Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 3 (1): 140-142, 2004
ISSN 1682-3974

Streptomyces Content of Agricultural Soils Decreases by Methyl *Tert*-butyl Ether (MTBE) Spills and Leakage, Causing Adverse Change in Soil Micro Flora

G.H. Shahidi Bonjar
Department of Plant Pathology, College of Agriculture, Bahonar University of Kerman, Iran

Abstract: Streptomyceticidal activity of Methyl *Tert*-butyl ether (MTBE) is being reported for the first time. Adverse effect of Methyl *Tert*-butyl ether (MTBE), the gasoline additive, against four soil-inhabitant *Streptomyces* spp. isolates and two plant root-pathogens was investigated. MTBE, an octane enhance is added to gasoline to reduce atmospheric concentrations of carbon monoxide and ozone. It contaminates soil and groundwater by fuel leaks and spills. *Streptomyces* spp. are of the major contributors to the biological buffering of soils by exerting beneficial and antagonistic activity against wide range of bacteria and fungi. 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.

Keywords: Soil micro flora, soil contamination, MTBE, antagonism

INTRODUCTION

Methyl *Tert*-butyl ether (MTBE) is added to gasoline by many oil companies 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[1-6] 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 especially on the growth of soil beneficial Streptomyces.

MATERIALS AND METHODS

Preparation of microorganisms: Pure cultures of *E. carotovora* Jones, *F. solani* Mart. and four *Streptomyces* spp. isolates (No. 32, 17, 66 and 73) obtained 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 non pathogenic but beneficial by the mentioned laboratory (personal communication).

Preparation of MTBE: The gasoline additive, MTBE was obtained from Oil Refinery of Abadan, Iran. Dilution series 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).

Corresponding Author: G.H. Shahidi Bonjar, Department of Plant Pathology, College of Agriculture, Bahonar University of Kerman, Iran

Culturing and assay method: The bacterium, *E. carotovora* was cultured on Mueller-Hinton-Agar medium (MHA, Merk, Germany). For assays, suspension of approximately $1.5x10^8$ cells ml^{-1} in sterile normal saline were prepared as described by Forbes *et al.*[14] and about 1.5 ml of it was uniformly seeded on MHA in 9x1.2 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. *F. solani*, the plant root pathogenic fungus, was cultured on Potato Dextrose Agar medium (PDA). For assays, suspension of fugal 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 h for *E. carotovora,* 3-5 days for *F. solani* and *Streptomyces* isolates. All samples were tested in triplicate. 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.

Elucidation of mechanism of action of MTBE: To deduce whether mechanism 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 arias of the corresponding isolates. All tests were performed in triplicates. Plates were incubated at 29°C for 3-5 days and then evaluated for the presence or lack of *Streptomyces* growth.

RESULTS

Inhibitory action of MTBE: No growth inhibition was observed in *E. carotovora* and *F. solani* but strong inhibitory effect of MTBE was noticed against *Streptomyces* isolates. Growth suppression of the four *Streptomyces* isolates by MTBE is presented in Table 1. The data indicate that Minimum inhibitory concentration (MIC) of MTBE is approximately 1/800 of the original MTBE against all used *Streptomyces* isolates.

Table 1: Growth inhibitory effect of MTBE serial dilutions against four *Streptomyces* sp. isolates, *Erwinia carotovora* and *Fusarium solani* tested in Agar-well diffusion method, indicated as diameter of growth-inhibitory zones in mm

	MTBE Dilutions					
Microorganisms	1:10	1:100	1:200	1:400	1:800	1:1600
Streptomyces sp. isolate No. 32	52	48	40	29	23	-
Streptomyces sp. isolate No. 17	33	27	25	20	14	-
Streptomyces sp. isolate No. 66	38	30	27	20	16	-
Streptomyces sp. isolate No. 73	32	24	20	16	14	-
Erwinia carotovora (Jones 1901), Bergey *et al.* 1923	-	-	-	-	-	-
Fusarium solani (Mart.) Sacc.	-	-	-	-	-	-

- = Zero growth, no inhibitory effect

Mechanism of action of MTBE: No growth was recovered in transfers from inhibition zones, indicating that mechanism of action of MTBE is streptomyceticidal upon isolates of this study. However, normal growth observed in case of controls.

DISCUSSION

Streptomyces spp. are of the major contributors to the biological buffering of soils and have roles in decomposition of organic matter conductive to crop production. However, these prokaryotes have been much studied as potential producers of antibiotics exerting antagonistic activity against wide range of soil bacteria and fungi[15,16]. Fading their role in the 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.[17,18]. 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.

ACKNOWLEDGMENT

Thanks to Research Affairs Office of Bahonar University of Kerman for support of this study. The spiritual result of this research is dedicated to the soul of Mr. A. Afzalipour founder of the University at Kerman, God may bless him.

REFERENCES

1. Johnson, R., J. Pankow, D. Bender, C. Price and J. Zogorski, 2000. MTBE, to what extent will past releases contaminate community water supply wells? Environ. Sci. Technol., 32: 210-217.
2. Hoffert, S.P., 1998. Haze of uncertainly surrounds gas additive. The Scientist, 12: 7.
3. Reuter, J.E., B.C. Allen, R.C. Richards, J.F. Pankow, C.R. Goldman, R.L. Scholl and J.S. Seyfried, 1998. Concentrations, sources and fate of the gasoline oxygenate methyle-*tert*-butyl ether (MTBE) in the multiple-use lake. Environ. Sci. Technol., 32: 3666-3672.
4. Brown, J.S., S.M. Bay, D.J. Greenstein and W.R. Ray, 2000. Concentrations of methyle-*tert*-butyl ether (MTBE) in inputs and receiving waters of Southern California. Southern California Coastal Water Research Project Authority, 1999-2000 Annual Report.
5. Bennett, G.F., 2001. MTBE: Effects on soil and groundwater resources. J. Hazardous Material, 88: 141-144.
6. An, Y., D.H. Kampbell and G.W. Sewell, 2002. Water quality at five marines in Lake Texoma as related to methyle-*tert*-butyl ether (MTBE). Environ. Pollution, 118: 331-336.
7. Caprino, L. and G.I. Togna, 1998. Potential health effects of gasoline and its constituents: A review of current literature (1990-1997) on toxicological data. Environmental Health Perspectives, 106: 115-125.
8. Williams-Hill, D., C.P. Spears, S. Prakash, G.A. Olah, T. Shamma, T. Moin, L.Y. Kim and C.K. Hill, 1999. Mutagenicity studies of Methyle-*tert*-butyl ether using the Ames tester strain TA102. Mutation Research, 446: 15-21.
9. Salanitro, J., L. Diaz, M. Williams and H. Wisniewski, 1994. Isolation of a bacterial culture that degrades methyl t-butyl ether. Applied Environ. Microbiol., 60: 2593-2596.
10. Hardison, L., S. Curry, S. Ciuffetti and M. Hyman, 1997. Metabolism of diethyl ether and cometabolism of Methyl tert-butyl ether by a filamentous fungus, a *Graphium* sp. Applied Environ. Microbiol., 63: 3059-3067.
11. Hanson, J., C. Ackerman and L. Scow, 1999. Biodegradation of methyl tert-butyl ether by a bacterial pure culture. Applied Environ. Microbiol., 65: 4788-4792.
12. Fortin, N., M. Morales, Y. Nakagawa, D. Focht and M. Deshusses, 2001. Methyl tert-butyl ether (MTBE) degradation by a microbial consortium. Environ. Microbiol., 3: 407-416.
13. Steffan, R., K. Mc Clay, S. Vainberg, C. Condee and D. Zhang, 2001. Biodegradation of gasoline oxygenates Methyl tert-butyl ether, Ethyl tert-butyl ether and Tert-amil methyl ether by propane-oxidizing bacteria. Applied Environ. Microbiol., 63: 4216-4222.
15. Gottleib, D., 1973. General Consideration and Implication of the Actinomycetales. In: 'Actinomycetales: Characteristics and practical importance'. (Ed. G. Sykes and F.A. Skinner), Academic Press: London, pp: 1-9.
16. Okami Y. and K. Hotta, 1988. Search and discovery of new antibiotics. In: Actinomycetes in Biotechnology. (Ed. M. Goodfellow, S.T. Williams and M. Modarski) Academic Press: London, pp: 33-67.
14. Forbes, B.A., D.F. Sahm, A.S. Weissfeld and E.A. Trevino, 1990. Methods for testing antimicrobial effectiveness. In: Bailey and Scott's Diagnostic Microbiology . (Eds. E.J. Baron, L.R. Peterson and S.M. Finegold), Mosby Co: St Louis, Missouri, pp: 171-194.
17. Demain, A.L., 1998. Induction of microbial secondary metabolism. International Microbiol., 1: 259-264.
18. Getha, K. and S. Vikineswary, 2002. Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f.sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process. Industrial Microbiol. Biotechnol., 25: 303-310.