Note

Isolation of *Zymomonas mobilis* Mutant Deficient in Tetrahydroxybacteriohopane BiosynthesisYasutaka TAHARA, Tomoyuki YAMASHITA,
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Tetrahydroxybacteriohopane (THBH), a pentacyclic triterpene derivative with a side chain of tetrahydroxypentane, occurs as the major constituent in the membrane lipids of *Zymomonas mobilis*,¹⁻³⁾ a Gram-negative bacterium having potential for commercial ethanol production.^{4,5)} Recently, Bringer *et al.* reported that the THBH content increased greatly only at the early stage of ethanol fermentation,¹⁾ and we found that most of the THBH accumulation took place in the cytoplasmic membrane of the organism, suggesting that THBH is closely connected with the ethanol fermentation.⁶⁾ As the next step to elucidate the THBH function, we attempted to isolate a mutant which is deficient in THBH biosynthesis as a nystatin-resistant mutant. This paper describes the isolation of such a mutant.

Nystatin, a polyene macrolide antibiotic, is known to be active on sterol-containing cells and by using nystatin-resistance as a screening indication, several mutants deficient in sterol biosynthesis have been isolated in yeasts.⁷⁻⁹⁾ So we first examined the sensitivity of *Zymomonas mobilis* having a sterol-like compound, THBH to nystatin. Growth of the organism was completely inhibited at 300 $\mu\text{g}/\text{ml}$, indicating that the reagent should be useful for obtaining THBH-deficient mutants of the organism as nystatin-resistant cells.

The parent strain, ST44, a methionine auxotroph derived from *Zymomonas mobilis* IFO 13756, was mutagenized with 50 $\mu\text{g}/\text{ml}$ of *N*-methyl-*N*-nitro-*N'*-nitroso-guanidine under the conditions described previously.¹⁰⁾ The mutagenized cells were cultured at 30°C for 24 hr in GYP medium¹¹⁾ containing 300 $\mu\text{g}/\text{ml}$ of nystatin, and then spread on an agar plate of the same GYP medium. Colonies forming much faster on the plate were isolated as candidates for THBH-deficient cells and their lipids were analyzed. Lipids were extracted by the method of Bligh and Dyer,¹²⁾ and then developed for THBH analysis on silica gel G thin-layer plates with a solvent system of CHCl_3 - CH_3OH - CH_3COOH (90:10:2, v/v). Spots on the plate were visualized by charring after

spraying with 50% H_2SO_4 , and mutants were selected by comparison of the THBH content in the parent cells with those in the candidates.

From among about 200 colonies assayed, two mutants having much less THBH, N85 and N103, were selected. In strain N103, very little of the spot of the THBH was observed on the thin-layer chromatogram, as shown in Fig. 1. Quantitative analysis by gas chromatography showed that the THBH content was reduced to 6% of that in the parent cells (Table I). On the contrary, the content

FIG. 1. Thin-layer Chromatograms of Lipids from Parent Strain ST44 (A) and Mutant Strain N103 (B).

The plate was developed with CHCl_3 - CH_3OH - CH_3COOH (90:10:2, v/v) and the spots were visualized by charring after spraying with 50% H_2SO_4 . THBH; tetrahydroxybacteriohopane described in the text.

TABLE I. HOPANOID CONTENTS OF PARENT STRAIN ST44 AND MUTANT STRAINS N85 AND N103

Cells were cultured statically at 30°C till the late log-phase in GYP medium.¹¹⁾ Lipids were extracted from lyophilized cells by the method of Bligh and Dyer.¹²⁾ THBH was measured as an acetylated derivative of 1-hydroxyethyl-29-hopane by gas chromatography with other hopanoids (hopene and hopane-22-ol),¹³⁾ by the method of Bringer *et al.*¹⁾

Strain	Hopene	Hopane-22-ol	THBH
$\mu\text{g}/\text{mg}$ of dried cell			
ST44	0.52	0.54	4.13
N85	0.62	0.96	1.10
N103	0.43	1.76	0.25

of hopane-22-ol of the mutant was increased by 3 times that in the parent cells, but the hopene content differed little between the parent and mutant strains. The THBH content in strain N85 was lowered to about 25% of that in the parent, also accompanied by an increase in hopane-22-ol (Table I). These results suggest that the genetic block of the mutants may be in an enzyme catalyzing the conversion of hopane-22-ol to THBH, although further analysis is needed for a definite conclusion.

In addition to *Zymomonas mobilis*, bacteria having THBH are comparatively common, including acetic acid bacteria, methylotrophs, purple non-sulfur bacteria, cyanobacteria, *Bacillus acidocaldarius*, and so on,¹⁴⁾ and THBH has been considered to help reinforce and stabilize the membrane, as sterols do in the membranes of eucaryotic cells.¹⁴⁻¹⁷⁾ However, the biosynthetic pathway and the role in the cell function of THBH remain unclarified. Mutants deficient in THBH as described here should be available for studying the function and biosynthesis of THBH. Wild type *Zymomonas mobilis* is tolerant to high glucose concentrations and high ethanol concentrations,⁴⁾ but the mutant N103 was sensitive to the former but tolerant to the latter, suggesting that in *Zymomonas mobilis* the THBH might be responsible for tolerance to a high glucose concentration.

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