NOVEL BIOSTIMULATION OF CONTAMINANTS CONTAINING ETHER LINKAGES

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Abstract

A novel biostimulation method based on the similarity between archaeal lipids and contaminants containing ether linkages, and the expectation that 1,4-dioxane-degrading microorganisms can degrade archaeal lipids, is proposed. The most general structure of an archaeal lipid, 2,3-di-*O*-phytanyl-*sn*-glycerol, was synthesized and added, as the sole source of carbon, to the culture medium for the 1,4-dioxane degrading fungus, *Cordyceps sinensis*. *C. sinensis* grew successfully on 2,3-di-*O*-phytanyl-*sn*-glycerol. Possibly the degradation of 1,4-dioxane and of 2,3-di-*O*-phytanyl-*sn*-glycerol are catalysed by the same enzymes. If soil and/or sediment microorganisms can be activated by 2,3-di-*O*-phytanyl-*sn*-glycerol, the degradation of 1,4-dioxane, and possibly dioxins and agro-chemicals, will be stimulated by its addition to polluted environments when the contaminating compounds contain ether linkages.

Introduction

Archaebacteria are the oldest microorganisms and are ubiquitous. They can be found in methane-producing ponds, halophilic lakes and thermophilic hot springs (*1*). Methane-producing archaebacteria have been used for the fermentative production of methane from fecal and/or organic wastes.

The lipid content of archea is relatively high (*2*), and their environmental biomass is large. The lipids differ from those of eukaryotes and prokaryotes in containg ether linkages. Many structural varieties have been found but they are mainly composed of 2,3-di-*O*-phytanyl-*sn*-glycerol. Lipids containing ether linkages also occur in humans and are involved with platelet activating factor (PAF), and therefore the degradation of lipids with ether linkages has been studied from a medical viewpoint. However, although such lipids are components of a substantial biomass, their microbial degradation in the environment, has not been studied in detail.

In contrast, the microbial degradation of the ether linkage in man-made chemicals has been the subject of many studies. Enzymes that can degrade man-made chemicals are adapted from those that act upon natural substances (*3*). For example, the natural enzymes lignin peroxidase, manganese peroxidase and laccase (*4*) can degrade natural biomass, lignin, and man-made agro-chemicals. Biodegradation using natural biomass has been

proposed for many applications including bioremediation. However, although there are many studies reporting the isolation from the environment of microorganisms capable of degrading man-made chemicals, there have been few studies about the relationship of the microorganisms to natural substances.

We have studied the degradation by microorganisms of chemicals containing ether linkages, namely, 1,4-dioxane and dioxins. We have previously isolated and identified a fungus, *Cordyceps sinensis*, which can degrade 1,4-dioxane and dioxins(*5, 6*). However we did not elucidate what natural substances containing ether linkages were utilized by this fungus. Such information will allow bioremediation, especially that involving biostimulation, using natural nutrition. The following studies were therefore carried out.

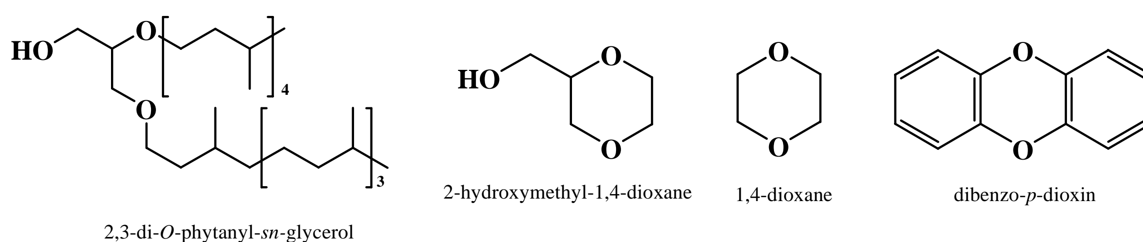

Figure 1. Compounds used for growth substrates for *Cordyceps sinensis*

Materials and Methods

Cultivation of *C. sinensis*: Basal salt medium (BSM) with 0.3% substrate (2,3-di-*O*-phytanyl-*sn*-glycerol or 1,4-dioxane or glucose) as the sole source of carbon (*7*) and without nitrilotriacetic acid. The pH was adjusted to 6.8 with HCl and/or NaOH. For the preparation of a solid medium, 1.5% agar was added to the BSM. For liquid cultivation, 3 ml of culture medium was placed in a 15 ml screw-capped vial with a Teflon inner liner, and auto-claved for 15 min at 121°C. Filter sterilized 1,4-dioxane and substrates were added. Cultures were grown at 30°C with constant stirring.

Analytical procedure: Each dried sample was extracted with an appropriate solvent. A portion of each extract was treated with *N*, *O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA, 1+4 v/v) for 1 hour at room temperature and a portion was left untreated. The solvent phases in all cases were analyzed by GC/MS (GC-MATE, JEOL Ltd, Tokyo, Japan, and an HP 6890, Agilent Technologies, CA, USA). Separations were carried out on a DB-17MS column (30 m x 0.25 mm i.d., J&W Scientific, CA. USA), and the analytes were ionized in EI mode. The oven temperature program was: 80°C (hold 2 min), 150° C (20°C/min) and 300°C (10°C/min). Structure confirmation of synthesized chemicals was by NMR spectroscopy (ECA800Spectrometer, JEOL Ltd, Tokyo, Japan).

Chemicals: 2,3-di-*O*-phytanyl-*sn*-glycerol was synthesized by the method of Aoki (*8*). Phytanic acid was synthesized by the method of Jellum (*9*). All other chemicals were of AR grade.

Results and Discussion

The isolate of *C. sinensis* can degrade 1.4-dioxane and 2-hydroxymethyl-1,4-dioxane and yield ethylene glycol and glycerol. It seems that the degradation products are metabolized to minerals (*6*) (*5*). These results indicated that when the ether linkage involved glycerols or ethylene glycols, all degradation products would be mineralized once the ether bond was broken by the fungal enzyme. Furthermore, there were indications that, in general, a broad spectrum of carbohydrates, proteins, and lipids serve as better substrates for *C. sinensis*.

Synthetic 2,3-di-*O*-phytanyl-*sn*-glycerol was added, as the sole carbon source, to the culture medium of *C. sinensis* And successful growth was observed. As shown in Figure 2, the growth of *C. sinensis* on 2,3-di-*O*-phytanyl-*sn*-glycerol was slightly delayed when compared with that on 1,4-dioxane or glucose , but the growth rate gradually increased. Overall, there were not major differences in the growth rates on the different substrates.

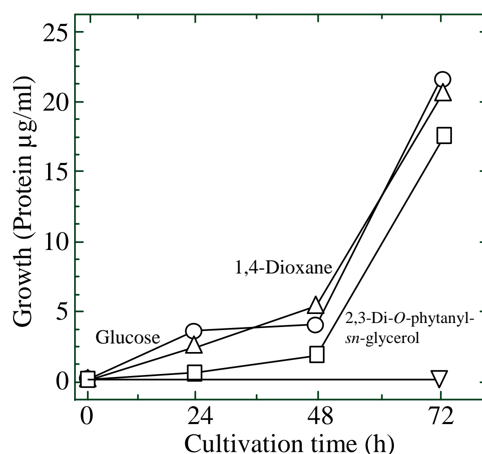

Figure 2. Time course for the growth of Cordyceps sinensis on the several substrates.

A proposed degradation pathway for 2,3-di-*O*-phytanyl-*sn*-glycerol is shown in Figure 3. Initially it was degraded to glycerol and phytanol; glycerol then disappeared from the chromatogram, phytanol decreased and phytanic acid appeared. The phytanic acid was further metabolized. It is possible that the further degradation yielded the aldehyde (B. Schink, personal communication). When PAF is synthesized in the human body, the degradation product is the aldehyde, and, in addition, mono-alkyl-glyceride is produced. If this reaction is followed in this case, mono-*O*-phytanyl-*sn*-glycerol will be produced from 2,3-di-*O*-phytanyl-*sn*-glycerol. However, we did not detect these chemicals. Further studies are needed to clarify this point.

In general, it appears that di (2-ethylhexyl) phthalate is degraded by fungal lipases (*10*). It is possible that 1,4-dioxane and 2,3-di-*O*-phytanyl-*sn*-glycerol are also degraded by lipases. If soil and/or sediment microorganisms can be activated by 2,3-di-*O*-phytanyl-*sn*-glycerol, the degradation of 1,4-dioxane, and possibly

dioxins and some agro-chemicals, will be stimulated by its addition to polluted environments when the comtaminating compounds contain ether linkages.

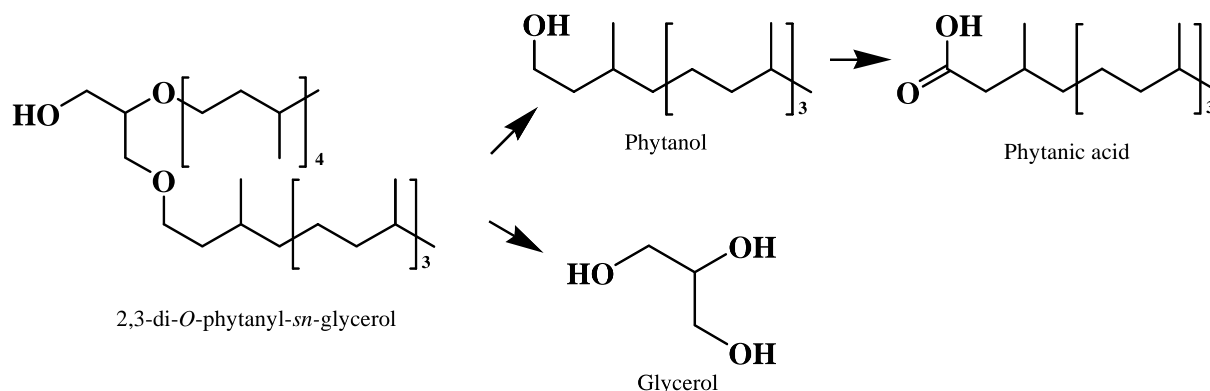

Figure 3. Proposed degradation pathway for 2,3-di-*O*-phytanyl-*sn*-glycerol

Acknowledgements

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