Original Research Paper

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Microbial Oxidation of Monoterpene By Soil Bacteria

Pharmaceutical sciences

KEYWORDS : Limonene,Carveol,Biotransformation,Carvone

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ABSTRACT

The present study involved in the isolation of microorganisms from petroleum contaminated soil that are able to biotransform limonene to various reaction products. Fermentation of limonene by isolated bacteria was conducted for 120 h at 30^{0}C at 150 rpm.Bioconversion products were identified as L-carveol, D-carvone,limonene glycol and other biotransformed products by Gas chromatography-Mass spectroscopic techniques

INTRODUCTION:

Bioactive monoterpenoid limonene is widely distributed in nature and signifies more than 90% orange peel oil and is cheap starting substrate available in citrus processing plants as a waste stream [1, 2].D-limonene has significant value in flavor industry as a precursor in synthesis of various aroma compounds [3]. Beyond their fragrance property its oxygenated derivative such as perillyl alchol,carveol,carvone,geraniol and menthol have shown anticancer activity[4] .These terpenoids are considered as Generally recognized as safe, obtained from natural source, can be added to foods[5]. Problems encountered in monoterpene bio transformations are i) chemical instability of monoterpents.ii) Low solubility of monoterpenes iii) volatility of monoterpenes.iv) Toxicity of monoterpenes v) Low product concentrations and product yield.vi) long incubation times.Monotepenes representing strong antimicrobial action however soil bacteria *pseudomonas* have ability to utilize and live on them [6].

MATERIALS AND METHODS:

Materials: Limonene procured from Hi-media labs, organic solvents procured from Sd-Fine chemicals of analytical grade. All culture media components were from Hi-media labs. Petroleum soil samples were collected at Nizamabad, telangana, India.

Composition of YM medium: gm/100ml distilled water (glucose-1, peptone-0.5, malt extract- 0.3, yeast extract-0.3, pH adjusted to 5). Composition of mineral salt media-(in g/L: (NH4)2SO4 = 5.00; (NH4)2HPO4 = 1.42; NaCl = 0.50; MgSO4.7H2O = 0.40; CaCl2 = 0.60; KCl = 2.15; FeSO4.7H2O = 0.01; ZnSO4 = 0.01; CuSO4 = 0.01; pH not adjusted).

Methods:

Isolation: Each sample collected above was transferred to 150 ml Erlenmeyer flasks containing 50 ml of sterile distilled water and kept for incubation for 24 hrs.After that a loopful of inoculums transferred into 150 ml of Erlenmeyer flasks containing 50 ml YM medium and 50 µL (0.1%, v/v) of d-limonene. After 48h or 7 days of cultivation in a rotary shaker at 30ºC/150 rpm, a loop of each flask was transferred to Petri dishes containing YM medium(2gm/100ml agar), following the streak plate method. The Petri dishes were incubated at 30ºC until complete colonies growth, limited to 7 days. Each different colony appeared, these were maintained on 18ml tubes with YM slant medium [8].

Selection: One loop of each isolated strain was transferred to a150 ml Erlenmeyer flask containing 10 ml of sterile YM medium and 200 µl (2%, v/v) of d-limonene. The flasks were inoculated and shaken on a rotary shaker (30ºC/150 rpm) for 48h. After this period, a 0.1µl sample of the culture broth was transferred to a Petri dish (YM medium) and homogenized with 'L' shaped rod .The culture growth was evaluated after 48h at 30ºC and all the strains that presented a satisfactory growth were considered resistant to 2ml of limonene in 100 ml (v/v) of medium[8].

Screening process: In the screening process all the isolated colonies considered resistant were screened as utilization of limonene as sole carbon source. Thus, one 100 µl aliquot of the culture broth from the last test was inoculated in a 150 ml Erlenmeyer flask containing 10 ml of mineral medium and 0.1 ml of (1%) d-limonene. After 48h incubation at 30ºC and 150 rpm, a 100 µl sample of the culture medium was homogenized in a Petri dish (YM medium) as already described. All the colonies that presented a satisfactory growth after 48h at 30ºC were considered as probable users of d-limonene as a sole carbon source [8].

Fig: 1.Isolate S-NP II in YM medium

Fermentation process: A loopful culture of strain S-NPII were transferred into 250ml Erlenmeyer flasks containing each 100ml of YM-media &cultured on a shaker for 24-48hrs at 150rpm.After 24 hrs of the growth 0.2 %(v/v) limonene was added to the broth as substrate under sterile conditions. The flasks were shaken at 30^{0}C for 5 days. The biotransformed products are collected by centrifugation at 10,000 rpm at 4^{0}C for 8 min.The biomass were discarded and supernatant was extracted with ethyl acetate The organic extract were combined and washed with distilled water for (3x10ml)dried over anhydrous sodium sulfate filtered using what man No.1 filter paper. The solvent was concentrated under reduced pressure to get crude reaction products [7].

Identification of products: Preliminary analysis of the reaction products was carried out by TLC on pre coated aluminum plates. Plates were placed in the solvent system N-Hexane: Diethyl ether (50:50, v/v) in the developing chamber. The plates were dried and visualized by iodine absorption [7].The products were identified by the gas chromatography-mass spectroscopy technique by comparing with the retention times in gas chromatogram and library mass spectra.

RESULTS AND DISCUSSION: Of Two soil samples S-NPI,S-NPI I i.e. S-NPII grew well with limonene 2% in YM media and 1% limonene in mineral salt medium as a sole carbon and energy source. TLC studies reported that the strain(S-NPII) was able to transform d-limonene to mixture of biotranformed products. The major products analysed after biotransformation process were limonene glycol, p-mentha 2, 8 dienol, L-carveol, D-carvone,alpha-terpeniol,Dihydrocarvone and Dihydrocarveol by Agilent series 6890.This may indicate that S-NPII utilizes different pathways for limonene conversion. Hydroxylation at C-6 carbon results in the formation of Carveol further reduction to carvone.Substitution of hydroxyl group on 1, 2-C results in the formation of limonene glycol which is major bioconversion product in S-NPII.

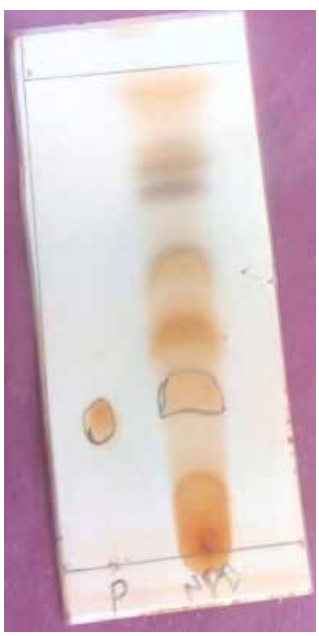

Fig 2. TLC of S-NP II

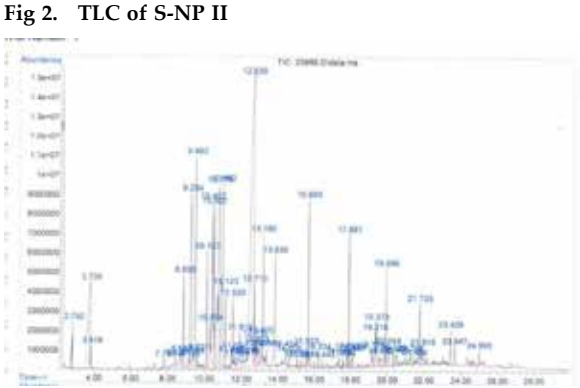

Fig:3 Gas chromatogram of S-NPII

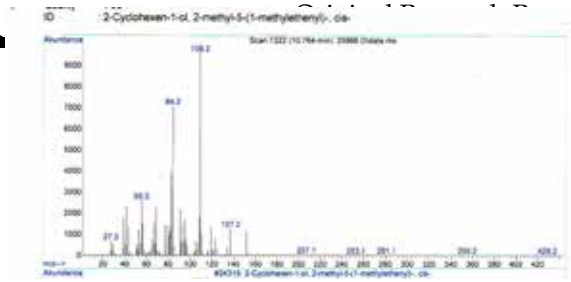

Fig 4.Gc-ms of carveol

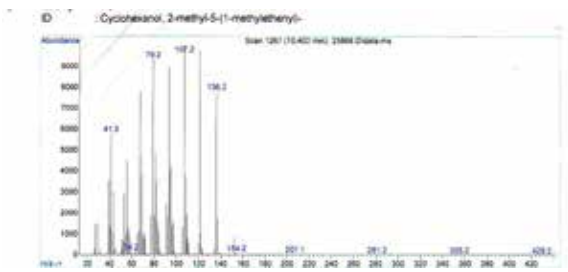

Fig.5:Gc-ms of dihydrocarveol

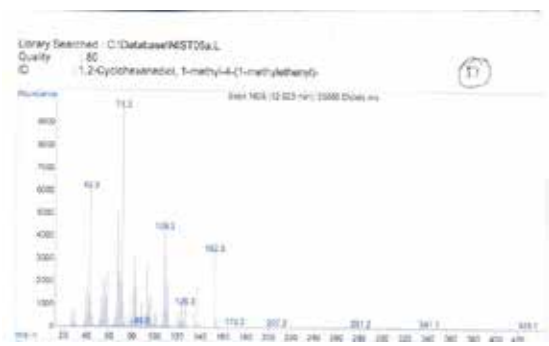

Fig.6:Gc-ms of limonene glycol

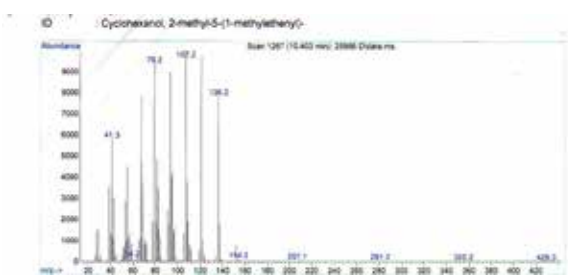

Fig.7: Gc-Ms of Iso dihydrocarveol

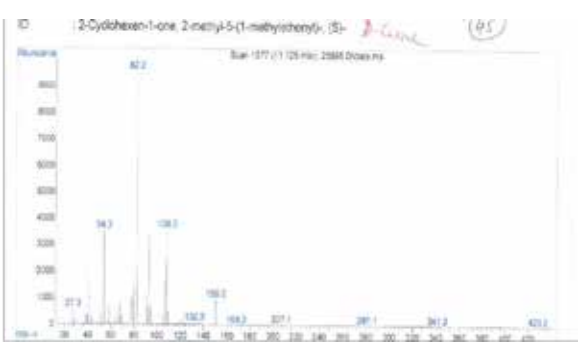

Fig.8: Gc-Ms of Carvone

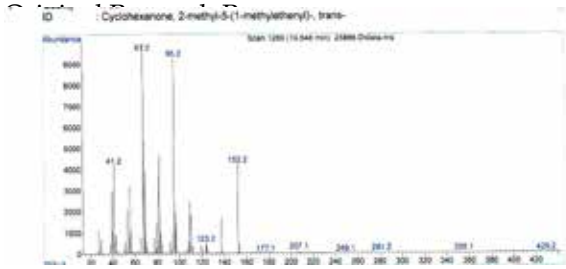

Fig. 9 Gc-ms data of dihydrocarvone

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Conflict of Interest: There is no conflict of interest by authors

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