Note

Use of Short-chain Amines and Amino Acids as Sole Sources of Nitrogen in a Marine Methylotrophic Bacterium, *Methylophaga* sp. AA-30

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The methylated amines such as monomethylamine (MA), dimethylamine (DMA), trimethylamine (TMA), and trimethylamine oxide (TMAO) seem to have widespread occurrence in marine environments as degradation, excretion, and metabolic products of marine animals. Our previous paper described the distribution of marine methylotrophs and showed that most of marine methylotrophs used MA as sole sources of carbon, nitrogen, and energy.¹⁾ Yamamoto *et al.* isolated similar marine methylotrophs and investigated the growth characteristics of the isolates.^{2,3)} We report here the use of nitrogen compounds as sole sources of nitrogen by a marine methylotrophic bacterium, *Methylophaga* sp. AA-30.

This bacterium isolated from coastal water is able to grow on methanol, MA, or fructose as the sole source of carbon and energy, but it is unable to grow on methane or on complex nutrient media supplemented or unsupplemented with NaCl. The bacterium is a strict aerobic Gram-negative rod that is motile by a single polar flagellum and requires cyanocobalamin, NaCl, and ferric ion for growth. Cells were broken by osmotic shock after washing with 0.5M NaCl. The guanine-plus-cytosine content of the DNA of the strain was 42.2 mol%. The bacterium appears to use the ribulose monophosphate pathway for carbon assimilation, because we found 3-hexulose phosphate synthase activity but not hydroxypyruvate reductase activity (data not shown). These results are consistent with the characteristics of *Methylophaga* described in 1985 by Janvier *et al.*⁴⁾ Strain AA-30 should belong to the genus *Methylophaga*.

The use of nitrogen compounds as sole sources of nitrogen by the strain was examined under the following conditions. The mineral medium used in this work contained the following per 1 l of solution made up with distilled water: Na₂HPO₄, 5 g; KH₂PO₄, 0.5 g; MgSO₄·7H₂O, 0.2 g; NaCl, 25 g; FeSO₄·7H₂O, 10 mg; CaCl₂·2H₂O, 5 mg; ash of yeast extract, 5 mg; CuSO₄·

5H₂O, 20 µg; ZnSO₄·7H₂O, 40 µg; H₃BO₃, 40 µg; NiSO₄·6H₂O, 40 µg; Na₂MoO₄·2H₂O, 40 µg; MnCl₂·4H₂O, 40 µg; CoSO₄·7H₂O, 40 µg; and cyanocobalamin, 1 µg. The mineral medium except for FeSO₄·7H₂O and the ash of yeast extract was adjusted to pH 7.5 with 1N HCl or 1N NaOH, sterilized by autoclaving at 120°C for

Fig. 1. Use of Nitrogen Compounds as Sole Sources of Nitrogen.

The culture was incubated by shaking at 30°C for 24 hr.

Fig. 2. Use of Amino Acids as Sole Sources of Nitrogen.

The L-form of the amino acids tested were used.

* 0.05% (w/v); ▨, 24 hr of cultivation; ■, 48 hr of cultivation.

Fig. 3. Use of Short-chain Amines as Sole Sources of Nitrogen.

The control culture was on the mineral medium containing one of these amines without methanol added as the source of carbon and energy, only MA supported the growth, the others did not.

▨, 24 hr of cultivation; ■, 48 hr of cultivation.

15 min and cooled. A filter-sterilized solution of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and ash of yeast extract was then added to the medium to give the composition stated above. Filter-sterilized methanol and filter-sterilized solutions of nitrogen compounds were added to the medium to a final concentration of 1% (v/v) and 0.2% (w/v), respectively. The ash of yeast extract was prepared by heating the pasty yeast extract kneaded with 0.1 N KOH solution at 550°C for 24 hr. The preculture of the strain was incubated at 30°C for 24 hr in shaken 50-ml Erlenmeyer flasks containing 10 ml of the medium using 0.05% $(\text{NH}_4)_2\text{SO}_4$ for nitrogen. A 0.1-ml inoculum of this preculture was then transferred aseptically to 50-ml Erlenmeyer flasks containing 10 ml of the medium. The flasks were incubated at 30°C on a reciprocal shaker. After incubating for 24 hr or 48 hr, the growth of cultures was measured spectrophotometrically at 610 nm.

The results on the use of nitrogen compounds as sole sources of nitrogen are shown in Figs. 1, 2, and 3. The bacterium had good growth with ammonium-nitrogen,

but poor growth on nitrate. Although the complex organic nitrogen compounds such as peptone and casamino acids did not support the growth of the bacterium as sole sources of carbon and energy, they support the growth as nitrogen sources. Consequently, the use of amines and amino acids as sole sources of nitrogen were investigated. The bacterium grew rapidly on methylated amines (such as MA, DMA, TMA, and TMAO), glutamine, asparagine, and lysine. When MA was added as the sole source of carbon, nitrogen, and energy, the bacterium grew, although the growth rate on MA only was lower than that on both methanol and MA. The organism appears to have, therefore, a metabolic pathway that assimilates these compounds not only as a source of nitrogen but also as sources of carbon and energy. The high availability of methylated amines as nitrogen sources may be attributable to metabolize methylated amine into available forms by the carbon-assimilating pathway before the use as a nitrogen source. The growth on the amino acids other than glutamine, asparagine, and lysine was appreciably lower than on glutamine, asparagine, and lysine. Forms of nitrogen other than α -amino groups, such as amide-nitrogen and ϵ -amino-nitrogen of lysine seem to be more effective for the use of the nitrogen. Recently Sugio *et al.* reported that the obligate chemolithoautotroph *Thiobacillus ferrooxidans* could use amino acids as sole sources of nitrogen and incorporate the amino acids into the cell.⁵⁾ Although the metabolism of these amino acids has not been examined, it would be interesting to find whether the residual portion of the carbon chain of the amino acids can be metabolized as a carbon source or whether they just accumulate in the culture.

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