the organism produced lecithinase that can be inhibited by antisera against *C. perfringens* (A) and *C. novyi* (A), did not produce pearly layer but did produce colonies, morphological characteristics of that of *C. sordellii* (5, 12). Although this strain was found to be indole negative when we tested the pathogenicity of *C. sordellii* (Sheep) and (Cattle) strains, they were found to be non pathogenic to mice. *C. novyi* type (A) and *C. novyi* type (B) were found to be pathogenic only when they were injected in higher doses.

On studying the properties of *C. sordellii* strains we found that the three strains, *C. sordellii* (Camel), *C. sordellii* (Cattle) and *C. sordellii* (Sheep) showed slight differences in their biochemical properties. *C. sordellii* (Camel) showed less fermentation activity for maltose and sucrose and the two strains *C. sordellii* (Camel) and *C. sordellii* (Cattle) were indole negative. However, even with known stock strains, the results of biochemical tests for *Clostridia* are often irregular and must be repeated several times before they can be accepted (16).

On testing the haemolytic of the three strains, strain *C. sordellii* (Cattle) was found to be more haemolytic than the other two strains. Camel erythrocytes were found to be more resistant to the haemolytic activity of *C. sordellii* strains than sheep or horse erythrocytes. The haemolysin produced by individual anaerobic species are not equally active against erythrocytes of animals (15). The agglutination titres obtained from testing individual rabbit serum, using the haemologous *C. sordellii* antigen were 256 for *C. sordellii* (Sheep), 256 for *C. sordellii* (Cattle) and 16 for *C. sordellii* (Camel).

Regarding other properties, the spores of *C. sordellii* (Camel) and *C. sordellii* (Sheep) were killed in 80 minutes at 100 °C. *C. sordellii* (Cattle) strain resists temperature of 100 °C for 80 minutes.

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On gas liquid chromatography, *Clostridium novyi* (A) and *C. novyi* (B) produced the same pattern of fatty acids. *Clostridium novyi* (A) was found to be more haemolytic for the sheep, horse and camel erythrocytes than *C. novyi* (B). The strains of *C. sordellii* tested, showed slight differences in their biochemical properties. *Clostridium sordellii* (Cattle) was found to be more haemolytic than the camel and sheep strains. Spores of the cattle strain were found to be more resistant to heat than the camel and sheep strains. Immunodiffusion studies proved that the three strains of *C. sordellii* are identical. On consulting GLC chromatogram, camel and sheep strains produced acetic acid, propionic acid and isocaproic acids. *Clostridium sordellii* (Cattle) produced higher percentages of acetic and butyric acids, but only a small amount of propionic acid and isocaproic acid. *Key words* : Cattle - Sheep - Camel - *Clostridium sordellii* - *Clostridium novyi* - Fatty acid.

La cromatografía en fase gaseosa evidenció la misma imagén de ácidos grasos para *Clostridium novyi* (A) y *Clostridium novyi* (B). Fué más hemolítico *C. novyi* (A) que *C. novyi* (B) para los eritrocitos de la oveja, del caballo y del dromedario. Las cepas de *C. sordellii* sometidas a prueba mostraron diferencias muy reducidas de las caracteristicas bioquimicas. *C. sordellii* (bovina) fué más hemolítica que las cepas ovinas y de dromedario. Las esporas de *C. sordellii*, cepas bovinas, fueron más resistentes al calor que las cepas ovinas y de dromedario. Según estudios en inmunodifusión, las tres cepas de *C. sordellii* son ligadas antigenicamente. Los cromatogramas de las cepas ovinas y de dromedario revelaron ácido acético, ácido propionico y ácido isocaproico. *C. sordellii* produjó un porcentaje elevado de ácido acético y de ácido butírico, pero una cantidad reducida de ácido propionico y de ácido isocaproico. *Palabras claves* : Bovino - Ovino - Dromedario - *Clostridium sordellii* - *Clostridium novyi* - Acido graso

REFERENCES

1. ABU-SAMRA (M. T.), EL SANOUSI (S. M.), IDRIS (S. O.), BAGADI (H. O.), A. SALAM (I. S.), ALI (B. H.), MUSA (B. E.). Infectious necrotic hepatitis (Black disease) among Sudanese sheep. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (4) : 422-429.
2. AL MASHAT (R. R.), TAYLOR (D. J.). Bacteria in enteric lesion of cattle. *Vet. Rec.*, 1983 a, **112** : 5-10.
3. BROOKS (J. B.), DOWELL (V. R.), FARSHY (D. C.), ARMFIELD (A. Y.). Further studies on the differentiation of *Clostridium sordellii* from *Clostridium bifermentans* by gas chromatography. *Can. J. Microbiol.*, 1970, **16** : 1071-1078.
4. BROOKS (J. B.), MOORE (W. F. C.). Gas chromatographic analysis of amines and other compounds produced by several species of *Clostridium*. *Can. J. Microbiol.*, 1969, **15** : 1433-1447.
5. BUCHANAN (R. E.), GIBBONS (N. E.), COWAN (S. T.), HALT (J. G.), LISTON (J.), MURRAY (R. G. E.), NIVEN (C. F.), RAVIN (A. W.), STANIER (R. Y.). Bergey's manual of determinative bacteriology. 8th. ed. Baltimore, MD, USA, Williams and Wilkins, 1974.
6. CLARK (F. E.), HALL (I. C.). A comparative study of *B. bifermentans* (Tiessier and Martelly), *B. contro sporogenes* (Hall) and certain closely related proteolytic anaerobes. *J. Bact.*, 1937, **33** : 23-24.
7. GAMEEL (A. A.), EL SANOUSI (S. M.), MOUSA (B.), EL OWNI (E. E.). Association of *Clostridium perfringens* type A *Clostridium sordellii* and *Aeromonas hydrophilia* with haemorrhagic enteritis in camels *(Camelus dromedarius)*. *Small Ruminant and Camel Research Group Document*, 1986, **12** : 50-55.
8. HOLDMAN (L. V.), MOORE (W. E. C.). Anaerobe laboratory manual. 3rd ed. Virginia, USA, The Virginia polytechnic Institute and State University, Black Surg, 1975.
9. LEWIS (G. M.), MACFARLANE (M. G.). The lecithinase of *C. bifermentans* toxin. *Biochem. J.*, 1953, **54** : 138-142.
10. SEIFERT (H. S. H.), RIEKE (K.), BOHNEL (H.). Differentiation of pathogenic clostridia from field material and from reference strains with the aid of the biometric analysis of quantitatively studied metabolic fatty acids patterns of the pathogen. *Anim. Res. Dev.*, 1982, **12** : 60-89.
11. SORDELLII (A.). Un anaérobie agent de gangrène gazeuse. *C. r. Séanc. Soc. Biol.*, 1922, **87** : 838.
12. STERNE (M.), BATTY (I.). Pathogenic *Clostridia*. London and Boston, Butterworth and Co. Ltd., 1975.
13. STEWART (S. E.). The titration of bifermentans antitoxin and the relationship between *C. bifermentans* and *C. sordellii*. *J. Bact.*, 1938, **35** : 13-14.
14. TATAKI (H.), HUET (M.). Valeur du test de l'uréase pour la différenciation de *C. sordellii* et *C. bifermentans*. *Annls Inst. Pasteur, Paris*, 1953, **84** : 890-894.
15. WILLIS (A. T.). Anaerobic infection. London, Publ. Hlth lab. services, H.M.S.O., 1972. (Monograph series).
16. WILSON (G. S.), MILES (A. A.). Topley and Wilsen's principles of bacteriology and immunity. 4th ed. London, Edward Arnold Ltd., 1961.