- Gardell, S., Heijkenskjöld, F. & Rochnorlund, A. (1950). *Acta chem. scand.* **4**, 970.
- Hanahan, D. J. & Olley, J. H. (1958). *J. biol. Chem.* **231**, 813.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Hardy, F. E. & Buchanan, J. G. (1963). *J. chem. Soc.* p. 3360.
- Hay, J. B., Archibald, A. R. & Baddiley, J. (1965). *Biochem. J.* **97**, 723.
- Kelemen, M. V. & Baddiley, J. (1961). *Biochem. J.* **80**, 246.
- Kenner, J. & Richards, G. N. (1954). *J. chem. Soc.*, p. 278.
- Kenner, J. & Richards, G. N. (1955). *J. chem. Soc.* p. 814.
- Knox, K. W. & Hall, E. A. (1965). *Biochem. J.* **96**, 302.
- LeCocq, J. & Ballou, C. E. (1964). *Biochemistry*, **3**, 976.
- Maley, F., Maley, G. F. & Lardy, H. A. (1956). *J. Amer. chem. Soc.* **78**, 5303.
- Naumova, I. B. & Zaretskaya, M. Z. (1964). *Dokl. Akad. Nauk S.S.S.R.* **157**, 207.
- Oeding, P., Myklestad, B. & Davison, A. L. (1967). *Acta path. microbiol. scand.* **69**, 458.
- Park, J. T. & Johnson, M. J. (1949). *J. biol. Chem.* **181**, 149.
- Ribi, E., Larson, C. L., List, R., Brown, W. & Goodie, G. (1959). *Proc. Soc. exp. Biol., N.Y.*, **100**, 647.
- Rosen, H. (1957). *Arch. Biochem. Biophys.* **67**, 10.
- Shabarova, Z. A., Hughes, N. A. & Baddiley, J. (1962). *Biochem. J.* **83**, 216.
- Shockman, G. D., Kolb, J. J. & Toennies, G. (1957). *Biochim. biophys. Acta*, **24**, 203.
- Slodki, M. E. (1966). *J. biol. Chem.* **241**, 2700.
- Suzuki, S. (1962). *J. biol. Chem.* **237**, 1393.
- Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.
- Whistler, R. L. & BeMiller, J. N. (1963). In *Methods in Carbohydrate Chemistry*, vol. 2, p. 477. Ed. by Whistler, R. L. & Wolfrom, M. L. New York and London: Academic Press Inc.
- Wicken, A. J. (1966). *Biochem. J.* **99**, 108.
- Wicken, A. J. & Baddiley, J. (1963). *Biochem. J.* **87**, 54.
- Wickerham, L. T. & Burton, K. A. (1962). *Bact. Rev.* **26**, 282.

APPENDIX

The Characterization of a *Micrococcus (Staphylococcus lactis)* sp. I3 with an Atypical Teichoic Acid in its Wall

By A. L. DAVISON

Microbiological Chemistry Research Laboratory, Department of Organic Chemistry, University of Newcastle upon Tyne, NE1 7RU

(Received 24 May 1968)

From a study of the wall and membrane teichoic acids in staphylococci and micrococci (Davison & Baddiley, 1963; Davison, Baddiley, Hofstad, Losnegard & Oeding, 1964; Davison & Baddiley, 1964) it was concluded that all contain membrane teichoic acid in which the polyol is glycerol. On the other hand, the presence and structure of the wall teichoic acids are characteristics of the different species. Thus walls of strains of *Staphylococcus aureus* contain ribitol teichoic acids in which the configuration of the *N*-acetylglucosaminyl substituents determines serological specificity. Similarly, in strains of *Staphylococcus epidermidis* the sugar is glucose and the polyol is glycerol; the configuration of the glucosyl substituents determines the serological specificity (Oeding, Myklestad & Davison, 1967).

Acetoin-negative cocci described collectively in these studies as *Staphylococcus lactis* did not conform to a systematic pattern in the composition of their walls; some contained no teichoic acid, whereas others contained either glycerol or ribitol teichoic acid. One freshly isolated coccus (strain I3)

contained in its wall a glycerol teichoic acid the structure of which differed fundamentally from the structures of all examples hitherto encountered. This organism has been the subject of much chemical and biochemical investigation in these Laboratories (Archibald, Baddiley & Button, 1965, 1968*a,b*; Button, Archibald & Baddiley, 1966; Blumsom, Douglas & Baddiley, 1966; Brundish, Shaw & Baddiley, 1967; Baddiley, Blumsom & Douglas, 1968). Its isolation and characterization are described.

Strain I3 is a Gram-positive catalase-positive coccus isolated from the uropygeal gland of an eviscerated chicken. The cocci are encapsulated and form tetrads. They grow in 15% (w/v) sodium chloride and do not produce coagulase and phosphatase. Glucose is oxidized when examined by the recommended method (Subcommittee on Bacteriological Nomenclature, 1965), indicative of a micrococcus. Similarly, acid is produced aerobically from mannitol, but not from lactose; no acetoin is formed from glucose. Since, however, cocci with these physiological characteristics (sub-group 5)

lack the overall homogeneity for delineation into valid species (Baird-Parker, 1965), this isolate is identified as *Micrococcus* sp. I3. However, as the organism was first described as *Staph. lactis* I3, and has been mentioned under this name in preliminary publications and reviews, the original name has been retained in the present series of publications.

The organism was grown in 101. batches with aeration (0.5 vol. of air/min.) and agitation (300 rev./min.) for 16 hr. at 37° (New Brunswick fermenter, type FS314) in a medium of the following composition (per 100 ml.): nutrient broth no. 2 (Oxoid), 2.5 g.; yeast extract (Difco), 0.5 g.; dipotassium hydrogen phosphate, 0.5 g.; glucose (A.R.), 1 g.; demineralized water; olive oil as antifoam (q.v.). Coccal walls were isolated by mechanical disintegration of an aqueous suspension (1 ml. of water/g. wet wt. of bacteria) of washed cocci in an MSK Braun homogenizer at 4000 rev./min. for 2 min. Walls were washed once or twice with cold saline (0.9% sodium chloride), recovered by centrifugation at 20 000 *g* for 20 min. at 2°, separated from any remaining cocci and deep-frozen overnight to prevent autolytic activity and to facilitate purification. Walls were further washed four times with saline and six times with water, then preserved by freeze-drying. Homogeneity was confirmed by electron microscopy and paper chromatography of wall hydrolysates.

In contrast with physiologically similar micrococci (strains 189, 234 and 238), walls of which contained glucosaminylribitol teichoic acids, walls

of strain I3 contained the polymer of glycerol phosphate and glucosamine phosphate (Davison & Baddiley, 1963; Archibald *et al.* 1968a). A similar but serologically distinct polymer has been found in walls of *Staphylococcus hyicus*; this polymer may therefore differ structurally from that of *Micrococcus* sp. I3 (Oeding *et al.* 1967).

REFERENCES

- Archibald, A. R., Baddiley, J. & Button, D. (1965). *Biochem. J.* **95**, 8c.
- Archibald, A. R., Baddiley, J. & Button, D. (1968a). *Biochem. J.* **110**, 543.
- Archibald, A. R., Baddiley, J. & Button, D. (1968b). *Biochem. J.* **110**, 559.
- Baddiley, J., Blumsom, N. L. & Douglas, L. J. (1968). *Biochem. J.* **110**, 565.
- Baird-Parker, A. C. (1965). *J. gen. Microbiol.* **38**, 363.
- Blumsom, N. L., Douglas, L. J. & Baddiley, J. (1966). *Biochem. J.* **100**, 26c.
- Brundish, D. E., Shaw, N. & Baddiley, J. (1967). *Biochem. J.* **105**, 885.
- Button, D., Archibald, A. R. & Baddiley, J. (1966). *Biochem. J.* **99**, 11c.
- Davison, A. L. & Baddiley, J. (1963). *J. gen. Microbiol.* **32**, 271.
- Davison, A. L. & Baddiley, J. (1964). *Nature, Lond.*, **202**, 874.
- Davison, A. L., Baddiley, J., Hofstad, T., Losnegard, N. & Oeding, P. (1964). *Nature, Lond.*, **202**, 872.
- Oeding, P., Myklestad, B. & Davison, A. L. (1967). *Acta path. microbiol. scand.* **69**, 458.
- Subcommittee on Bacteriological Nomenclature (1965). *Int. Bull. bact. Nomencl.* **15**, 109.