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Biotransformation of one monoterpene by sporulated surface cultures of *Aspergillus niger* and *Penicillium* sp.

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In this study, biotransformation of menthol by sporulated surface culture of *Aspergillus niger* and *Penicillium* sp. was studied. The main bioconversion product obtained from menthol of *A. niger* was *cis*-*p*-menthan-7-ol and the main products obtained by surface *Penicillium* sp. were limonene, *p*-cymene and γ-terpinene using sporulated surface culture. The pathways involved in the biotransformation of menthol by *A. niger* and *Penicillium* sp. to main products are also discussed.

Keywords: *Aspergillus niger*; *Penicillium* sp.; biotransformation; bioconversion; limonene; *p*-cymene; γ-terpinene; *cis*-*p*-menthane-7-ol

1. Introduction

The work related to the bioconversion of monoterpene alcohols by *Aspergillus niger* and *Penicillium* sp. was investigated. In recent years, the biotechnological production of natural aromatic chemical (NACS) has been stimulated by consumer demand for natural and healthy products (Sehilling & Hauslre, 1997).

This interest in natural flavours instead of synthetic flavours has led to increasing research to focus on the microbial production of the so-called 'bioflavours' (Berger, Drawert, & Tiefel, 1992; Drawert, 1988; Schreier, 1989, 1992). Nearly 80% of the flavours and fragrances used worldwide are produced chemically (Demyttenaere & Kimpe, 2001).

The bioconversion of geranyl and neryl acetate by *A. niger* has been described (Madyastha & Krishna Murthy, 1988a, 1988b). The main reaction found was hydrolysis of terpene acetates to the corresponding alcohols, followed by further hydroxylation experiments using liquid cultures of *A. niger*. Citral transformation to menthols by liquid phase over Ni supported H–MCM-41 and H–Y, has also been examined by a group in Finland (Arvela et al., 2005).

Biotransformation of some monoterpenes (geraniol, nerol and citral) by *A. niger* has been studied. Linalool, α-terpineol and limonene were the main products obtained from

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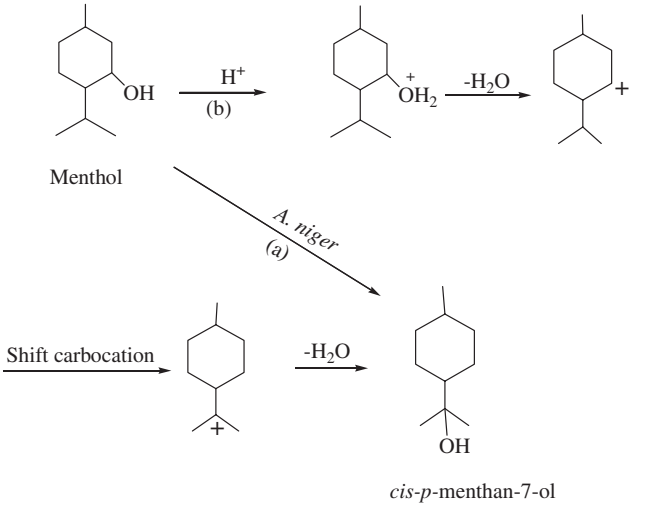


Scheme 1. Biotransformation of menthol by *Aspergillus niger* (a); biosynthesis of menthol to *cis*-p-menthan-ol (b).

nerol and citral by sporulated surface culture (Demyttenare, Herrea, & Kimpe, 2000). Using a surface culture of the organism and adding a methanolic solution of the terpene after a good mat had been developed, the evidence was found to suggest that geraniol was converted to linalool and partially oxidized to citral (Wood, 1969).

In this article, the biotransformation of a monoterpene (menthol) by sporulated surface cultures of *A. niger* strain and *Penicillium* sp. strain is compared.

2. Experimental

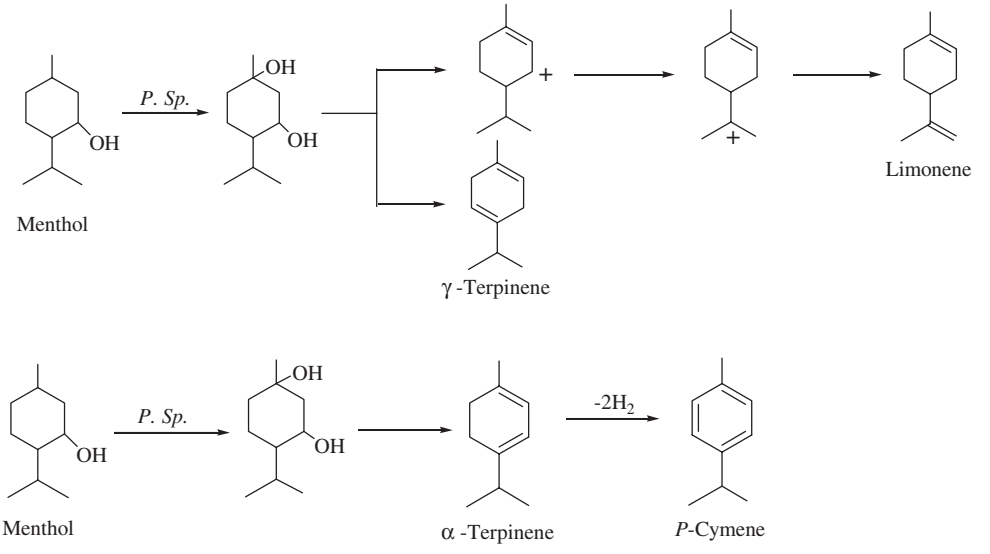
2.1. *Microorganisms*

A strain of *Penicillium* sp. and *A. niger* was isolated from the soil of our laboratories in Tehran prefecture, and was identified according to its physiological and morphological characteristics. *Aspergillus niger* (P.T.C.C.5011) and *Penicillium* sp. (P.T.C.C.5074) were identified according to its Persian-Type Culture-e Collection, Iranian Research Organization for Science & Technology, Tehran, Iran. A spore suspension of *A. niger* and *Penicillium* sp. was prepared in nutrient broth solution for inoculating fungi in Petri dishes.

2.2. *Growth medium and conditions*

For the isolation, growth and conservation of the fungi in Petri dishes one and the same solid medium was used: Sabouraud Dextrose Agar (SDA) medium contained mycological peptone 1.0%, glucose 4.0% and agar 1.5%.

The solid agar medium was inoculated with spores of *A. niger* and *Penicillium* sp. First, germination of the spores and mycelial growth took place, then the growth medium was stored at room temperature. After 1 week, the surfaces of Petri dishes were covered with spores and the biotransformation reaction started.



Scheme 2. Biotransformation of menthol by *Penicillium* sp.

2.3. *Experiments with spore suspension*

In both spores were recovered from 1-week-old surface cultures of *A. niger* and *Penicillium* sp. grown in Petri dishes on SDA. This was done by adding 10 mL of a sterile Tween 80 solution and 0.2% Tween 80 in distilled water to each culture, bringing the spores into suspension. A total spore suspension of 50 mL was obtained and shaken in a 250 mL conical flask.

To this spore suspension of 1 mL, a solution of 5% menthol in ethanol was added, and the suspension was placed on a shaker at 180 rpm. In each process, it was taken out and extracted with Et_2O three consecutive times after 7 days, and the products were directly analysed by GC ad GC/MS.

2.4. *Analysis of the sample with GC/MS*

Analysis was performed using a Hewlett–Packard 6890 with a DB-5 capillary column (30 m × 0.25 mm; film thickness 0.25 μm) and programmed as follows: 60°C for 5 min and programmed to 220°C at a rate of 4°C/min. The flow rate to helium as carrier gas with (2 mL min^{-1}) MS were taken at 70 eV. The retention indices of C_9–C_{28} *n*-alkanes were computer matched with the Wiley 275 Library, as well as by comparison of their mass spectrums with those of authentic samples or with data already available in the literature (Adams, 2001; Davies, 1990).

3. Results and discussion

In this experiment, the biotransformation of menthol by sporulated surface cultures of *A. niger* (P.T.C.C.5011) and *Penicillium* sp. (P.T.C.C.5074) grown on some medium culture flasks was monitored similarly for only 1 week. Cultures were grown in Petri dishes on solid medium Sabouraud Dextrose Agar (SDA) containing menthol. After incubation,

SDA culture was extracted – see Section 2. It was noticed that after 3 days the cultures with 0.05 menthol were fully grown and sporulation had occurred. The cultures with 0.1% menthol covered only a part of the surface. The suspension was extracted with Et_2O three consecutive times and directly analysed by GC and GC/MS.

In these analyses various chemicals were obtained. The main products obtained in the bioconversion of *A. niger* of menthol and bioconversion of *Penicillium* sp. of menthol were *cis*-*p*-menthan-7-ol and, limonene, *p*-cymene and γ-terpinene, respectively. From the data in Scheme 1 it can be concluded that menthol has been converted much more than limonene. Scheme 2 shows that menthol can be bioconverted by *A. niger* to *cis*-p-menthan-7-ol. Pot synthesis of *p*-cymene directly from menthol can, however, be a difficult task because it includes selective dehydration of menthol (Scheme 1). Synthesis of limonene and γ-terpinene from menthol showed converted dehydration by tertiary hydrogens.

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