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Biotransformation of furfural and 5-hydroxymethyl furfural by enteric bacteria

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SUMMARY

A survey was conducted with seventeen enteric bacterial strains (including the genera *Klebsiella, Enterobacter, Escherichia, Citrobacter, Edwardsiella* and *Proteus*) to examine their ability to transform furfural and 5-hydroxymethyl furfural (5-MHF). The enteric bacteria were able to convert furfural to furfuryl alcohol under both aerobic and anaerobic conditions in a relatively short incubation time of 8 h. 5-HMF was transformed by all the enteric bacteria studied to an unidentified compound postulated to be 5-hydroxymethyl furfuryl alcohol, which had an absorbance maximum of 222 nm. These bacteria did not transform furfuryl alcohol or 2-furoic acid. The enteric bacteria did not use furfural, 5-HMF, furfuryl alcohol or 2-furoic acid as sole source of carbon and energy. Biotransformation of furfural and 5-HMF was accomplished by co-metabolism in the presence of glucose and peptone as main substrates. The rate of transformation was similar under both aerobic and anaerobic conditions. These transformations are likely to be of value in the detoxification of furfurals, and in their ultimate conversion to methane and CO_2 by anaerobic digestion.

INTRODUCTION

Naturally produced furan derivatives are frequently found in plants [18]. There are about 350 naturally occurring furanoid and benzofuran compounds present in the environment [8]. Furfurals are the most widely distributed simple furan in nature, and can be produced readily from lignocellulosic plant material such as oat husks or maize cobs by treatment with mineral acids, such as hot sulfuric acid [19]. Under this condition, the hemicelluloses are hydrolyzed and the pentoses are dehydrated to furfural and the hexoses are dehydrated to 5-hydroxymethyl furfural (5-HMF). Furfural is also formed by the Maillard reaction [15]. Many furans in cooked food arise from the pyrolytic breakdown of sugars. Furfural and its 5-substituted derivatives are strong flavor components of foods [8] and have been reported to occur in bread, popcorn, sterilized apple juice, cooked meat, and distilled alcoholic drinks [18].

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Only a few microbial transformations of furfural are known. The $2e^-$ reduction of furfural to furfuryl alcohol by *Saccharomyces* has been reported [12]. Under aerobic conditions, *Acetobacter ascendens* dismutes the aldehyde to alcohol and acid [12]. Abdulrashid and Clark [1] have demonstrated that a mutant strain of *Escherichia coli* can grow with 2-fuoric acid as the sole carbon and energy source. Hong et al. [11] described *Pseudomonas* strain FS-1, which degraded 2-furoic acid. Koenig and Andreesen [13] reported aerobic degradation of 2-furoic acid by *Pseudomonas putida* Fu-1. Degradation of furfural under anaerobic conditions by sulfate-reducing bacteria has been reported earlier by several workers [6,7,9]. There was no report in the literature on the transformation of 5-HMF by bacteria.

Furfural is inhibitory to microorganisms at low concentrations, 1–12 mM [16], yet many wastes that contain furans are otherwise appropriate for biological treatment, especially anaerobic digestion. Furfural is present at a concentration of approx. 30 mM in wastewater from the pulp and paper industry [5], and is also formed during the heat treatment of municipal wastes [14]. Apart from furfural, these wastewaters also contain other furan derivatives such as 5-HMF, 2-furoic acid and furfuryl alcohol in significant quantities. Even though some bacteria are known to degrade furfural, the presence of furfural at high concentration (>10 mM) in wastewater inhibits these

bacteria [7]. Due to the report that a mutant strain of *E. coli* could grow on 2-furoic acid as the sole carbon and energy source [1], and since enteric bacteria are present in all sewage wastes, and in most other anaerobic digestion systems, we investigated the ability of a variety of enteric bacteria to transform or degrade furans. This paper describes their inhibition by some furans, their ability to transform furfural and 5-HMF, and their inability to grow on any furans as the sole carbon and energy source.

MATERIALS AND METHODS

Bacteria

Seventeen strains of enteric bacteria, *Klebsiella pneumoniae* H strain, *K. pneumoniae* UI 495, *Enterobacter aerogenes* ATCC 13048, *Enterobacter cloacae* ATCC 13047, *Enterobacter cloacae* H strain, *Escherichia coli* ATCC 1175, *E. coli* Snyder strain, *E. coli* phage host, *E. coli* B-06 Walker, *E. coli* 055 B5, *E. coli* B5 Cas, *Citrobacter freundii*, *C. freundii* Quinn strain, *Edwardsiella* sp., *Proteus vulgaris*, *P. mirabilis* and *P. mirabilis* H strain, were obtained from the Department of Microbiology culture collection of the University of Iowa, Iowa City, IA, USA.

Media and growth conditions

The medium contained the following components in grams per liter of distilled water: K_2HPO_4 (7.0), KH_2PO_4 (3.0), $MgSO_4$ (0.1), NaCl (0.1), $(NH_4)_2SO_4$ (0.25), sodium succinate (0.2), peptone (0.5), yeast extract (0.1) and glucose (0.5); pH was adjusted to 7.0 by the addition of Na_2CO_3. An anaerobic culture tube (No. 2048-00150, Belco glass, Vineland, NJ) was used for both aerobic and anaerobic growth. Each tube contained 10 ml of medium. The tube was made anaerobic (in the case of anaerobic experiments) by air evacuation with a water vacuum and using N_2/CO_2 (80:20, v/v) to replace the air, similar to methods described by Balch and Wolfe [3]. The stock solution (100–1000 mM) of furfural, 5-hydroxymethyl furfural, furoic acid and furfuryl alcohol as prepared in a culture bottle, filter sterilized and made anaerobic using argon gas as described above. The desired concentration of furan was injected into the experimental tube. A 10% inoculum was added to each tube. The tubes were covered with aluminum foil to prevent photoreaction and were incubated at 37 °C with the tubes horizontal in a gyratory shaker at 150 rpm with a stroke length of 1.5 cm. All data are the averages of duplicate tubes.

Analytical methods

Culture turbidity was measured by absorbance at 600 nm with a Spectronic 20 spectrophotometer (Bausch and Lomb). Concentrations of furans in the samples were determined with a Perkin Elmer UV/Vis Lamda 3A spectrophotometer. The absorbance maxima of furans were 282, 276, 244 and 215 nm for 5-hydroxymethyl furfural, furfural, 2-furoic acid and furfuryl alcohol, respectively. The concentration of furans in the sample was calculated using standards of known concentration. HPLC was used to confirm furfural and 5-HMF transformation. A Waters Model 510 HPLC with a Waters 49D multi-wavelength detector was used. The column was a reverse phase C-18 μ Bondpack. Injection volumes, via an autosampler, were 5 and 10 μl for the standards and sample solutions, respectively. Elution was with a gradient of solvent A (0.9 ml of H_3PO_4 per liter of water) vs. solvent B (acetonitrile/water, 80:20, v/v, containing 0.9 ml H_3PO_4 per liter). Flow rate was 1 ml/min, at room temperature.

Chemicals

All furans were purchased from Aldrich Chemical Company (Milwaukee, WI). All other chemicals were of reagent grade.

RESULTS

Initial experiments with 10 mM concentrations of furfural, 5-HMF, furfuryl alcohol and furoic acid in the glucose-peptone medium demonstrated that all 17 strains of enteric bacteria studied were able to grow in the presence of these furans under both aerobic and anaerobic conditions. When glucose and peptone were omitted, there was no growth under either condition. The furans could not be used as the sole source of carbon and energy for growth. However, all strains growing on glucose-peptone medium transformed approx. 95% of the furfural and 5-HMF within 24 h, as monitored by the decrease in absorbance at 276 and 282 nm, respectively; none of the strains transformed furfuryl alcohol or 2-furoic acid, even after 14 days of incubation (data not shown).

In Table 1, growth by selected enteric bacteria, and their percentage transformation of furans are presented. Bacterial growth was significant, and after 8 h of incubation all but *Proteus mirabilis* transformed $> 67\%$ of the furfural and $> 57\%$ of the 5-HMF; several strains transformed $> 89\%$ of the furans. Using growth curves with various concentrations of furans, we constructed plots of percent maximal growth (growth after 12 h of incubation) vs. the furan concentration. From these plots, we estimated the concentration of furfural and 5-hydroxymethyl furfural that lead to 50% inhibition (IC_{50}). The IC_{50} values of furans for the various enteric bacteria are given in Table 1. Most of the bacteria had an IC_{50} for furfural above 25 mM and for 5-hydroxymethyl furfural around 20 mM. Presence of high concentrations of 2-furoic acid and furfuryl alcohol (100 mM) did not inhibit the growth of the enteric bacteria (data not shown).

TABLE 1

Percent transformation of furans and IC_{50} values for furans under anaerobic conditions**

Bacteria	Growth* (A_{600})	% Transformation		IC_{50} (mM)	
	Fur	Fur[a]	5-HMF	Fur	5-HMF
Escherchia coli ATCC 1175	0.70	91.5	85.0	35	21
Enterobacter aerogenes	0.52	90.6	89.7	39	20
Citrobacter freundii	0.58	89.9	82.6	32	21
Klebsiella pneumoniae H strain	0.90	88.5	86.9	36	18
Edwardsiella sp.	0.44	57.8	94.4	25	26
Proteus vulgaris	0.79	68.4	57.9	17	15
Proteus mirabilis	0.60	51.1	16.5	15	10

[a] Initial furan concentration was 10 mM. Glucose served as main substrate. All strains were incubated for 8 h.
Fur = furfural; 5-HMF = 5-hydroxymethyl furfural.
IC_{50}, refer to the text for details.
* Similar growth was seen for 5-HMF.
** Data represent mean of two values.

An example of the spectral change of furfural by a culture of *Klebsiella pneumoniae* is shown in Fig. 1. The furfural-supplemented 0-h culture showed an absorbance maximum at 276 nm, identical to the standard furfural. The 24-h processed culture [9] showed a maximum absorbance at 215 nm, identical to the standard furfuryl alcohol. HPLC analysis (data not shown) showed a furfural peak at 3.1 min and the 24-h product had an identical retention time (2.5 min) compared to furfuryl alcohol, confirming this was the product of furfural reduction.

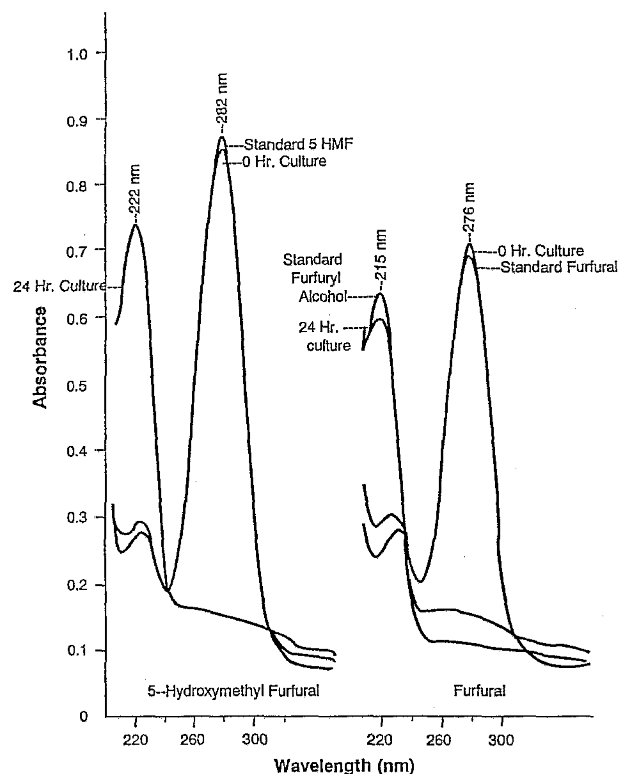

Fig. 1. UV/Vis spectrum of the 0- and 24-h samples of *Klebsiella pneumoniae* grown with 10 mM of furfural or 5-hydroxymethyl furfural under anaerobic conditions.

An example of the spectral change of 5-HMF is also given in Fig. 1. The 5-HMF-supplemented culture showed the expected absorbance maximum at 282 nm, and after 24 h virtually all 5-HMF had been converted to a species with an absorbance maximum at 222 nm. This represents a 60 nm decrease, compared to the 61 nm decrease when furfural is reduced to its alcohol form, and thus suggests that 5-hydroxymethyl furfuryl alcohol is the transformation product. HPLC analysis of this transformation revealed a retention time of 2.7 min for 5-HMF and 2.3 min for the transformation product. This reduction in retention time of 15% is similar to the 19% reduction in retention time above of furfuryl alcohol compared to furfural (chromatogram not shown). The spectral and chromatographic information is consistent with the reduction of 5-HMF to 5-hydroxymethyl furfuryl alcohol.

DISCUSSION

This study shows that furfural and also most likely 5-hydroxymethyl furfural are reduced to their corresponding alcohols by enteric bacteria. This metabolic transformation appears to make use of the aldehyde as an electron acceptor rather than functioning as the first step in a degradative sequence by enteric bacteria. Oxidation of the aldehyde to 2-furoic acid would be a more logical initial

step in degradation, especially under aerobic conditions. Attempts to isolate heterotrophic bacteria capable of anaerobic growth with furfural as the main carbon source have been unsuccessful, except when sulfate is included as an electron acceptor [7]. However, the practical implication of this observation in the biological treatment of wastewater is significant. Wastewater from the paper and pulp mill industry [4], and oat and corn processing industries can contain high concentrations of furfural and 5-HMF. Furfural wastes can be successfully treated by anaerobic digestion by the mixed bacterial consortium [4,10,14] but one problem with the treatment of furfural-containing waste is the toxicity of furfural to bacteria that degrade furans. Furfural can be used as a germicide [19] and its inhibits the growth and fermentation of microorganisms at relatively low concentrations of 1 to 12 mM [2,16,17]. When present at a sufficiently low concentrations (5–10 mM) furfural can be completely converted to acetate by sulfate-reducing bacteria, *Desulfovibrio* sp. and can be used by these bacteria as the sole carbon source for growth [6,7,9]. Furfural at concentrations of above 10 mM inhibits the activity of *Desulfovibrio* sp. but it tolerates a higher concentration of furfuryl alcohol (50 mM) in the culture media [7]. Thus, our study suggests this toxicity problem is at least partly overcome in wastewater treatment plants because of the presence of enteric bacteria which detoxify the furans.

Similarly, 5-hydroxymethyl furfural was detoxified by the enteric bacteria and converted to an intermediate compound which is less toxic. The sulfate-reducing bacteria present in the wastewater treatment plant convert furfuryl alcohol to acetate and CO_2 [5], and ultimately the major products are methane and CO_2, as the result of methanogenic bacteria. It is not yet known which organisms can mineralize 5-HMF or 5-hydroxymethyl furfuryl alcohol.

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REFERENCES

1 Abdulrashid, N. and D. Clark. 1987. Isolation and genetic analysis of mutants allowing the degradation of furans and thiophenes by *E. coli*. J. Bacteriol. 169: 1267–1271.

2 Azhar, A.F., M.K. Berk, A.R. Colcord, R.S. Roberts and G.V. Corbitt. 1981. Factors affecting alcohol fermentation of wood acid hydrolysate. Biotechnol. Bioeng. Symp. 11: 293–300.

3 Balch, W.E. and R.S. Wolfe. 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethane sulfonic acid (HS-CoM) dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. 32: 781–791.

4 Benjamin, M.M., S.L. Woods and J.F. Ferguson. 1984. Anaerobic toxicity and biodegradability of the pulp mill waste constituents. Water Res. 18: 601–607.

5 Brune, G., S.M. Schoberth and H. Sahm. 1982. Anaerobic treatment of an industrial wastewater containing acetic acid, furfural and sulphite. Process. Biochem. 17: 20–35.

6 Brune, G., S.M. Schoberth and H. Sahm. 1983. Growth of a strictly anaerobic bacterium on furfural (2-furaldehyde). Appl. Environ. Microbiol. 46: 1187–1192.

7 Boopathy, R. and L. Daniels. 1991. Isolation and characterization of a furfural degrading sulfate reducing bacterium isolated from an anaerobic digester. Curr. Microbiol. 23: 327–332.

8 Dean, F.M. 1963. Naturally Occurring Ring Compounds. Butterworths, London, pp. 1–15.

9 Folkerts, M., U. Ney, H. Kneifel, E. Stackebrandt, E.G. Witte, H. Forstel, S.M. Schoberth and H. Sahm. 1989. *Desulfovibrio furfuralis* sp. nov., a furfural degrading strictly anaerobic bacterium. Syst. Appl. Microbiol. 11: 161–169.

10 Harris, W.L., R.A. Wirtz, S. Sung and R.R. Dague. 1991. Anaerobic Filter Treatment of Furfural Byproduct Wastewater. Proceedings of the 46th Industrial Waste Conference, West Lafayette, IN.

11 Hong, S.W., H.E. Han and K.S. Chae. 1981. Detection of furfural and 2-furoic acid in bacterial cultures by HPLC. J. Liquid Chromatogr. 4: 285–291.

12 Kieslich, K. 1976. Microbial Transformation of Nonsteroid Cyclic Compounds. Thieme Verlag, Stuttgart.

13 Koenig, K. and J.R. Andreesen. 1989. Molybdenum involvement in aerobic degradation of 2-furoic acid by *Pseudomonas putida* Fu-1. Appl. Environ. Microbiol. 55: 1829–1834.

14 McCarty, P.L., L.Y. Young, D.C. Stuckey and B.B. Healy Jr. 1977. Heat treatment for increasing methane yields from organic materials. In: Microbial Energy Conversion (Schlegel, H.G. and Barnea J. eds.), pp. 179–199, Pergamon Press, Oxford.

15 Monnier, V. 1990. Nonenzymatic glycosylation, the Maillard reaction and the aging process. J. Gerontol. 45: B105–B111.

16 Morris, J.A., A. Kheltry and E.W. Seitz. 1978. Antimicrobial activity of aroma chemicals and essential oils. J. Am. Oil. Chem. Soc. 56: 595–603.

17 Soboleva, G.A., V.I. Golubkov and A.M. Vitrinskaya. 1983. Effect of furfural on the cytochrome system of yeasts. Mikrobiologiya 42: 441–444.

18 Trudgill, P.W. 1984. The microbial metabolism of furans. In: Microbial Degradation of Organic Compounds. (Gibson, D.T. (ed.)) pp. 295–307, Marcel Dekker, New York.

19 Windholz, M., M. Budvari, L.Y. Stroumtsos and M.N. Fertig. 1976. The Merck Index, 9th edn. Merck and Co., Raway, NJ.