

dence of growth. This process was repeated on four separate occasions without success. Apparently the manipulation of cultures prior to storage had a detrimental effect on the perpetuation of the spirochetal strains.

The survival of the smaller oral treponemes, strains N39 and N36, for 74 months under these conditions adds credence to the assumption that the spirochetal granules may be germinative units in the life cycle of the spirochetes. It is a possibility that these granules may be resting bodies formed in response to adverse environmental conditions with reduction of their metabolic activities to a minimum with retention of reproductive capacities. Additional studies are in progress to determine how long spirochetal cultures can survive under the conditions described in these experiments.

## THE USE OF A SYNTHETIC RESIN IN ANAEROBIC MEDIA

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Relatively few convenient media are available for the cultivation of anaerobes and some of these are unsuitable for particular applications. For example, it has been observed that the commonly used medium incorporating thioglycolate is inhibitory to some organisms in the presence of carbohydrate. The commercial availability of an oxygen adsorbing synthetic resin, "duolite S-10"<sup>1</sup> (Mills and Dickinson: *Ind. Eng. Chem.*, **41**, 2842, 1949), suggested the possible use of such a resin in media for culturing anaerobes. This insoluble resin can be autoclaved satisfactorily and is noninhibitory to the organisms tested.

For testing anaerobic media a variety of *Clostridium* species were used: *C. thermosaccharolyticum*, *C. acetobutylicum*, *C. chauwei*, *C. perfringens*, *C. sporogenes*, *C. pasteurianum*, and *C. putrificum*. The basal medium consisted of peptone, phosphate, and a carbohydrate. To this were added one or more of the following in various combinations: 0.1 per cent agar, 0.1 per cent sodium thioglycolate, and the resin (about 1 g wet weight per 10 ml).

Growth experiments were successful using basal medium containing both agar and the resin, and basal medium plus resin alone. In the latter medium, a flocculent growth was obtained directly above the resin layer. It was possible to separate the growth from the resin by shaking the test tube, thus dispersing the cells throughout the medium. The cells could then be removed easily by decantation or with a pipette since the resin settled almost immediately.

Media containing thioglycolate gave poor or no growth with *C. thermosaccharolyticum*, *C. chauwei*, *C. putrificum*, and *C. perfringens*.

Although "duolite S-10" was the only resin used in these experiments, it seems reasonable that other anion exchange resins of amine type would perform equally

<sup>1</sup> Purchased from the Chemical Process Company, Redwood, California.

well. Reduced copper is deposited on the anion exchange resin by treatment with a cupric salt followed by an alkaline reduction. The resulting metal-resin complex reacts readily with dissolved oxygen in much the same manner as the familiar cuprous copper-ammonia solutions which have been used for oxygen removal in gas analysis. The advantage of the metal-resin complex is its very small dissociation in neutral or alkaline media. Consequently, there is negligible leakage of harmful copper ions into properly buffered media.

The anion exchange resin used in these experiments, "duolite S-10," must be reactivated before use since it is in the oxidized state when received. This may be conducted easily in the laboratory by first soaking and washing the resin with distilled water. Each 500 ml portion of wet resin is then treated with two liters of a solution containing 0.5 M sodium hydrosulfite and 1.25 M sodium hydroxide. Apparently, sodium hydrosulfite is the only appropriate reducing agent. Each 500 ml portion of wet resin is then washed with approximately 5 liters of distilled water which has been previously boiled to remove oxygen. The reactivation steps may be conducted either in a continuous column or in beakers by adding successive small portions of liquid with stirring followed by decantation. The reduced resin, which has undergone a color change from a blue-green in the oxidized state to a metallic purple in the reduced state, is then stored under oxygen-free water until used.

It is felt that there are many special cases of anaerobic growth problems to which the use of the resin may be applied.

## STARCH PRODUCTION IN THE GENUS TRICHOSPORON

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A variety of microorganisms produce starch in the culture medium if the H-ion concentration in the medium during growth drops below pH 5.0. Among the true mycelial fungi this phenomenon has been reported for species of *Aspergillus*, *Penicillium*, and *Dematium* by Lindner *et al.* (quoted by Schmidt: *Biochem. Z.*, **153**, 223, 1925). Among yeasts a single group only has been described so far as giving the starch reaction (Aschner, Mager, and Leibowitz: *Nature*, **156**, 295, 1945). This group which can be characterized as nonfermenting capsulated yeasts is included at present in Lodder's monograph in the genus *Torulopsis*. The present paper deals with starch production in the genus *Trichosporon* in which this phenomenon has not been described yet.

We examined 20 strains comprising 13 species and 4 undetermined species of the Culture Collection of the Laboratory of Mycology of the Instituto Oswaldo Cruz, 2 strains of *Trichosporon cutaneum* which were received by courtesy of