

## Biological Activity of Methyl tert-butyl Ether in Relation to Soil Microorganisms has a Negative Environmental Impact

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**Abstract:** Fuel oxygenates are added to gasoline to enhance combustion efficiency of automobiles and reduce air pollution. Methyl tert-butyl ether (MTBE) is the most commonly used oxygenate because of its low cost, high-octane level and ease of blending with gasoline. However, due to its water solubility, high mobility and low biodegradability it leaches in soil subsurface at the speed of groundwater. Amending gasoline with MTBE has made a widespread contamination of groundwater, surface waters in coastal environments and at low levels in well water. Although current public concern about MTBE contamination is widely discussed, but its adverse effects on soil micro flora is not yet understood. Soil Streptomyces are beneficial to soil productivity and are of the major contributors to the biological buffering of soils having antagonistic activity against wide spectrum of pathogenic bacteria and fungi. Streptomycetidal activity of Methyl tert-butyl ether (MTBE) is being reported here. Adverse effect of MTBE against four soil-inhabitant *Streptomyces* spp. isolates and two plant root-pathogens was investigated. To elucidate antimicrobial activity of MTBE, it was tested against four soil isolates of *Streptomyces*; a plant bacterial-pathogen, *Erwinia carotovora* and a plant root fungal-pathogen, *Fusarium solani*. MTBE did not reveal any growth inhibitory-activity against *E. carotovora* and *F. solani* but showed strong inhibitory effect against *Streptomyces* spp. isolates. The Minimum Inhibitory Concentration (MIC) was 1/800 of the original MTBE. Fuel leaks and spills can adversely suppress or eliminate the *Streptomyces* role in the soil causing alteration in the balance of soil micro flora. This change will lead to domination of microorganisms with adverse biological or ecological effects. Fortunately, major oil companies have decided to phase out MTBE from automobile fuels because of its adverse effect on environment and human health.

**Key words:** Soil Micro Flora, Soil Contamination, MTBE, Antagonism

### INTRODUCTION

Since 1992, Methyl tert-butyl ether (MTBE) has been added to gasoline worldwide in order to enhance combustion efficiency and reduce air pollution. It is the most commonly used oxygenate because of its low cost, high-octane level and ease of blending with gasoline [1]. Due to its water solubility, high mobility and low biodegradability it leaches in soil subsurface at the speed of groundwater. Amending gasoline with MTBE has made a widespread contamination of soil, groundwater and surface waters in coastal environments and at low levels in wells water [2-6]. The carcinogenic effect of MTBE has been observed in animals and furthermore, its metabolites have shown mutagenicity effects in the Ames bacterial assay [7, 8]. Although current public concern about MTBE contamination is widely discussed and is at the focus of environmental scientists, but its adverse effects on agricultural soil micro flora is not yet understood. To reduce the problem of MTBE contamination, several workers reported laboratory methods of remediation, especially bioremediation [9-13] however, these methods are not practically established for wide spread use in the natural

environment. At the present study, to investigate inhibitory effect of MTBE against some microorganisms of agricultural soils, four soil-inhabitant *Streptomyces* sp. isolates, *Erwinia carotovora* a bacterial and *Fusarium solani* a fungal, plant root-pathogens were tested by *in vitro* assays. The aim of the study was to elucidate if contamination of agricultural soils with MTBE can cause adverse effects in soil environment especially on the activity of soil *Streptomyces*. These findings support the wide range of reports on harmful effects of MTBE in environment [14-18].

### MATERIALS AND METHODS

**Microorganisms:** The tested micro organisms, *Erwinia carotovora* Jones, *Fusarium solani* Mart. and four *Streptomyces* spp. isolates were prepared from the Research Laboratory of Department of Plant Pathology, College of Agriculture, Bahonar University of Kerman, Iran. The *Streptomyces* isolates were isolated from soil, identified at Genus level and proved beneficial but non pathogenic.

Table 1: Differential Antimicrobial Activity of MTBE Serial Dilutions on four *Streptomyces* sp. Isolates, *Erwinia carotovora* and *Fusarium solani*. The Numbers Indicate the Diameter of Inhibition Zones in mm. The Test was an *in vitro* Bioassay Performed by Agar Diffusion Method

Microorganisms	MTBE Dilutions					
	1:10	1:100	1:200	1:400	1:800	1:1600
<i>Streptomyces</i> isolate No. 18	30	28	22	16	14	-
<i>Streptomyces</i> isolate No. 51	51	41	35	22	17	-
<i>Streptomyces</i> isolate No. 59	44	39	30	18	15	-
<i>Streptomyces</i> isolate No. 71	41	32	34	19	25	-
<i>Erwinia carotovora</i>	-	-	-	-	-	-
<i>Fusarium solani</i>	-	-	-	-	-	-

-= Zero growth, no inhibitory effect

**MTBE:** Serial dilutions of 1:10, 1:100, 1:200, 1:400, 1:800 and 1:1600 of MTBE were prepared in dimethyl sulfoxide (DMSO): methanol (1:1, v/v) solvent (DM solvent), sealed and kept refrigerated before use.

**Bioassay Methods:** *Erwinia carotovora*, the bacterium, cultured on Mueller-Hinton-Agar medium (MHA, Merk, Germany). For assays, suspension of approximately  $1.5 \times 10^6$  cells  $\text{mL}^{-1}$  in sterile normal saline were prepared as described by Forbes *et al.* [19] and about 1.5 mL of it was uniformly seeded on MHA in 9 x 1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers and MTBE dilutions administered to fullness in the corresponding wells.

*Fusarium solani*, the plant root pathogenic fungus, was cultured on Potato Dextrose Agar medium (PDA). For assays, suspension of fungal spores was uniformly seeded on PDA medium using sterile cotton swabs and assayed as mentioned.

*Streptomyces* isolates were cultured on Casein Glycerin Agar medium (CGA). For assays, suspensions of spores were uniformly seeded on CGA using sterile cotton swabs and assayed as above.

Culture plates were incubated at 29° C for 24 hr for *E. carotovora*, 3-5 days for *F. solani* and *Streptomyces* isolates. Bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm. DM solvent controls were included, although no antimicrobial activity noted in the solvent employed for the test. All samples were tested in triplicate and the mean values were recorded.

**Mode of Action of MTBE Inhibitory Effect:** To determine if mode of action of MTBE is Streptomyceticidal or Streptomycetistatic, by using transfer needles smears from MTBE inhibitory zones of all sensitive isolates were streaked to new plates of CGA medium aseptically. As controls, similar transfers were made from non-inhibitory areas of the corresponding isolates. Plates were incubated at 29°C

for 3-5 days and then evaluated for the presence or lack of *Streptomyces* growth. All tests were performed in triplicates.

## RESULTS AND DISCUSSION

**Differential Antimicrobial Activity of MTBE:** Strong inhibitory effect of MTBE was noticed against *Streptomyces* isolates, but no growth inhibition was observed in *E. carotovora* and *F. solani*, the plant pathogens. Inhibitory effects of MTBE on four *Streptomyces* isolates are presented in Table 1.

**Minimum Inhibitory Concentration (MIC) of MTBE:** Minimum Inhibitory Concentration (MIC) of MTBE was 1/800 of the original MTBE against all of the tested *Streptomyces* isolates.

**Mode of Action of MTBE:** In all triplicate tests, no growth was recovered in transfers from inhibition zones, representing that apparently mode of action of MTBE is streptomyceticidal upon isolates of this study. Normal growth observed in case of controls. However, further investigation is in process for ruling out this type of physiological response.

It is well known that *Streptomyces* spp. are of the major contributors to the biological buffering of soils exerting antagonistic activity against wide range of soil bacteria and fungi, have roles in decomposition of organic matter conducive to crop production and are potential producers of antibiotics [20, 21]. Diminishing their role in soil can alter the balance of soil micro flora and dominate microorganisms with hazardous biological or ecological effects. *E. carotovora* and *F. solani* are of major plant-root pathogens which under normal conditions are partially suppressed by antagonistic activity of soil *Streptomyces* spp. [22, 23]. Spread of MTBE in agricultural soils occurs by spills or leakage of gasoline in the vicinity of reservoirs or gasoline pumps constructed in the fields during in and out refills. From there, by many ways as irrigation, runoffs after precipitations, field animals, soil levelers and contaminated mud on field machine-tires, MTBE

spreads around the field. As a result, concentration of MTBE increases in soil upon time causing suppression of these beneficial microorganisms. Consequently, the increased soil contamination by MTBE can lead to reduction in soil fertility, eruption of harmful microorganisms causing detrimental changes in soil health and fertility. For better elucidation of its adverse effects and spectrum of bioactivity, further inhibitory and cytotoxic activities of MTBE should be investigated against wider range of soil-inhabitant organisms. As expressed by many researchers and public media, MTBE may be one of the greatest unfolding environmental challenges in America's history [15-18]. Eliminating it from fuel and its remediation from soil and water should receive special attentions from governmental and research agencies in the affected countries throughout the globe.

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