growth medium. Growth was not appreciably affected by the detergents (0.0001%) except for dodecyl and octadecyl sulfate, which showed some inhibition. It is apparent that enzymatic activity decreases as the number of carbons in the alkyl chain is increased. Equivalent concentrations are without effect on preformed penicillinase. However, the same pattern of inhibition occurred when the detergent concentration was increased 100-fold (0.01%).

The effect of alkyl sulfates on penicillinase induction was tested. The cells used for induction were grown in Trypticase Soy Broth (BBL) containing these detergents. A similar pattern of inhibition occurred (Fig. 1). Penicillinase induction was markedly inhibited if not stopped completely with the C_{12}, C_{14}, and C_{17} compounds.

The inhibition of preformed penicillinase by high concentrations of detergent is most likely due to the well-known protein-denaturing property of synthetic detergents. However, at the lower concentrations, which were used in the growth medium, the mode of action of surface-active agents is unknown. Although growth was not significantly affected when the detergents were present in the media at low concentrations, it is possible that other biosynthetic properties not essential for growth were also inhibited. The decrease in penicillinase synthesis that occurs with the use of alkyl sulfates having an increasing number of carbons is in accord with Steinhardt's (J. Res. Natl. Bur. Std. **28**:293, 1942) conclusion that there is an increase in anion affinity with the rise in chain length within a homologous series of anionic detergents.

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DETERMINATION OF CARBOHYDRATE METABOLISM OF MARINE BACTERIA

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Preparations of the O-F medium of Hugh and Leifson (J. Bacteriol. **66**:24, 1953), using either natural sea water or artificial sea water, failed to support growth of a number of marine bacteria. The bromothymol blue indicator was toxic for many of these bacteria, and its pK value too low. Since phosphate could not be used because of precipitation with the sea water salts, the pH was not sufficiently stable. The following medium, which I shall name marine oxidation-fermentation medium or MOF, has proven satisfactory for the determination of the carbohydrate metabolism of a large number of marine bacteria of various kinds.

For the sake of uniformity, the medium is preferably prepared with artificial sea water rather than with natural sea water. The incidence of marine bacteria which fail to grow in artificial sea water media must be very small, since I have never encountered any in my studies to date. The artificial sea water may be used as is or diluted one-half with distilled water. I prefer the latter since the bacteria have grown equally well, and the medium is more clear and more sensitive to small amounts of acid without loss of stability. Sea water normally has a pH around 8, and it seems logical to adjust the medium to this pH. Several indicators were compared, and phenol red was chosen as most satisfactory. In the concentration used (0.001%), phenol red appeared to have no toxic effect. In sea water without much buffer material, the pH rises considerably on autoclaving. On storage, however, the pH drops. For this reason it was found advantageous to buffer the medium slightly and tris(hydroxymethyl)aminomethane (tris) buffer was found to be very satisfactory for this purpose. The peptone concentration was kept as low as possible for adequate growth, and a small amount of yeast extract was added to supply any accessory growth factors which might be required.

The MOF medium has the following composition: Casitone (Difco), 0.1%; yeast extract, 0.01%; ammonium sulfate, 0.05%; tris buffer, 0.05%; agar, 0.3%; phenol red, 0.001% (1.0 ml of 0.1% aqueous solution per 100 ml of medium);

HCl to pH 7.5 (about 0.3 ml of 1 N HCl per 100 ml of medium); artificial sea water, half strength; carbohydrate, 0.5 to 1.0%.

The ingredients may be dissolved in the half-strength sea water, the pH adjusted to 7.5 (orange color), and the solution sterilized by autoclaving. The pH after autoclaving should be about 8. If much above this, adjust with sterile HCl. My own preference is to dissolve the ingredients in distilled water in double strength, adjust the pH to 7.5, and autoclave. An equal volume of artificial sea water is autoclaved separately and the two solutions mixed aseptically. The carbohydrate in 10 to 20% solution in distilled water is sterilized by autoclaving or, if necessary, by filtration and then added aseptically to the sterile base. I prefer to place the medium aseptically in tubes (13 × 100 mm), with 2.5 to 3 ml per tube. The solid medium is stab-inoculated.

To differentiate between fermentation and oxidation of carbohydrates, two glucose tubes are inoculated with each culture; one of these is covered with melted sterile petrolatum to form a layer about 1 cm thick. Carbohydrate fermenters acidify the medium in both tubes but oxidizers acidify only the open tube. As with nonmarine bacteria in the O-F medium of Hugh and Leifson, marine bacteria are encountered which produce neither a clear-cut acid reaction nor a clear-cut alkaline reaction. Such more or less neutral reactions are limited to the nonfermenters and interpretation is a problem. If the medium is made more sensitive by reducing the buffer concentration or the peptone concentration, the uninoculated medium may turn acid on incubation and lead to false-positive tests. A control inoculation of the medium without the carbohydrate may sometimes be necessary for comparison of acidity.

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