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Symbiotic Biodegradation of Furfural by Some Bacteria

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數種의 細菌共存에 依한 Furfural의 分解

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ABSTRACT

Three *Pseudomonas* spp. and one *Zoogloea* sp. which could decompose the furfural were isolated from the enriched undefined cultures of soil. In the decomposition of furfural they demonstrated protocooperation and synergism, utilizing 2-furoic acid and a certain sudstance containing primary amine group as metabolites transformed from the furfural. Thus the furfural was subject to complete oxidation, which resulted in decolorization by mutural interactions. The decomposition was more efficient in mixed cultures than in a single culture.

INTRODUCTION

Furfural and 5-hydroxymethyl furfural are formed by acid dehydration of pentoses and hexoses, and decome dark brown in air exposure(Wolfrom, *et al.*, 1949).

Furfural has bacterial mutagenicity in *Salmonella typhimurium* TA100 and carcinogenicity in animal, and destroys the liver function in mouse(Zdziennicka, *et al.*, 1978).

It is problem how the furfural is decomposed by microorganisms in nature because the furfural is also an important colol pollutant. A few studies were reported about the decomposition of furan ring by microorgaisms. Kakima, *et al.*(1964) suggested for the first time that the cleavage of furan ring resulted in oxidation of 2-furoic acid to glutamate by *Pseudomonas* sp. Its metabolic pathway in *Pseudomonas* F2 was proposed by Trudgill (1969). Morimoto, *et al.*(1969) reported the conversion of furfuryl alcohol and furoic acid by yeasts, i.e., *Saccharomyces cerevisiae*, *Saccharomyces soja* and *Saccharomyces rouxii* without the cleavage of furan ring. But it was not yet reported that the furan ring of furfural was directly cleavaged by the microorganisms.

In the decomposition of furfural, it can be considered that the furfural itself or the transformed derivatives of furfural is degraded by the microorganisms. If so, more than one microorganism can be needed for the complete oxidation of furfural.

Therefore, it is expected that the decomposition of furfural necessitate mixed cultures, in view point of the treatment of color-tint waste water due to various industries dealing with foods, alcohol beverages, pulps and *etc.*

This paper presents the investigation about bio-

degradation of furfural by some dacteria in soil.

MATERIALS AND METHODS

Culture Medium

One litre of the basal medium contained: KH_2PO_4, 1.0g; K_2HPO_4, 2.0g; NaCl, 0.2g; $CaCl_2$, 0.01g; $MgSO_4 \cdot 7H_2O$, 0.2g; KNO_3, 1.0g; furfural, 0.5g.

Pseudomonas S1 was cultured in the basal medium, and *Pseudomonas* FSI and *Zoogloea* A8 were cultured in the basal medium supplemented with 0.3g of yeast extract per litre.

In particular, *Pseudomonas* O1 was cultured in the basal medium added 2-furoic acid instead of furfural or in Millipore filtrate(0.45 μm) of *Zoogloea* A8.

Isolation and Identification of Microorganisms

Undefined cultures which could utilize the furfural as sole carbon source were obtained from soil in University Campus. The utilization of furfural was proved by High Pressure Liquid Chromatography (Waters Associates Inc., Milford, Mass. 01757, USA). These cultures were enriched for three days at 37°C in G24 Environmental incubator Shaker(New Brunswick Scientific Inc., Edison N.J., USA). A few colonies were isolated on the agar plates solidified with 15g of agar and 0.3g of yeast extract and identified as *Pseudomonas* sp. (strains; S1, FSI, O1) and Zoogloea sp. (strain; A8) according to the 8th edition of Bergey's manual of Determinative Bacteriology(1974).

Estimation of Eurfural and its Products

All of culture filtrates were obtained by using Millipore filter(0.45μm, Millipore Corp., Bedford, Mass. USA) and used for analysis of High Pressure Liquid Chromatography(HPLC).

Furfural and 2-furoic acid were detected by HPLC(Hong, *et al.*, 1980) under the following conditions:

column; μ Bondapak C_{18}(Waters Associates)
solvent; methanol/water(70/30)
flow rate; 1.0m*l*/min
detector; UV Model 440, 254nm

Concentrations of furfural are expressed as relative peak height by HPLC.

Estimation of Cell Turbidity

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

Detection of the other product was carried out by comparing it with authentic 20 amino acids on thin-layer chromatograms in solvent system, n-butanol/acetic acid/water(4 : 2 : 1 v/v). After one-dimensional development the chromatograms were heated to remove most of the solvent. They were sprayed with ninhydrin reagents: A solution containing a mixture of 0.5g of ninhydrin in 10m*l* of methanol and 20mg of $SnCl_2 2H_2O$ in 6.25m*l* of 0.1M citrate buffer, pH5.0, in a final volume made to 100m*l* with methanol(Gaitonde,*et al.*,1967).

RESULTS AND DISCUSSION

1. Characteristics of isolated bacteria

Pseudomonas S1: cells single, rods. Motile by polar flagella; multitrichous. Gram-negative. Strict aerobes. Catalase positve. Denitrification without gas production.

Pseudomonas FSI: Cells single, rods. Motile by a polar flagellum. Gram-negative. Strict aerobes. Catalase positive. Oxidase positive. Denitrification without gas production. Dimensions, generally 0.6~1.2μm by 0.6~3.6μm.

Pseudomonas O1; Cells single, rods. Motile by a polar flagellum. Gram-negative. Strict aerobes. Catalase positive. Oxidase negative. Denitrification without gas production. Dimensions, generally 0.6~1.2μm by 0.6~2.4μm.

Zoogloea A8; Cells rod shaped, range 0.6~0.

9μm by 0.6~2.4μm. Motile with polar monoflagellation. Gram negative. Catalase positive. Strict aerobes. Not gelatin hydrolytic. Nitrates not reduced to nitrites. Finger-like growth. Oxidase negative.

2. Detection of Products in Culture Filtrates

Pseudomonas S1, *Pseudomonas* FS1 and *Zoogloea* A8 were isolated from undefined cultures on agar plates adding 0.3g of yeast extract per liter. *Pseudomonas* S1 could grow in the basal medium. On the contrary, *Pseudomonas* FS1 and *Zoogloea* A8 could not grow in the basal medium, but they could do in the basal medium added yeast extract.

It is considered that *Pseudomonas* FS1 and *Zoogloea* A8 require a certain substance(s) produced by *Pseudomonas* S1 from the fact that they were strictly dependent on the presence and absence of yeast extract and grew in the filtrate of *Pseudomonas* S1.

In order to detect a certain substance(s), culture filture filtrate of *Pseudomonas* S1 were obtained by Millipore filtrate and evaporated at 100°C and condensated about 10 times. The condensate showed typical color with ninhydrin reaction. The condensate was developed on thin layer chromatography (TLC) with 19 standard amino acids as showm in Figure 1.

Glutamine, asparagine and condensate did not developed on TLC. The substance in condensate was hydrolysed with 6N HCl at 121°C for 30min, since the substance could be converted into glutamic acid or aspartic acid by acid hydrolysis if the substance might be either glutamine or asparagine. As shown in Figure 1, the hydrolyzed substance showed a different Rf value, compared with standard amino acids. This indicated that the condensate contained a primary amine different from 19 standard amino acids. Again the filtrate in which two third of furfural remained after

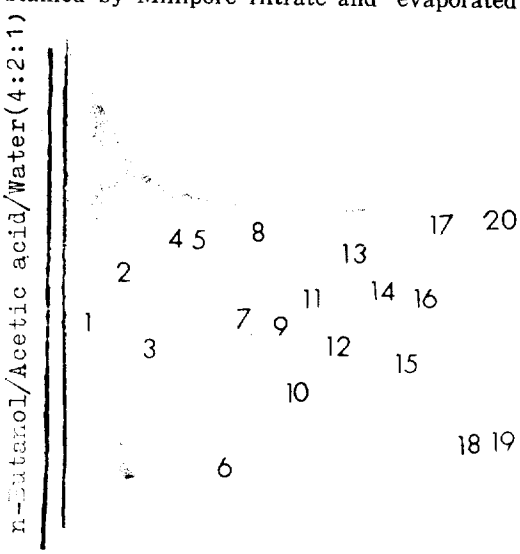

Fig. 1 One-dimensional separation of standard amino acids and hydrolyzed condensate
1. Gln 2. hydrolyzed condensate 3. Glu 4. Ile 5. Leu 6. Lys 7. Met 8. Trp 9. Ser 10. Pro 11. Thr 12. Cys 13. Val 14. Asn 15. Gly 16. Ala 17. Phe 18. His 19. Arg 20. Thr

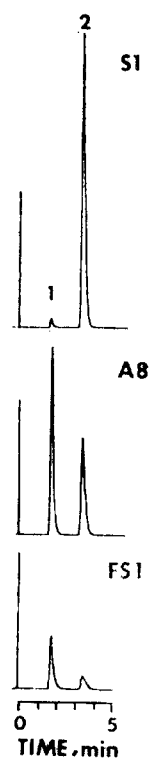

Fig. 2. Chromatograms of HPLC on the decomposition of furfural after growth of *Zoogloea* A_8 and *Pseudomonas* FSI in the culture filtrate of *Pseudomonas* SI
1. furoic acid 2. furfural

Pseudomonas S1 was cultured for 36 hours, was prepared to examine the utilization of this amine in the condensate by *Pseudomonas* FSI and *Zoogloea* A8.

As shown in Figure 2, the latter microorganisms were cultured in the prepared filtrate without addition of any other ingredients. F-urfural and 2-furoic acid were detected by HPLC. *Pseudomonas* FSI could utilize both f-urfural and 2-furoic acid, whereas *Zoogloea* A8 rather accumulated 2-furoic acid.

Culture filtrate of two microorganisms also showed a negative reaction with ninhydrin reaction. It means that they utilized amine as a growth factor under the same environmental condititions as that of undefined cultures.

3. Interactions of Bacteria in Furfural Biodegradation

As shown in Fig. 3, when each culture of three bacteria was compared with the undefined cultures, the undefined cultures showed not only the highest turbidity but also more rapid degradation of furfural.

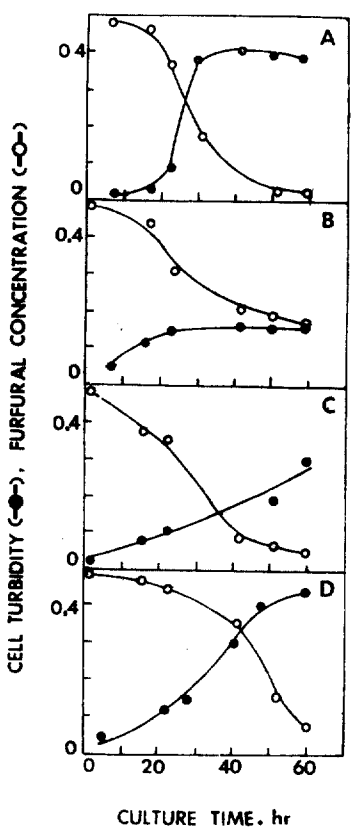

Fig. 3. Furfural decomposition and cell turbidity of isolated bacteria
A : undefined cultures B : *Zoogloea* A_8
C : *Pseudomonas* FSI D : *Pseudomonas* S1

As shown in Fig. 2, in mineral salts medium contained yeast extract *Pseudomonas* FS1 transformed furfural to 2-furoic acid and then reutilized it as a carbon source(Hong, *et al.*, 1980) and *Zoogloea* A8 accumulated 2-furoic acid without further assimilation(Byun, *et.al.*, 1980). *Pseudomonas* S1 could utilize furfural and produce a substance having primary amine group.

However,in the culture filtrate of undefined cultures 2-furoic acid was not accumulated. It is suggested that the other microorganisms could be present which are capable of utilizing 2-furoic acid, which remained in the individual culture filtrate of *Zoogloea* A8. As descrибed by Trudgill(1969). we also obtained a similar strain, i.e., *Pseudomonas* O1 which could utilize 2-furoic acid as a carbon source.

The mixed cultures of the isolated four bacteria demonstrated the quite similar growth curve and decomposition of furfural to those of undefined cultures, in spite of that other microorganisms are present in undefined cultures.

Therefore we consider that the above men-

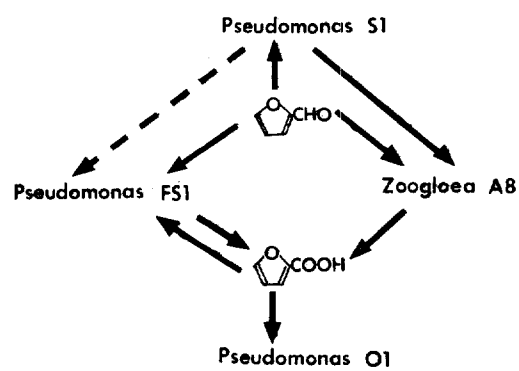

Fig. 4. Schematic diagram in interactions between four bacteria during the decomposition of furfural

tioned microorganisms played an important role for the complete oxidation of furfural.

Fig. 4 illustrates biological interactions of four bacteria in the decomposition of furfural. Lynch *et al.*, (1979). defined that synergism is association of organisms having complementary activities resulting in greater formation of products than by component organisms growing alone. Odum(1971) defined that protocooperation is to benefit by the association of both populations not to be obligatory in its relations.

Pseuomonas S1 decomposes furfural and pr oduced a certain substance(unknown amine), which is required by *Zoogloea* A8 for the bioconversion of furfural into 2-furoic acid.

This transformed 2-furoic acid cad be utilized by *Pseudomodas* S1, but is not obligatory for this strain. Therefore *Pseudomonas* S1 and *Zoogloea* A8 interact as protocoperation.

Then 2-furoic acid transformed by *Zoogloea* A8 is also utilized by *Pseudomonas* O1. Therefore, the cleavage of furan ring of furfural by three microorganisms is enhanced more efficiently than two microorganisms. Interaction of three microorganisms is referred to synergism. On the other hand, the interactions between *Pseudomonas* FS1 and other three microorganisms are not yet known.

In conclusions, it was pronounced that the furfural was decomposed more efficiently by coexistance of protocooperation and synergism, resulting in the biological elimination of a toxicant and decolorizaton.

적 요

Pseudomonas sp. 세균주와 *Zoogloea* sp. 한 균주를 토양에서 분리하였으며 이들 균주는 furfural로부터 전환된 2-furoic acid와 primary amine group을 포함한 어떤 물질을 매개체로 이용함으로서 상호간에 procooperation과 Synergism을 보여주었다. 이에 의하여 furfural이 완전히 산화됨과 동시에 탈색이 되는데 그 효과는 단일균주보다 혼합배양이 보다 효과적이었다.

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