The freshly inoculated fermentation medium had 7.5 mg of maltose per 5 ml. The fermented medium after 24 hours at 70 C contained 14.6 mg of maltose per 5 ml. The dissolved ethanol precipitate had 3.9 mg per 5 ml. Since it was mixed with the substrate in equal portions, the mixture at zero hour contained 1.95 mg of maltose per 5 ml. This amount was deducted from each determination.

It must be emphasized that this experiment was conducted under conditions which were not necessarily optimum for the enzyme. Preliminary results with enzyme preparations obtained from the medium of cells grown at 65 C and tested at 85 C showed much better results than those previously described. It is hoped that even better activity will be obtained as soon as the optimum conditions have been worked out.

The identity of the enzyme preparation presents a problem. It might be *alpha*-amylase, maltase, or a mixture of enzymes. Whatever the case, an active enzyme preparation has been isolated from the medium of stenothermophilic bacteria at 70 C, the upper limit of growth for this particular strain; and activity at 90 C was ascertained.

THE ACTION OF MEMBERS OF THE GENUS ACHROMOBACTER ON TRIMETHYLAMINE OXIDE AND RELATED COMPOUNDS

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Members of the genus *Achromobacter* have considerable significance as causes of spoilage of fresh foods, including especially marine products. Identification of an unknown organism as an *Achromobacter* and its separation from closely related forms are sometimes difficult. The work here reported was conducted to ascertain whether the action of members of the genus on trimethylamine oxide, choline, and betaine might have some diagnostic importance, as has been found to be the case among the *Enterobacteriaceae*. The factors involved in the production of the amine from the various compounds tested were also studied.

Twenty cultures were selected for study: *Achromobacter stationis*, *A. thalassius*, *A. aquamarinus*, *A. lipidis*, *A. butyri*, *A. guttatum*, *A. hartlebii*, *A. lipolyticum*, *A. amylovorum*, *A. liquefaciens*, *A. alcaliaromaticum*, *A. delicatulum*, *A. superficiale*, *A. eurydice*, and 6 unnamed species isolated from marine sources and designated as strains C96, M8, M14, M23, N43, and N45. *Shigella alkalescens* and *S. dysenteriae* were used as positive and negative controls, respectively, on trimethylamine oxide reduction.

Replicate cultures to test the ability of each species to reduce trimethylamine oxide were grown in a medium of the following composition: peptone "C" 0.5 per cent, NaCl 0.5 per cent, $MgSO_4 \cdot 7H_2O$ 0.1 per cent, KH_2PO_4 0.1 per cent, glucose 0.25 per cent, and trimethylamine oxide 0.1 per cent. pH adjusted to

7.2. Sterilization was at 15 lbs steam pressure for 15 minutes. Cultures in the medium without trimethylamine oxide were set up in parallel as controls. After 48 hours' incubation at 30 C the cultures were checked for trimethylamine by the qualitative method of Wood and Baird (J. Fish. Res. Bd. Can., **4**, 267, 1943). Five of the twenty species studied, namely *A. delicatulum*, *A. amylovorum*, *A. lipidis*, and *Achromobacter* strains C 96 and M 23, showed the ability to produce trimethylamine from the oxide. Quantitative studies with the positive strains revealed that the amine was produced in good yield. *A. delicatulum* produced 268 μg, *A. amylovorum* 17 μg, *A. lipidis* 180 μg, *Achromobacter*, strain C96 205 μg, and *Achromobacter* strain M 23 200 μg of trimethylamine N per ml of culture filtrate.

A reducing species, *A. delicatulum*, was used to study the rate of reduction. A nonreducing species, *A. aquamarinus*, was carried in parallel as a control. Replicate Thunberg tubes containing the following constituents were set up for each culture: 2 ml of washed cell suspension; 1 ml of 0.15 M phosphate buffer, pH 7.0; 1 ml of 0.1 M solution of the hydrogen donator to be tested; 1 ml of 0.1 M solution of trimethylamine oxide. The hydrogen donators used were glucose, lactate, and acetate. The tubes were evacuated with a water pump, placed in a 30 C water bath, and at 2 hour intervals, for 16 hours, tubes were withdrawn and analyzed for trimethylamine by the colorimetric method of Dyer (J. Fish. Res. Bd. Can., **6**, 351, 1945). Reduction of the oxide was found to be a linear function of time. The rate of reduction was most rapid with glucose and slowest with lactate.

Studies by the Thunberg technique on the activation of trimethylamine oxide established that reducing strains have a heat labile enzyme which activates trimethylamine oxide, rendering it susceptible to the reducing systems of the cell. This finding is in agreement with that of Tarr (J. Fish. Res. Bd. Can., **4**, 367, 1939) for micrococci of marine origin.

Choline, acetyl choline, and betaine were tested as sources of trimethylamine by growing the organisms in the following medium: peptone "C" 0.5 per cent, NaCl 0.5 per cent, $MgSO_4 \cdot 7H_2O$ 0.1 per cent, KH_2PO_4 0.1 per cent, compound under study 0.5 per cent. The cultures were set up in replicate and tested qualitatively after 48 hours at 30 C.

None of the species gave a positive test for trimethylamine from betaine, and only 2 of the 20 tested, *A. delicatulum* and *Achromobacter*, strain C96, produced the amine from choline and acetyl choline. Trimethylamine was isolated as the hydrochloride from the culture filtrates of the 2 positive species. The amount of trimethylamine produced from choline in the presence of excess $CaCO_3$ by each of these strains approached the theoretical maximum after only 24 hours at 30 C, being respectively 1,920 mg and 1,870 mg per liter of medium. Production of the amine was progressively depressed by increasing amounts of glucose in the medium, up to complete inhibition by 1 per cent.

It was noted that acid was produced by these species in the choline broth; the pH fell from 7.2 to 4.5, whereas the pH of the cholineless medium was 7.4 after 18 hours. Preliminary tests with lanthanum nitrate reagent on filtrates of 18 hour cultures were positive for acetate, and the Duclaux constant of fractions

collected by steam distillation of a culture indicated that acetic was the only volatile acid present. Melting points of 145 C and 115 C, respectively, were found for the *p*-toluidide and anilide derivatives of the acid present, values which agree well with the 147 C and 114 C given for these derivatives of acetic acid by Cheronis and Entrikin (Semimicro Qualitative Organic Analysis, T.Y. Crowell Co., New York, 1947). The identity of the volatile acid as acetic was further confirmed by melting points of 146 C and 116 C of a mixture of the derivatives of the culture acid with similar derivatives of a known sample of acetic acid.

The formation of acetic acid, together with the fact that available carbohydrate in the medium inhibits the production of trimethylamine, suggests that the breakdown of choline is probably effected by the utilization of the alcoholic side chain as a source of energy, setting trimethylamine free as a result of the oxidative cleavage of the carbon-nitrogen bond.

From the results obtained in this study it appears that the ability to produce trimethylamine from trimethylamine oxide, and choline may have some significance as a taxonomic character within the genus *Achromobacter*.