Anaerobic Transformation of Furfural by *Methanococcus deltae* ΔLH

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Methanococcus deltae ΔLH was grown on H₂-CO₂ in the presence of various concentrations of furfural. Furfural at higher concentrations, namely, 20 and 25 mM, inhibited growth of this organism. At concentration of 5 and 10 mM, no inhibition of growth was observed. The other methanogens in this study were not inhibited by 10 mM furfural. Among the methanogens tested, *M. deltae* was capable of transforming furfural, whereas *Methanobacterium thermoautotrophicum* Marburg, *Methanosarcina barkeri* 227, *Methanococcus thermolithotrophicus*, and *Methanobrevibacter ruminantium* lacked this capability. One hundred percent removal of furfural was observed within 48 h of incubation in *M. deltae* cultures. The end product observed during furfural metabolism was furfuryl alcohol. An almost stoichiometric amount of furfuryl alcohol was produced by *M. deltae*. This transformation is likely to be of value in the detoxification of furfural and in its ultimate conversion to methane and CO₂ by anaerobic digestion.

Furan compounds and their derivatives are commonly found in plants (19). There are 350 naturally occurring furan compounds present in the environment (8). The most widespread simple furan in the environment is furfural. Furfural is produced from lignocellulosic plant materials by treatment with acids such as sulfuric acid (20). Furfural is also formed by the maillard reaction (15).

Only a few microbial transformations of furfural are known. *Saccharomyces* spp. reduce furfural to furfuryl alcohol (11). Under aerobic conditions, many bacteria transform or degrade furfural (1, 10, 12). Degradation of furfural under anaerobic conditions by sulfate-reducing bacteria has been reported earlier (2, 6, 9). Boopathy (4) demonstrated the transformation of methylfurfural compounds to furfural by *Methanococcus* sp. strain B. Some methanogens are known to grow on alcohols such as 2-propanol and 2-butanol in the presence of CO₂ (21). There is no report in the literature on the metabolism of furfural by methanogenic bacteria. Many industrial wastewaters containing furans can be treated by using anaerobic digesters (5). Methanogens are a very important group of bacteria involved in the anaerobic digestion process of many industrial and municipal wastewater treatment processes. Furfural is present in wastewater from the paper and pulp industries (5) and is also found in municipal wastes (14). We investigated the metabolism of furfural by methanogens under anaerobic conditions, and the results showed that *Methanococcus deltae* has the ability to transform furfural to furfuryl alcohol.

Organisms and growth conditions. *M. deltae* ΔLH was obtained from L. Daniels, University of Iowa. *Methanobacterium thermoautotrophicum* Marburg, *Methanococcus thermolithotrophicus*, *Methanosarcina barkeri* 227, and *Methanobrevibacter ruminantium* were from the methanogen collection in our laboratory. *M. deltae* and *M. barkeri* 227 were grown on H₂-CO₂ (80:20, vol/vol) as described previously (18). *M. thermoautotrophicum*, *M. ruminantium*, and *M. thermolithotrophicus* were grown on H₂-CO₂ as described by Daniels et al. (7). The

incubation temperature was 65°C for *M. thermolithotrophicus* and *M. thermoautotrophicum* and 37°C for the other strains. The stock solution of furfural was prepared in deionized water, and it was filter sterilized and made anaerobic by using argon gas (3). The desired concentration of furfural was added to each culture tube anaerobically. Cultures in each experiment were initiated with a 10% inoculum from a mid-log-phase starter culture. The tubes were covered with aluminum foil to prevent photoreaction and were incubated in a gyratory shaker kept at 150 rpm. A duplicate control without bacterial cells was used throughout the experiment. The concentrations of furfural used in the growth studies were 5, 10, 15, 20, and 25 mM. All data are the averages of results for duplicate tubes.

Analytical methods. Culture turbidity was measured by absorbance at 600 nm with a Spectronic 20 spectrophotometer (Bausch and Lomb). The concentrations of furfural and furfuryl alcohol in culture samples were analyzed with a Perkin-Elmer UV/Vis Lambda 3A spectrophotometer after removal of the cells by centrifugation. The absorbance maximum of furfural was 276 nm, and the absorbance maximum of furfuryl alcohol was 215 nm. The concentrations of furans in each sample were calculated by using standards of known concentrations. The confirmation of the production of the metabolite furfuryl alcohol was done by using high-pressure liquid chromatography with a Waters Associates (Milford, Mass.) liquid chromatograph equipped with two model 6000A solvent pumps, a model 490E programmable multiwavelength detector set 215 nm, a data module, and a model 600E system controller. The column was a reverse-phase C18 μBondapak. Volumes injected via an automatic sampler were 10 μl. Elution was with a gradient of solvent A (0.9 ml of H₃PO₄ per liter of water) versus solvent B (acetonitrile-water [80:20, vol/vol] containing 0.9 ml of H₃PO₄ per liter). The flow rate was 1 ml/min at room temperature.

Chemicals. Furfural and furfuryl alcohol were purchased from Aldrich Chemical Company (Milwaukee, Wis.). All other chemicals were of reagent grade.

Bacterial growth. Growth of *M. deltae* cultures in the presence of 5 or 10 mM furfural was slightly better than that of cultures that received no furfural, indicating possible stimulation of growth by furfural at low concentrations (Fig. 1). On the other hand, cultures that received 20 or 25 mM furfural were severely inhibited, and moderate inhibition was observed at 15 mM. Furfural is inhibitory to many microorganisms at

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FIG. 1. Growth of *M. deltae* in the presence of various concentrations of furfural. H_2 - CO_2 (80:20, vol/vol) served as a substrate. Symbols: ■, uninoculated control; ●, no furfural; ▲, 5 mM furfural; ◆, 10 mM furfural; □, 15 mM furfural; ○, 20 mM furfural; △, 25 mM furfural. Data represent average results for two culture tubes.

concentrations ranging from 1 to 12 mM (16). Some enteric bacteria are inhibited at concentrations of 15 to 30 mM (3). Furfural can be used as a germicide (20), and it inhibits the growth and fermentation of microorganisms. In this study we observed that growth of *M. deltae* is not inhibited by furfural at a concentration of 10 mM.

Metabolism of furfural. We studied the fate of furfural in the culture medium during the growth of *M. deltae*. The culture sample was analyzed by UV spectrophotometry. An example of the spectral change of furfural mediated by *M. deltae* is shown in Fig. 2. The furfural-supplemented 0-h culture showed an absorbance maximum at 276 nm, which is identical to that of standard furfural. The 24-h culture sample showed a maximum absorbance at 215 nm, which is identical to that of standard furfuryl alcohol. The production of furfuryl alcohol in the cultures was confirmed by high-pressure liquid chromatography analysis.

The levels of furfural and furfuryl alcohol in the cultures of *M. deltae* were monitored, and the data are presented in Fig. 3. In the control where there were no cells, the concentration of furfural remained at the initial level of 10 mM throughout the experiment, indicating that there were no physical and chemical changes taking place in the parent compound. In the active culture with *M. deltae*, the concentration of furfural rapidly dropped from 10 to 0 mM within 48 h of incubation. At the same time, the concentration of furfuryl alcohol in the cultures increased from 0 to 9.75 mM. There was an almost stoichiometric amount of furfuryl alcohol produced from furfural by *M. deltae*. The transformation of furfural was directly attributable to the bacterial growth, indicating that biotransformation of furfural was achieved by the growing cells. We also observed the furfural transformation reaction when *M. deltae* was grown with formate, and this showed that the transformation of furfural is not hydrogen dependent (data not shown).

This study shows that furfural is reduced to furfuryl alcohol by *M. deltae*. This metabolic transformation appears to make use of the aldehyde as an electron acceptor. This observation is unique to *M. deltae* among the methanogens tested. We stud-

FIG. 2. UV-visible spectrum of the 0- and 24-h samples of *M. deltae* grown in the presence of 10 mM furfural under anaerobic conditions. H_2 - CO_2 (80:20, vol/vol) served as a substrate.

ied various methanogens, including *M. barkeri*, *M. thermoautotrophicum*, *M. thermolithotrophicus*, and *M. ruminantium*, and none of these bacteria exhibited this metabolic transformation of furfural (results not shown); growth of these species was not inhibited by 10 mM furfural but was inhibited by 15, 20, and 25 mM furfural. The cultures of *M. deltae* grown with higher concentrations of furfural (15, 20, and 25 mM) showed 30 to 50% transformation of furfural even though the higher concentrations inhibited the growth. *M. deltae* did not use furfural as a substrate. Growth was not observed when furfural was used as the sole carbon source (data not shown); *M. deltae* simply reduced furfural to furfuryl alcohol. The spectral analysis showed that *M. deltae* reduced furfural to furfuryl alcohol by $2e^-$ reduction as shown in Fig. 4. A similar reductive transformation of furfural was observed earlier in *Saccharomyces* cultures (11) and in various enteric bacteria (3). The use of aldehyde as an electron acceptor has been shown to occur in *Desulfovibrio desulfuricans* under nitrate-reducing conditions (17) and in some acetogenic bacteria such as *Peptostreptococcus productus* and *Clostridium thermoaceticum* (13). The prac-

FIG. 3. Levels of furfural and furfuryl alcohol in culture samples of *M. deltae* incubated in the presence of 10 mM furfural. H_2 - CO_2 served as a substrate. Symbols: ■, furfural in uninoculated control; ●, furfural in *M. deltae* culture; ▲, furfuryl alcohol in *M. deltae* culture. There was no production of furfuryl alcohol observed in the uninoculated control. Data represent average results for two culture tubes.

tical implication of this observation for the biological treatment of furfural-containing wastewater may be significant. Wastewaters from the pulp and paper mill industries (5) and oat- and corn-processing industries (3) contain furfural as one of the waste products. Furfural waste can be successfully treated by anaerobic digestion with mixed bacterial consortia (5). Furfuryl alcohol is one of the intermediates in the furfural degradation pathway of sulfate-reducing bacteria (2, 9). This study shows that methanogens, especially *M. deltae*, may play a role in the furfural-to-furfuryl alcohol transformation step, which will enhance the overall metabolic capacity of other bacteria, such as sulfate-reducing bacteria, in anaerobic digesters. Sulfate-reducing bacteria metabolize furfural and furfuryl

FIG. 4. Furfural reduction to furfuryl alcohol by $2e^-$ reduction.

alcohol to acetic acid (2, 9), which can be eventually converted to methane and CO_2 by acetate-utilizing methanogens.

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