KOR. JOUR. MICROBIOL. Vol. 17, No. 4, 193~197, 1979

Bioconversion of Furfural into 2-Furoic acid by *Zooglea* sp.

Byun, Kyu Hee, Hong Eui Han, Soon Woo Hong and Yung Chil Hah

(Department of Microbiology,
Seoul National University)

Zooglea sp.에 依한 furfural에서 2-furoic acid로의 生物學的 轉換

卞 圭 熙·韓 弘 毅·洪 淳 佑·河 永 七

(서울大學校·自然大學·微生物學科)

ABSTRACT

Attempts were made to elucidate the process of biodegradation of furfural.

Zoogloea sp. isolated from soil could convert furfural into 2-furoic acid by a certain enzyme (s) and also accumulated it extracellularly. This substance was extracted with diethyl ether and identified with U.V. spectrophotometry, high pressure liquid chromatography (HPLC) and IR spectrophotometry.

INTRODUCTION

Furfural is a solvent widely used in petroleum and chemical industries, and also present as a toxicant in waste water of the sugar-using industries in large quantities.

Furfural becomes dark brown in air exposure and is reported to be bacterial mutagen and carcinogen(Zdzienicka, *et al.*, 1978).

It has been assumed that furfural was not easily bioconverted or biodegraded by microorganism for its toxicity.

On the other hand, Trudgill(1969) demonstrated the metabolic pathway of furoic acid into glutamate by *Pseudomonas* F2 which can not metabolize furfural as a carbon source.

Furfural can, however, be converted into mainly furfuryl alcohol and a bit of furoic acid by *Saccharomyces cerevisieae* in the medium containing molasses and Koji extract(Morimoto, *et al.*, 1968).

Problems arise from the direct decomposition of furfural in ecosystem, or aldehyde group of furfural may be converted into either hydroxyl group or carboxyl group which can be easily attacked by microorganism.

Therefore, biodegration of furfural probably necessiates mixed populations of microbes (Han, *et al.*, 1980).

In this paper we describe the isolation of microorganisms capable of oxidizing aldehyde group of furfural and identification of oxidized substance from furfural in culture medium.

MATERIALS AND METHODS

1. Isolation and cultures of microorganism

Microorganism was isolated from soil of the University Campus. Isolation medium contains per liter: KH_2PO_4, 1.0g; K_2HPO_4, 2.0g; $CaCl_2$, 0.02g; $MgSO_47H_2O$, 0.2g; KNO_3, 1.0g; furfural, 500mg; yeast extract, 0.3g; agar, 15g.

For liquid culture isolated strain was cultured in the isolation medium without addition of agar and with addition of proline instead of yeast extract.

Liquid cultures were carried out with New Brunswick fermenter Bioflo Model C30 (New Brunswick Scientific, New Brunswick, N.J.,U.S.A.). Culture conditions were: agitation, 250 rpm; temperature, 35°C; pH, 7.0; aeration, 0.3 liter per minute; working volume, 350m*l*.

Identification of isolated microorganism was followed by Bergey's Manual of Determinative Bacteiology(1974).

2. Estimation of Biomass

Biomass was estimated as turbidity at 610 nm using CE 272 Linear Readout Ultraviolet spectrophotometer(Cecil Instruments, Cambridge, England).

3. Detection of furfural and 2-furoic acid in the culture filtrate

In order to estimate furfural and its products furfural and 2-furoic acid were scanned and detected by Perkin-Elmer Model 139 U.V.-visible spectrophotometer(Hitachi, Ltd.,Tokyo, Japan) and high pressure liquid chromatography(HPLC, Waters Associates Inc., Milford, Massachusetts, U.S.A.) under the conditions: column, μ-Bondapak C18; solvent, methanol/water(10/90); flow rate, 1.0m*l*/min; U.V. detector, Model 440, 254nm.

Each change of furfural and 2-furoic acid during the culture development was estimated as measurement of absorbance at 277nm and 245nm using CE 272 Linear Readout Ultraviolet spectrophotometer(Cecil Intruments, Cambridge, England).

Purified product in culture filtrate was identified with authentic 2-furoic acid by IR spectrophotometry (Beckman, IR-20A).

4. Purification of 2-furoic acid in culture filtrate

Cell-free filtrate was prepared by centrifuging for 15 min at 5000rpm(Hitachi Automatic Refrigerated Centrifuge, Hitachi, 20PR-5) and millipore filtration(0.45 μm, Millipore Corp., Bedford, Mass.). Thereafter cell-free filtrate was treated with cation exchanger(Amberite IR-120) followed by anion exchanger(Amberite IR-400) equilibrated with pH 3.0 so as to remove the mineral salts.

The effluent was extracted with diethyl ether, and nonaqueous phase of diethyl ether was decanted and then evaporated completely at 35°C to obtain yellow powder, which dissolved in distilled water adding a small amount of active carbon and again filtered by Millipore filter.

This filtrate was acidified with 2N HCl(ca. pH 3.0) followed by extraction with diethyl ether and evaporation to dryness at 35°C. The obtained white powder was identified by IR spectrophotometry.

RESULTS AND DISCUSSION

1. Characteristics of isolated *Zoogloea* sp.

The characteristics of isolated microorganism were Gram negative; cell shape, rod; size, 0.6x0.9 um (—) 0.6x2.4um; motile by polar flagellum; catalase positive; oxidase negative; nitrate not reduced to nitrite or to gaseous nitrogen.

In shake cultures starlike flocs were formed

in arginine medium(arignine HCl; 0.05%, $MgSO_4 \cdot 7P_2O$; 0.07%, K_2HPO_4; 0.2%, KH_2PO_4; 0.1%, $FeSO_4 \cdot 5H_2O$ trace; vitaminB_{12}; 2ng/ml, distilled water). Thus genus of isolated micr-oorganism was classified as *Zoogloea* sp. and identification of species is under the further study. *Zoogloea* sp. which was isolated from undefined cultures of soil could grow in the medium containing yeast extract, but not do

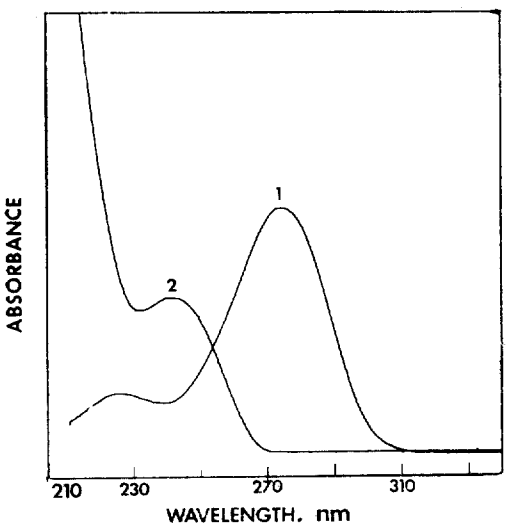

Fig. 1. UV scanning spectra of E_{245} and furfural in the culture filtrate of *Zoogloea* A_8
1. furfural 2. E_{245}

in the medium without yeast extract. Growth of *Zoogloea* A_8 was also observed in each m-edium supplemented with 0.1g of each amino acid.

In stead of yeast extract 0.1g of proline was substituted in order to simplify the cul-ture medium. It was, however, ambiguous w-hether furfural was assimilated by *Zoogloea* A_8 or not, when *Zoogloea* A_8 propagated in the medium with proline plus furfural.

Thus its culture filtrate was scanned within UV range of 210nm to 330nm wavelength.

2. Detection and identification of extracellu-lar product formed by *Zoogloea* sp

As shown in Fig. 1, two maximum peaks appeared in the culture filtrate. One peak at 277nm was that of furtural and the other at 245nm was unknown.

This unknown substance (E_{245}) might be p-roduced from furfural by *Zoogloea* A_8 and still remained after even complete exhaustion of furfural.

The culture filtrate was positive with Ant-hrone reagent indicating the presence of furan derivative.

E_{245} substance was adsorved at pH 7.0 by anion exchanger(Amberite IR-400). So it was considered to be anionic at this pH. This su-bstance can be assumed to be 2-furoic acid s-ince in general aldehyde is oxidized to carbo-xyl group.

Then E_{245} substance was purified as descri-bed in method. It was confirmed that the pu-rified E_{245} had the same peak and retention time as those in culture filtrate by means of UV scanning and HPLC (Fig. 2).

IR spectra indicated that an unknown subs-tance was 2-furoic acid comparing with auth-entic 2-furoic acid(Fig. 3).

Quantitative changes of furfural and 2-fur-oic acid during the culture development were expressed as measurement of absorbancy at 277nm and 245nm by UV spectrophotometer, respectively.

As shown in Fig. 4, while furfural decrea-sed, acid and biomass increased. Therefore f-urfural was certainly transformed to 2-furoic acid extracellularly by *Zoogloea* A_8.

2-Furoic acid was not assimilated furtherm-ore and-accumulated in culture medium.

Such bioconversion of furfural into 2-furoic acid seems to be enzymatic catalysis reaction by enzyme(s), for extracellular protein(s) increased gradually while furfural was conve-rted to 2-furoic acid. We investigate about p-urification and kinetics in detail.

Trudgill(1969) and Han, *et al*,(1980) report-ed that 2-furoic acid could be oxidized by *P-seudomonas* sp.

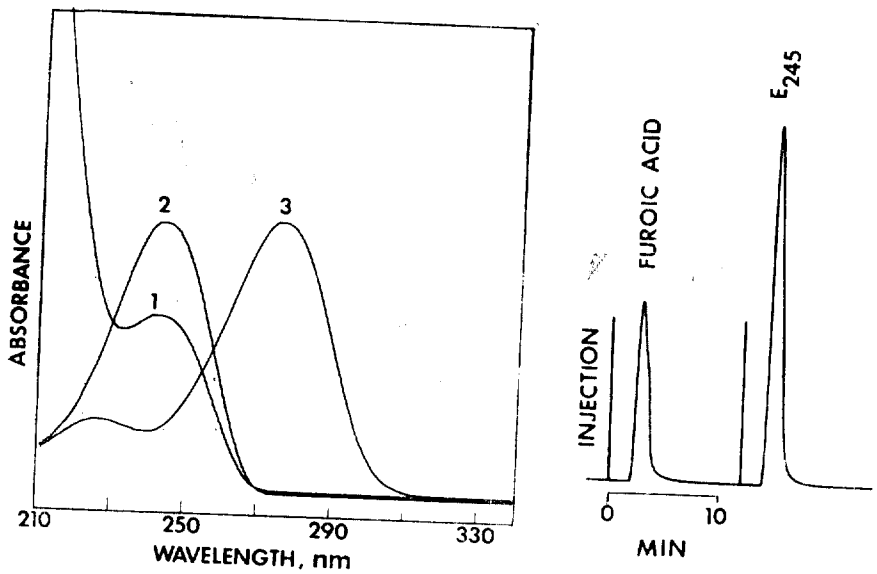

Fig. 2. a) UV scanning spectra of purified E_{245}(1), furoic acid(2), and furfural(3)
b) The liquid chromatographic analysis of purified E_{245} and furoic acid

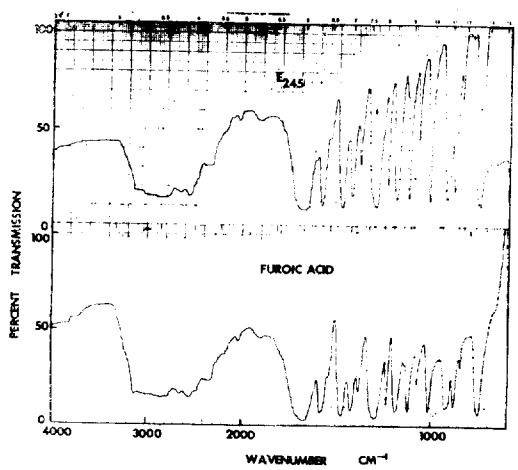

Fig. 3. IR spectra of purified E_{245} substance and 2-furoic acid

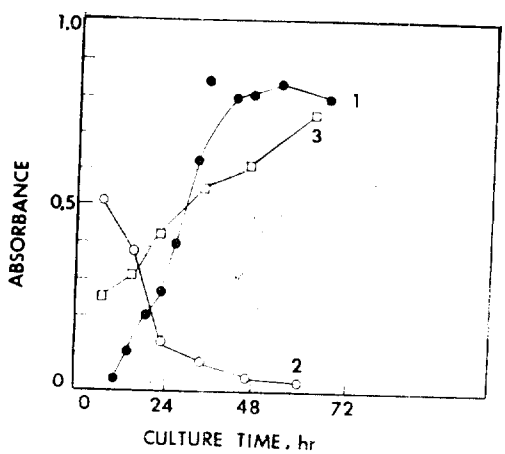

Fig. 4. Bioconversion of furfural into E_{245} in the culture development of *Zoogloea* A_8
1. Biomass 2. Furfural 3. E_{245}

After accomplishing microbial transformation to 2-furoic acid from furfural, furfural can be assimilated via 2-furoic acid to glutamate or other metabolite(s)by several microorganisms.

Thus we take account of that this study may be significant not only in the metabolism of toxicant but in waste water treatment.

摘 要

본 연구는 furfural의 생물학적 분해에 대한 과정을 연구하였다.
토양에서 분리한 *Zoogloea*. sp는 어떤 효소에 의하여 furfural을 2-furoic acid로 전환시켜 체외에 축적시켰다

생물학적으로 전환된 2-furoic acid는 ether로 추출하여 U.V. spectrophotometer, HPLC, IR로 물질구조를 규명하였다.

ACKNOWLEDGEMENT

Authors wish to thank Dr. Park, Man Ki, College of Pharmacy, Seoul National University for helpful advice for IR spectrophotometry and to Mr. Tae Yong Kim for performing enthusiastic experiments.

REFERENCES

1. R.E. Buchanan and N.E. Gibbons(1974), *Bergey's Manual of Determinative Bacteriology*, 8*th* ed., The Williams and Wilkins company, Baltimore, p.249~250.
2. Han, H.E., S.W. Hong and Y.C. Hah (1980), Symbiotic biodegradation of furfural by soil bacteria (unpublished)
3. Hong, S.W., H.E. Han and K.S. Chae(1980), Detection of furfural and 2-furoic acid in bacterial cultures by high pressure liquid chromatography (unpublished)
4. Morimoto, S., T. Hirashima and M. Ohashi (1968), Studies on fermentation products from aldehyde by microorganisms, *J. Ferment. Technol.* 46(4) : 276~287.
5. Trudgill, P.W. (1969), The metabolism of furoic acid by *Pseudomonas* F2, *Biochem. J.* 113 : 577.
6. Zdzienicka, M., B. Tudek, M.Zielenska and T. Szymczyk (1978), Mutagenic activity of furfural in *Salmonella typhimurium* TA100, *Mutation Research* 58 : 205~209.