species of *Rhodotorula* that form tough polysaccharide capsules. The press was found to be far superior in both the time required and the degree of breakage to both the colloid mill and sonic oscillation. The press has further been used successfully for the disruption of *Chlorella vulgaris* and *Lactobacillus plantarum*. In both cases, active enzyme preparations were obtained.

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**TWO VARIANTS OF STAPHYLOCOCCUS AUREUS WOOD 46 (NCTC 7121) DIFFERING IN RESPECT TO ALPHA TOXIN PRODUCTION**

E. KJEMS

*Streptococcal Department, Statens Seruminstitut, Copenhagen, Denmark*

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*Staphylococcus aureus* strain Wood 46 has been used for several years at Statens Seruminstitut, Copenhagen, for the production of staphylococcal alpha toxin. The method described by Jensen and Maaløe (Acta Pathol. Microbiol. Scand. 27:313, 1950) has been used with one modification: the crude toxin was filtered through a Berkefeld filter instead of a Seitz filter.

Owing to difficulties in the production of the toxin, a new sample of the same strain (Wood 46, NCTC 7121) was requested from NCTC, Colindale, England.
This strain appeared to consist of a mixture of two variants: 4 of 20 subcultures from single colonies were coagulase-negative; the remaining 16 were coagulase-positive.

The two variants differed also in respect to toxin production. The hemolytic zones produced on 5% rabbit blood agar by the coagulase-negative variant were smaller than the zones produced by the coagulase-positive one. This property was shown to be constant in five successive subcultures. In fluid medium, the coagulase-negative variant did not produce any alpha toxin, whereas the coagulase-positive one produced twice the amount produced by the strain from NCTC. Consequently, the coagulase-positive variant was chosen for further alpha toxin production.

The new strain from NCTC as well as the isolated variants of the strain were found to be of the same phage type: 42D, at a routine test dilution of $\times 1000$ (Rosendal et al., Acta Pathol. Microbiol. Scand. 58:72, 1963).

The two variants have been taken into the NCTC under the following numbers: coagulase-positive variant, NCTC 10,344; coagulase-negative variant, NCTC 10,345.

GROWTH OF PATHOGENIC LEPTOSPIRAE BY REPLACING RABBIT SERUM WITH MYCOBACTERIUM PHLEI OR MYCOBACTERIUM SMEGMATIS

ICHII MIFUCHI, MASAHARU HOSOI, AND YASUTAKE YANAGIHARA

Department of Microbiology, Shizuoka College of Pharmacy, Shizuoka, Japan

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Studies on the nutritional requirements of leptospiroae have been reported by many investigators, but the growth factors of pathogenic leptospiroae have not yet been ascertained. Rabbit serum, which is the most important constituent in the medium, is still necessary at present for the good growth of pathogenic leptospiroae. Accordingly, biochemical studies have been impeded because of the difficulties of mass cultivation.


Thus, cultural experiments with pathogenic leptospiroae were attempted with cells of acid-fast bacilli, which are known to be abundant in lipid content, in place of rabbit serum.

Mycobacterium phlei or M. smegmatis grown on the surface of Sauton synthetic liquid medium at 37 C for 7 to 10 days were harvested, washed with physiological saline, and added to the basal medium (Korthof basal medium, lacking rabbit serum, supplemented with: asparagine, 50 mg; vitamin B$_1$ hydrochloride, 200 $\mu$g; vitamin B$_2$ hydrochloride, 200 $\mu$g; vitamin B$_3$, 0.2 $\mu$g; niacinamide, 200 $\mu$g; calcium pantothenate, 200 $\mu$g; glucose, 667 $\mu$g; MgSO$_4$·7H$_2$O, 30 mg; and phenol red, 5 mg per liter) in a concentration of 2 and 4 mg/ml as semidried bacilli. This medium containing cells of mycobacteria was sterilized at 100 C for 30 min or 121 C for 10 min. Leptospira icterohaemorrhagiae Mikawajima and L. canicola Utrecht were grown in Korthof medium, washed twice with the basal medium by centrifugation (4200 $\times$ g, 30 min), and inoculated to the test medium containing acid-fast bacilli. Incubation temperature was 30 C. Growth of leptospiroae was estimated with a Petroff-Hauser counting chamber.

Growth curves of leptospiroae are shown in Fig. 1 and 2. By addition of dead cells of M. phlei or M. smegmatis, the growth of L. canicola was supported as well as by serum. L. icterohaemorrhagiae also showed relatively good growth in the medium with added mycobacterial cells, although its growth was somewhat inferior to the growth in serum medium. However, at the second transfer culture in the same medium, it grew as well as in serum. No other microbes except mycobacteria could support the growth of L. icterohaemorrhagiae.