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REGULAR ARTICLE

Novel biotransformation of menthone by microbial strains and vermicompost microbial consortium

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Abstract

The aim of the study was to evaluate the ability of *Trichoderma harzianum* and *Pseudomonas fluorescence* and vermicompost microbial consortium to biotransform the monoterpene menthone. The different microbial transformation was carried out in different media including the control flask. Samples of different cultures were taken after every 24 hours, extracted with n-hexane and analysed by GC and GC-MS. The chemical structure of the bio transformed products were identified by GC and GC-MS. All the microbial strains led to the decomposition of menthone with time. The most valuable transformation was the production of menthol by vermicompost microbial strain. The obtained data indicated that vermicompost microbial consortium is a good biocatalyst to convert the ketonic group into hydroxyl group and showed the importance of various microbial strains in the biotransformation of the menthone.

Key words: Biotransformation, GC, GC-MS, *Trichoderma harzianum*, *Pseudomonas fluorescence*, vermicompost microbial consortium

Introduction

In present scenario terpenes are extensively used due to their potent potential in the prevention and therapy of several diseases including cancer. As natural insecticides, their antimicrobial properties which are frequently employed in storage of agricultural products and as building blocks for the synthesis of many highly valuable compounds (Lilly, 1994). Monoterpenes in plants are known to have mainly ecological roles in acting as deterrents against feedings by herbivores, as defences and attractants for pollination. In mammals, higher terpenes are involved in stabilizing cell membranes, metabolic pathways and as regulators of enzymatic reactions (Langenheim, 1994).

The transformation using microbes enables the preparation of optically pure compounds (Nakamura et al., 1989; Suga and Hirata, 1990). Microbial transformation of terpenes is extensively studied because of production of new metabolites with enhanced biological activity (Abraham et al., 1985). In addition, regio and stereoselective introduction at inactivated carbon atom by biotransformation is achieved which is otherwise difficult with chemical methods (Holland, 1992). These terpenes and their derivatives can be utilized as synthetic intermediates and chiral synthons for assymetric synthesis (Azerad, 2000). The main focus of our investigation was performed to study the biotransformation of menthone by microbial strains and vermicompost microbial consortium into certain valuable product that

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can beneficial for human and to develop inexpensive technology for utilization of agro industrial waste from mentha industries in the tarai region of Uttaranchal with prime objective to isolate some effective organism capable for biotransformation.

Materials and methods

Source of terpene (menthones)

Standard terpenes, menthone, isomenthone and menthol (Sigma Aldrich) were procured with the courtesy of Professor S. H. Walia, Division of Agricultural Chemicals, (IARI) New Delhi.

Microrganism and vermicompost

The microbial culture of *Trichoderma harzianum* and *Pseudomonas fluorescens* used for biotransformation was obtained from Department of Microbiology, G. B. Pant University of Agriculture & Technology, Pantnagar with the courtesy of Dr. Laxmi Tiwari, (Assistant Professor). Vermicompost was procured from University dairy from Nagla and Farm yard manure was procured from Garden section G.B. Pant University, Pantnagar.

Culture medium

The culture of microorganism was performed in potato medium which was made of the following composition (L): 20g glucose and 200g potato. the culture media was sterilized in an autoclave at 122°C.

Biotransformation procedures

Sterilised PDB (Potato Dextrose Broth) in 150 ml flask were used for pouring the medium. In each conical flask, about 125 ml sterilized medium was aseptically poured near burner flame in a sterilized laminar air flow chamber and menthone (0.2ml) was added into the flasks through the syringe. After adding menthone, microbial culture of *Trichoderma harzianum, Pseudomonas fluorescence,* 10g and 15g of vermicompost microbial consortium was inoculated in different flask and then kept in incubator shaker at 37°C and 120 rpm respectively. The samples were kept in incubator shaker for 5 hours. 50ml of sample was drawn out periodically from each to check the bioconversion, after 24, 31, 48, 54 and 70 hours respectively. These samples were extracted using n-hexane in a separating funnel. Extraction was done three times with 10 ml n-hexane and the hexane extracts were combined. The hexane extract was dried over anhydrous Na_2SO_4 before GC analysis. Selected samples were subjected to GC and GC-MS analysis to identify the products (Bansal et al., 2006; Singer et al., 2001).

Identification and quantification of product

GC analysis

Gas chromatographic analyses were carried onto a Thermoseries CERES 800 plus gas chromatograph with FID fitted detector using DB-5 capillary column (non-polar, 30 m × 0.32 mm id., 0.25 μm film thickness) as described previously (Sethi et al., 2015).

GC–MS analyses

Gas chromatography–mass spectrometry (GC–MS) analyses were carried out on a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, USA), fitted with an HP-5 (5% phenyl methylpolysiloxane, 30 m × 0.32 mm i.d., 0.25 μm film thickness) capillary column coupled with a model 5973 mass detector as described previously (Sethi et al., 2015). Individual components were identified by Wiley or NIST database matching, comparison of retention times and mass spectra of constituents with Adams database (Adams, 2007) and other literature.

Result and discussion

Analysis of sample by GC

The standards were analysed by GC for their purity. The retention times and purity. The data has been recorded in Table 1 and Fig. 1a and 1b. In order to check the formation of menthol, a mixture of standard menthone, isomenthone and menthol was also injected Fig. 1c.

Table 1. The retention times, KI and purity % of various standard.

Compounds	KI (lit)	Retention time (min)	Purity %
Menthone	761	17.90	78.5
Isomenthone	785	18.43	20.2
Menthol	809	18.910	100%

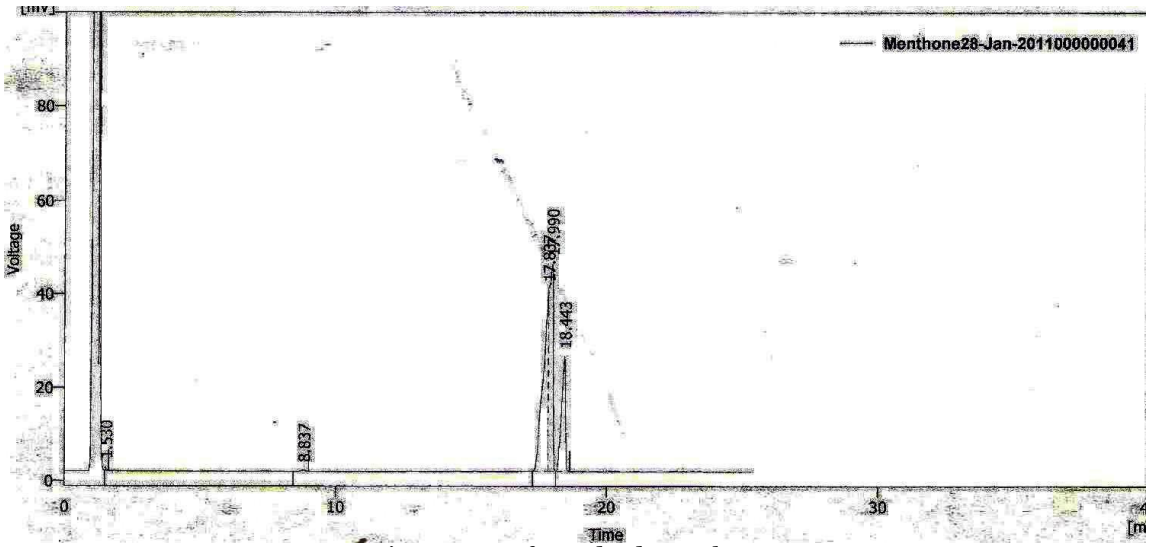

Fig. 1a. GC of standard menthone.

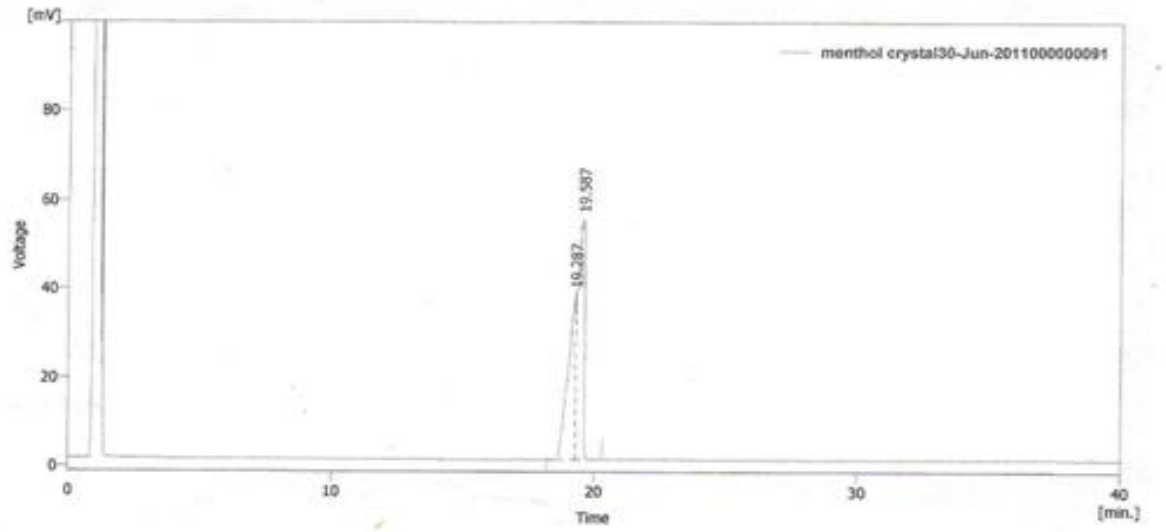

Fig. 1b. GC of standard menthol.

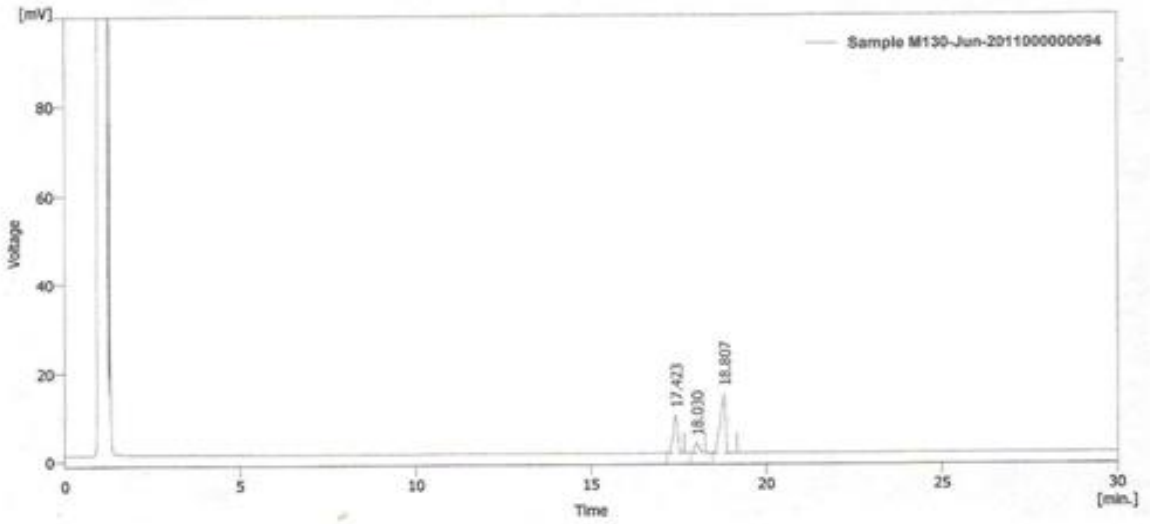

Fig. 1c. GC of mixture of menthone, isomenthone and menthol.

Biotransformation studies of menthones by *Trichoderma harzianum*

It was observed that with time, menthones were degraded slowly by *Trichoderma harzianum*. If menthone/ isomenthone is considered 100% at 0 hours, these were degraded to 88.65% at 161 hours. It was observed that with inoculation time, the ratio of menthone /isomenthone the ratio dropped from 3.94 to 2.00 upto 72 hours of incubation there after increased to 2.95% at 96 hours and dropped to 2.33 at 161 hours. In GC-MS analysis, the presence of menthone and isomenthone could be established.

Biotransformation of menthones by *Pseudomonas fluorescens*

It was observed that *P. fluorescens* is capable of degrading menthones and uses it as a carbon source. It is degraded upto 48.5% in 48hours and completely degraded within 72 hours. The variation in menthone/ isomenthone ratio was quite erratic. In 12 hours, it decreased from 3.94% to 2.71% again increased after 18hour to 24 hours from 3.07% to 3.63 and decreases to 2.29 in 48 hours.

Biotransformation studies using microbial consortium

Biotransformation studies of menthones using vermicompost microbial consortium were designed. Two experiments with microbial culture from 15gm and 10gm vermicompost were used for culture and biotransformation experiments. Using culture from 15gm vermicompost, it was observed that in 12 hours, 50% of menthones were degraded. These were completely degraded within 24hours. The menthone/isomenthone ratio did not vary significantly in 12 hrs.

When experiments with culture from 10 gm of vermicompost were used for biotransformation studies, it was observed that the menthones were degraded to 50% in 12hours and 26% in 24 hours. Traces and upto 2.1% of menthol was also formed (Fig 2a) formation. However, all the products were degraded within 48 hours. Menthone/isomenthone ratio also decreased from 3.94 to 3.91 at 12 hours and 1.88 at 24 hours (Fig 2b).

Pure microbial culture of *T. harzianum* a fungus, *P. fluorescens* a bacteria and microbial consortium from vermicompost were taken for the experiments. Though *P. fluorescens* and *T. harzianum* were found to degrade menthones but no useful product was observed to be formed. Only in case of microbial consortium from vermicompost, the conversion of menthone to menthol was observed on the basis of GC analysis.

Conclusion

It is well known that vermicompost is rich in *actinomycetes* which are capable of production of secondary metabolites as well as capable of biotransformation to useful products. Further studies are required to isolate and screening pure microbial cultures from vermicompost in order to check their capability for biotransformation of terpenoids. The isolated biotransformed compounds can be screened for their optical purity and biological activity. The outcome of the results if useful can be further taken up for fermentation technology to scale them up to the industry.

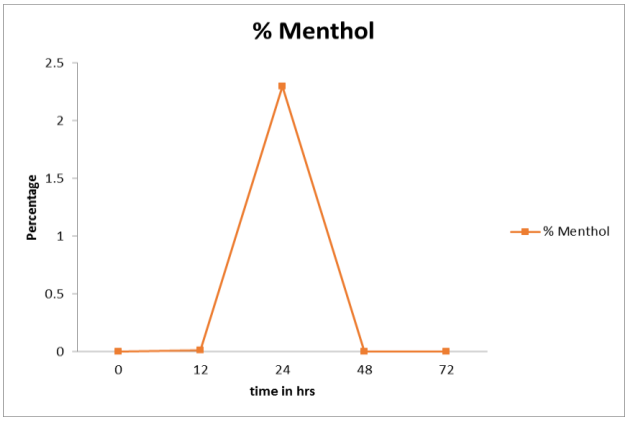

Fig. 2a. Biotransformation of Menthones to menthol by vermicomposte (10 gm).

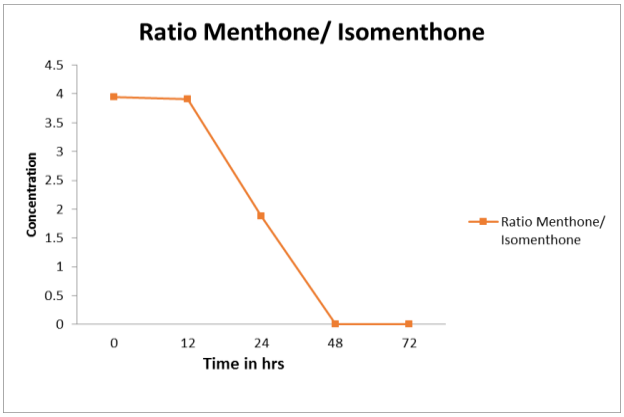

Fig. 2b. Biotransformation of Menthones by vermicompost (10 gm).

Author Contribution

All authors contributed equally in the present study. All authors approved the final version of the manuscript for publication. Authors are thankful to G. B. Pant University of Agriculture and Technology, Pantnagar, India for providing lab facilities.

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