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Biosynthesis of Biotin from Dethiobiotin by the Biotin Auxotroph *Lactobacillus plantarum*

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***Lactobacillus plantarum* requires biotin for growth. We show that in the presence of high levels of the biotin biosynthetic precursor, dethiobiotin, *L. plantarum* synthesizes biotin and grows in medium with dethiobiotin but without biotin. *Lactobacillus casei* also grew under similar conditions.**

Lactobacillus plantarum (formerly *Lactobacillus arabinosus*) has long been used as an assay organism for biotin (10), since it requires biotin for growth. However, dethiobiotin (desthiobiotin), the biosynthetic precursor of biotin, can promote growth of *L. plantarum* in the presence of biotin (2, 6). Here we show that some lactobacilli grow without biotin if the medium contains at least 1 μM dethiobiotin and that growth is due to synthesis of biotin from dethiobiotin (Fig. 1).

Materials and methods. *L. plantarum* was obtained from the American Type Culture Collection, and *Lactobacillus casei* 7469 was from Karl Dawson. Biotin assay medium was from Difco or formulated from laboratory chemicals. D-biotin and DL-dethiobiotin were from Sigma Chemical Corp.

Inocula were prepared from cultures grown at 30°C for approximately 24 h in biotin assay medium (10 ml) with biotin (5 nM) except where indicated. Cells were harvested by centrifugation, washed three times with 0.9% NaCl, and then diluted 1/100, thus producing the inoculum (20 μl). Growth tests were performed in capped tubes containing biotin assay medium (10 ml) and biotin or dethiobiotin. Cell growth was carried out at 30 or 37°C and was monitored by A_{660}.

Biotin, biotin sulfoxide, and dethiobiotin were separated on a column (1.5 by 16 cm) of Dowex 1-X8 (formate form) by elution with a formic acid gradient from 0 to 0.24 M followed by 100 ml of 0.5 M formic acid (Fig. 2). Fractions were analyzed by the standard bioassay (10). Biotin sulfoxide, dethiobiotin, and biotin eluted as seen previously (7). Fractions containing dethiobiotin but lacking biotin and biotin sulfoxide were pooled, concentrated, and then rechromatographed to assure purity. Some preparations employed a larger column with analogous conditions.

For recovery of biotin from cell protein, 10 liters of biotin assay medium supplemented with purified dethiobiotin (10 μM) and a second 10 liters of this medium, not supplemented with dethiobiotin, were prepared. Both media were inoculated with 15 ml of *L. plantarum* culture and were incubated at 30°C for 72 h. Cells were harvested, washed three times in 0.9% NaCl, resuspended in water, and boiled for 15 min. Precipitated protein was collected by centrifugation. Bound biotin was released by resuspending the precipitate in 6 N H_2SO_4 and autoclaving for 1 h. The solutions were neutralized with 6 N NaOH, filtered, and then assayed for biotin.

The purity of *L. plantarum* cultures was confirmed before and after all experiments.

Results. Figure 2 shows a typical chromatogram of the purification of commercially obtained dethiobiotin superimposed on a chromatogram of the purification of commercially obtained biotin. All commercial preparations of dethiobiotin tested contained biotin and biotin sulfoxide. Figure 2 shows that dethiobiotin alone can promote the growth of biotin. Note also that the effect of dethiobiotin on growth is slight at 24 h but pronounced at 96 h.

In other experiments (not shown), dethiobiotin proved to have inhibiting and stimulating effects on the growth of *L. plantarum*. At concentrations greater than 5 μM, at 17 and 36 h, dethiobiotin was inhibitory, whereas the growth-promoting effect of dethiobiotin was not seen until approximately 36 h. Dethiobiotin is similarly inhibitory to growth of *L. casei* (3). At 72 h, growth over the basal level was seen even in cultures grown with dethiobiotin but without biotin. Also, all cultures with dethiobiotin greater than 4.0 μM, whatever the biotin level, exhibited maximal growth.

An additional experiment (not shown) demonstrated that the growth-promoting effect of dethiobiotin was not due to either biotin or some unknown factor. *L. plantarum* was grown in biotin assay medium with 1 μM dethiobiotin. This amount of dethiobiotin is growth limiting, so any biotin-like nutrient used in trace amounts would be depleted in the medium following cessation of growth. After 72 h of growth, the dethiobiotin in the medium was recovered as described previously for biotin (1) and used to supplement the medium of additional cultures of *L. plantarum*. The growth response to dethiobiotin depleted of possible trace nutrients was identical to that seen before depletion.

Another set of experiments confirmed that significant biotin was not being carried over into successive cultures from the initial inoculum. The inoculum was grown with 500 pM biotin prepared as described in "Materials and methods" above and inoculated into a second culture, which contained only purified dethiobiotin (5 μM) as supplement. The culture was grown, harvested, washed, and used as an inoculum, which was prepared and grown as just described, for three sets of culture conditions. One set of tubes contained 400 pM biotin, the second set of tubes contained 2 μM purified dethiobiotin, and the third set of tubes contained 2 μM purified dethiobiotin and 20 pM biotin. The tubes were inoculated and then incubated at 30°C. Periodically, tubes were analyzed for growth (Fig. 3). It may be seen that the slower growth of the dethiobiotin-containing cultures (Fig. 2) was due to a longer lag period. After the lag, cells grew at approximately the same rate as in biotin-containing cultures. This result was seen only when the inoculum was from a culture that had entered stationary phase. When the inoculation was prepared with cells in exponential

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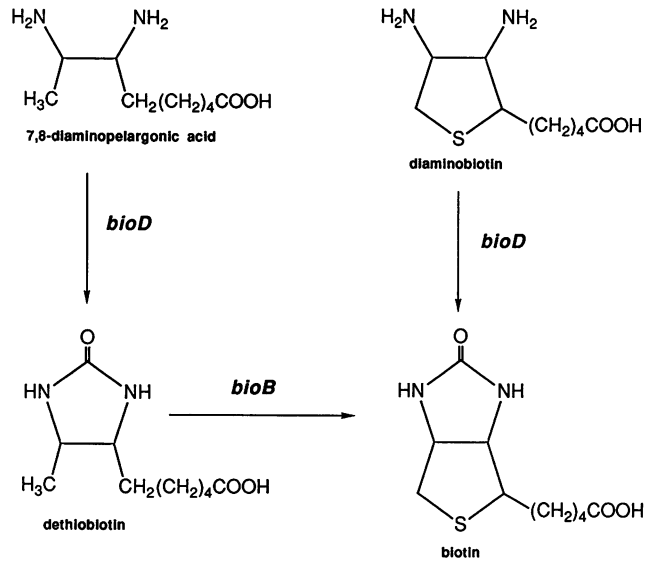

FIG. 1. Final steps in the biosynthesis of biotin. Synthesis of biotin from diaminobiotin, although nonphysiological, is catalyzed by the *bioD* gene product (5, 8).

growth, there was no lag. Addition of biotin (20 pM) to cultures also containing 2 μM purified dethiobiotin shortened the lag, thus proving that the lag is due to an inability to synthesize biotin from dethiobiotin efficiently. Cells from a dethiobiotin-containing culture were harvested at 90 h and used as an inoculum for an additional set of growth experiments identical to those shown in Fig. 3, and the results were the same as those shown in Fig. 3. Even if one assumed that all of the biotin in the medium of the inoculum were internalized because of the 1/200,000 dilution during preparation of the inoculum, only 2.5×10^{-17} mol of biotin would be transferred to the second inoculum, and 1.25×10^{-22} mol of biotin, which is a factor of 4×10^{10} less than that required for growth, would have been carried over into the third set of cultures (Fig. 3).

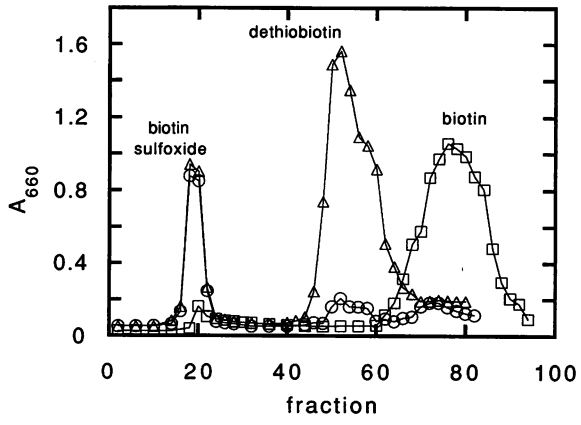

FIG. 2. Growth response (A_{660}) of *L. plantarum* to biotin sulfoxide (peak response at fraction 19), dethiobiotin (peak response at fraction 52), and biotin (peak response at fraction 86), separated by chromatography on a column of Dowex 1-X8 as described in "Materials and methods." For purification of commercial DL-dethiobiotin, which contains small amounts of biotin sulfoxide and biotin, growth was measured after 48 (○) and 96 (△) h after incubation. For purification of commercial biotin, which contains some biotin sulfoxide, growth was measured after 24 h of incubation (□). In each of these chromatograms, a portion of each even-numbered fraction was assayed at the indicated times for its ability to promote the growth of *L. plantarum*.

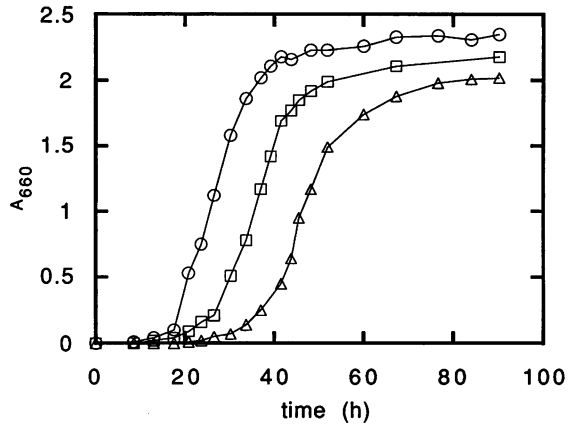

FIG. 3. *L. plantarum* cultures supplemented with either biotin (0.4 nM) (○), dethiobiotin (2 μM) (△), or biotin (20 pM) and dethiobiotin (2 μM) (□).

A final set of experiments demonstrated how dethiobiotin elicits its effects on *L. plantarum*. Two possibilities are envisioned. In one hypothetical case, dethiobiotin would be converted to biotin by the known reaction carried out by most microorganisms (Fig. 1). The high level of dethiobiotin would be required either to induce biotin synthesis or to facilitate dethiobiotin uptake by the cells. In the second case, dethiobiotin would replace biotin as a coenzyme on biotin enzymes, so high levels of dethiobiotin might be needed to overcome the specificity of the holoenzyme synthetase for biotin.

L. plantarum grown in 10 liters of medium with or without dethiobiotin was recovered in amounts of 10.0 and 0.9 g, respectively. Covalently bound biotin was released from cell protein as described in "Materials and methods" above. Biotin assays determined that the amounts of bound biotin released from cells grown with and without dethiobiotin were 700 and 13 pmol, respectively. The more than 50-fold increase in biotin recovered from cells grown with dethiobiotin over that from the control cultures can be attributed to biotin synthesis by *L. plantarum* from added dethiobiotin, which allowed more extensive growth. Dethiobiotin was not replacing biotin on biotin enzymes, since only 10 pmol of dethiobiotin could have been recovered from 10 g of *L. plantarum* (4, 9; our unpublished results). The conditions of our assay would have detected dethiobiotin only if it were present in an amount approximately 1,000 times higher. The growth characteristics of *L. plantarum* in the bioassay of the hydrolysate (not shown) are those of a biotin-supplemented culture and not those of a dethiobiotin-supplemented one, where most of the growth occurs after 36 h. Therefore, growth of *L. plantarum* with dethiobiotin occurs because the organism is synthesizing biotin from dethiobiotin.

L. casei and *L. plantarum* can use diaminobiotin (Fig. 1) as a biotin substitute (8). The explanation for this, made 25 years later, was that the gene that encodes dethiobiotin synthetase (Fig. 1) is transcribed and that a functional protein is produced (5). Dethiobiotin synthetase acts on diaminobiotin, producing biotin, similarly to the usual mode of action of the enzyme, which produces dethiobiotin from 7,8-diaminopelargonic acid (5). We found that *L. casei* also grew in the absence of biotin but in the presence of dethiobiotin, as did *L. plantarum*.

Discussion. Lactobacilli colonize nutritionally rich environments, where there is no selective pressure to maintain the metabolic pathways responsible for the synthesis of those nutrients found in adequate amounts. Consequently, over time many of those metabolic pathways have become nonfunctional. This has apparently happened with biotin biosynthesis in lactobacilli. However, although they are evidently unable to synthesize one or more of the early precursors of biotin and so cannot synthesize biotin under normal environmental conditions, at least two of the later metabolic steps are still functional.

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