STUDIES ON THE METABOLISM OF PHOTOSYNTHETIC BACTERIA

VI. Metabolism of Isopropanol by a New Strain of Rhodopseudomonas gelatinosa

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The demonstration that a simple organic molecule could serve solely as a source of hydrogen concomitant with the photosynthetic reduction of CO_2 was achieved first by Foster (1940, 1944), who showed that certain strains of *Rhodopseudomonas* species[2] were able to convert isopropanol to acetone with the simultaneous assimilation of carbon dioxide. The quantitative relationship expressed by the equation

$$\underset{\text{isopropanol}}{2CH_3CHOHCH_3} + CO_2 \rightarrow \underset{\text{acetone}}{2CH_3COCH_3} + \underset{\text{cell material}}{(CH_2O)} + H_2O$$

provided strong evidence that isopropanol acted solely as a hydrogen donor. This stoichiometry did not exclude the possibility, however remote, that turnover of isopropanol carbon with endogenous material might also occur. It appeared desirable therefore to re-examine the conversion of isopropanol to acetone using isotope-labeling techniques.

While at the Hopkins Marine Station, Pacific Grove, California, one of us (J.M.S.) attempted to repeat the observations of Foster using the particular strains of *Rhodopseudomonas* species the latter had isolated. It was soon discovered that the bacteria had lost the ability to effect this transformation. Efforts to readapt the organisms to the use of isopropanol were unsuccessful.

In the present paper is described the isolation of a new strain of *Rhodopseudomonas gelatinosa*, characterized by its ability to metabolize isopropanol and acetone. By the use of cells uniformly labeled with radioactive carbon 14 it has been verified that isopropanol is dehydrogenated directly to acetone without destruction and reconstitution of its carbon skeleton. Further studies on the metabolism of acetone will be reported in a subsequent publication.

EXPERIMENTAL METHODS AND RESULTS

Culture conditions. All cultures in liquid media were grown under anaerobic conditions at 30 C with continuous illumination from a bank of incandescent lamps. Sufficiently anaerobic conditions were maintained by using bottles completely filled with the growth medium and sealed with ground-glass stoppers.

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[2] These strains were later identified as *Rhodopseudomonas capsulatus* and *Rhodopseudomonas spheroids* by C. B. van Niel (private communication).

Enrichment and isolation procedure. The basic medium employed in the specific enrichment cultures had the following composition: tap water, 1 liter; $(NH_4)_2SO_4$, 1 g; $MgCl_2$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.04 g; KH_2PO_4, 2 g; 6 N H_3PO_4 3.6 ml; thiamine hydrochloride, 4 mg; nicotinic acid, 4 mg; biotin, 5 μg; bicarbonate solution (5 g $NaHCO_3$ plus 5 g $KHCO_3$ per 100 ml), 40 ml; and 98 per cent isopropanol, 1.5 to 3.0 ml (final isopropanol concentration 0.1 to 0.2 per cent). The use of the three growth factors listed above was suggested by the findings of Hutner (1946), who showed that all representatives of the *Athiorhodaceae* could be cultivated in strictly synthetic media, containing one or more of these three compounds. The basal mineral medium including the three growth factors was autoclaved separately from the bicarbonate solution. When the two components were combined, the resulting medium had a pH of 7 to 7.5. The isopropanol was added directly to this solution without sterilization.

The details of the enrichment procedure were as follows: A 60-ml glass-stoppered bottle, completely filled with the foregoing medium, was inoculated with several grams of mud from a small shallow lake in St. Louis County. The bottle was sealed with a ground-glass stopper and incubated in the light until sufficient growth had occurred (10 days). Two ml of this culture were used to inoculate fresh growth medium that was then incubated in the same manner. In each case development of the photosynthetic purple bacteria was accompanied by the appearance of green algae. Consequently, two more transfers were made in the same medium, but with the addition of 0.05 per cent Na_2S to inhibit the growth of the algae. Finally, two transfers were made in a medium that was identical with the basal except $(NH_4)_2SO_4$ was replaced by NH_4Cl to eliminate the sulfate reducers as well as the sulfur-purple bacteria (*Thiorhodaceae*).

The isolation of pure strains of bacteria from the final highly enriched culture was accomplished by the "shake" culture method (van Niel, 1931) using a medium composed of tap water, 0.3 per cent Difco yeast extract, and 1 per cent agar. At least three additional series of "shake" cultures were made for each of the representatives present in the first series before it was deemed to be a pure strain. During the course of this work it was discovered that growth of the isopropanol-oxidizing strain was accelerated by the addition of 0.3 per cent Difco peptone. Thereafter the agar medium used in shake cultures and for the maintenance of stock cultures in deep stabs contained 0.3 per cent peptone as well as 0.3 per cent yeast extract.

A previous attempt to achieve enrichment of isopropanol-oxidizing photosynthetic organisms using 0.1 per cent liquid yeast autolyzate as a source of growth factors was unsuccessful. The yeast autolyzate provided suitable substrates for the rapid development of other representatives of the *Athiorhodaceae* so that bacteria growing more slowly on isopropanol always constituted a small minority of the bacterial flora.

Identification of the isopropanol-oxidizing bacterium. The bacterium isolated by the foregoing procedure is a rod-shaped organism, actively motile in young cultures. It forms highly mucoid masses in all liquid growth media. In liquid cultures the bacterial masses display a delicate peach color, except in yeast

extract or peptone media in which they appear a dirty brown. The bacterium is able to liquefy gelatin, identifying it with *Rhodopseudomonas gelatinosa* according to the scheme of classification of the *Athiorhodaceae* proposed by van Niel (1944). Its ability to grow in propionate medium is the only character at variance with van Niel's description of this species.

General biochemical characteristics. A preliminary survey was made of the substances suitable for growth, using the basal medium cited above, fortified with 200 mg of dehydrated yeast extract per liter of water to accelerate growth. The various substrates were added to concentrations of 0.1 to 0.5 per cent. Controls with no substrate were included in each set of cultures to determine the extent of growth on the small amount of yeast extract present.

The results of this survey indicate that this strain can metabolize isopropanol *n*-butanol, *n*-hexanol, acetone, ethylmethyl ketone, acetic acid, propionic acid, butyric acid, and acetoacetic acid in addition to those substrates listed by van Niel (1944) for *Rhodopseudomonas gelatinosa*. It is unable to utilize 2-butanol and 2-hexanol. Thus, the biochemical characteristics of this bacterium differ significantly from those of the *Rhodopseudomonas* species isolated by Foster (1944). The latter species attacked secondary alcohols in general and were unable to metabolize ketones.

Conversion of isopropanol to acetone. In spite of the fact that acetone was metabolized rapidly by this bacterium, an appreciable amount was found to accumulate during growth on isopropanol. To determine the stoichiometric relationship between the amounts of isopropanol and CO_2 consumed and the amount of acetone produced, 60-ml glass-stoppered bottles were filled with isopropanol medium and half of the bottles were inoculated with one drop of a dense liquid culture. The stoppers were sealed with paraffin to prevent loss of volatile substances, and the cultures were incubated for 2 to 3 weeks so that a large fraction of the alcohol was consumed. At the conclusion of the incubation period the cells were removed by centrifugation and the supernatant liquid was analyzed for total carbonate, isopropanol, and acetone. Similar analyses were performed on the uninoculated controls in order to obtain the corresponding initial values.

The analytical procedures employed in these determinations were the same as those used by Foster (1940). Total carbonate was determined by the method of Peters and van Slyke (1932), isopropanol by oxidation with excess standard dichromate solution in sulfuric acid followed by iodometric determination of the unreacted dichromate, and acetone by the Messinger-Goodwin iodometric method (Goodwin, 1920). Under the conditions of the Messinger-Goodwin procedure, isopropanol was found to react to a small extent depending on its concentration. The appropriate corrections were applied.

The results of these determinations are given in table 1. As is to be expected for an organism capable of metabolizing both isopropanol and acetone, there is no definite relationship between the amount of alcohol consumed and the amount of acetone produced.

To determine whether acetone was formed in these organisms by the direct

dehydrogenation of isopropanol, suspensions of bacterial cells, labeled with carbon 14, were incubated in the presence of unlabeled isopropanol and the acetone produced was examined for radioactive carbon. If all or part of the acetone were formed indirectly from normal cellular constituents, e.g., decomposition of acetoacetic acid, then carbon 14 would appear in the acetone because the cellular carbon was uniformly labeled.

Uniform labeling of the cells was accomplished by growing the bacteria in the presence of $NaHC^{14}O_3$ using the basal isopropanol medium fortified with 0.5 per cent yeast extract to induce rapid growth. The culture was incubated for 4 days and the cells were harvested by centrifugation under aseptic conditions. A sample of the acetone produced was obtained by distilling a portion of clear supernatant growth medium directly into 2 ml of a saturated solution

TABLE 1

Conversion of isopropanol to acetone by Rhodopseudomonas gelatinosa

CULTURE	INITIAL ISOP. CONC. (mg/ml)	ISOPROPANOL CONSUMED		CO_2 CONSUMED mM/ml	ACETONE PRODUCED	
		mg/ml	mM/ml		mg/ml	mM/ml
1	1.56	1.23	0.0205	0.0208	0.52	0.0090
2	2.20	1.38	0.0230	0.0162	0.60	0.0103
3*	1.67	1.56	0.0260	0.0186	0.61	0.0106
4†	2.20	0.92	0.0153	0.0083	0.43	0.0074

* Very old culture.

† The initial concentration of bicarbonate was limiting in this culture.

TABLE 2

Conversion of isopropanol to acetone by Rhodopseudomonas gelatinosa uniformly labeled with C^{14}

	ACETONE FROM GROWING CELLS	ACETONE FROM RESTING CELLS	BACTERIAL CELLS
Counts per min per mg carbon..	0(<5)	0(<5)	1,580

of 2,4-dinitrophenylhydrazine in 2 N HCl. The precipitate of acetone-2,4-dinitrophenylhydrazone was washed thoroughly with cold 2 N HCl and water, slurried in 1 ml of 95 per cent ethanol, and evaporated on a weighed aluminum disk under an infrared lamp. A small portion of cells was washed twice with water and mounted on an aluminum disk in a similar manner.

The remaining cells were washed under aseptic conditions and resuspended in isopropanol medium from which growth factors and ammonium salts were omitted to maintain the cells in a resting state. An aliquant of this suspension was shown, manometrically, to metabolize isopropanol actively. The suspension was then incubated anaerobically in the light for 2 days and a portion of the acetone produced was isolated as above. The samples thus prepared were assayed for carbon 14 by means of a thin mica window, bell-jar-type Geiger-Mueller counter.

After the radioactive assay of carbon 14 was complete, the melting points of the acetone derivatives were compared with that of authentic acetone-2,4-dinitrophenylhydrazone. The various samples melted at the theoretical value (124 to 125 C) either individually or when intimately mixed with the authentic derivative.

The results, presented in table 2, reveal that no carbon 14 was incorporated in the acetone although the cellular carbon had a high specific activity. This fact demonstrated unambiguously that all the acetone originated by a direct dehydrogenation of isopropanol.

SUMMARY

A new strain of *Rhodopseudomonas gelatinosa* has been isolated by an enrichment procedure specific for photosynthetic isopropanol-oxidizing bacteria, particularly representatives of the *Athiorhodaceae*. This strain is characterized by its ability to metabolize acetone and ethylmethyl ketone as well as isopropanol. The conversion of isopropanol to acetone was studied by means of isotopic-labeling techniques and found to proceed by direct dehydrogenation.

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