Biotransformation of (–)–menthol by spores of *Mucor ramannianus* and study of the pathways involved

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Summary

The biotransformation of (–)–menthol by *Mucor ramannianus* was studied. It was carried out with sporulated surface cultures of *Mucor ramannianus*. The main bioconversion products obtained from (–)–menthol were *trans-p*-menthan-8-ol, *trans*-menth-2-en-1-ol, sabinane, *p*-menthane-3,8-diol, isomenthol, and 1,8-cineole, also resulting in higher yields. Biotransformation with sporulated surface cultures was also monitored in Petri dishes and the same solid medium, Sabouraud Dextrose Agar (SDA), was used. In the solid agar medium inoculated with spores of *Mucor ramannianus,* first germination of the spores and then mycelial growth took place. After 1 week, the surfaces of Petri dishes were covered with spores and biotransformation reaction had started. However, there is no report on the biotransformation of (-)-menthol using *Mucor ramannianus*. Six isolates (93.6%) found *Mucor ramannianus* as a biocatalyst and biotransformation of (–)–menthol was investigated. The pathways involved in the biotransformation of (–)–menthol by two main products are also discussed.

Key words: *Biotransformation, bioconversion, Mucor ramannianus, fungi, menthol*

INTRODUCTION

Monoterpenes are important, abundant natural resources supplying the flavour and food industry with solid raw material. Due to their significant bioactivities, some monoterpenes were used as drugs and were found to have a wide range of beneficial effects on human health. Recently, there has been significant progress in the monoterpene microbial biotransformation. The work related to the bioconversion of monoterpene alcohols by *Mucor ramannianus was* investigated. In recent years, the biotransformation of some monoterpenes with fungus has been reported: (±)piperitone [1-3], piperitenone[4],(+)–isopiperitenone [4], karahanenone [5], (–)–camphorquinone [6], (–)–isopinocampheol [7] and(+)–isopinocampheol [7] by *Rhizoctonia solani* and also biotransformation of (+)–and (–)–menthol by larvae of common cutworm (*Spodoptera litura*) [8], l-menthol by *Rhizoctonia solani* [9], (–)–piperitone, (+)– and (–)–carvone, (–)–menthone, (+)–pulegone and (–)–verbenone [10], (–)–menthol have been investigated by using nine isolates of *Rhizoctonia solani* as biocatalyst [11].

The bioconversion of geranyl and neryl acetate by *A. niger* has been described [12-13]. The main found reaction was hydrolysis of terpene acetates to corresponding alcohols, followed by further hydroxylation experiments, with liquid cultures of *A. niger being* used. Monoterpene (citral) transformation to menthols by liquid phase over Ni supported H-MCM-41 and H-Y, has also been examined by a group from Finland [14].

Biotransformation of some monoterpenes by *A. niger* was studied. Linalool and α-terpineol as well as limonene were the main products obtained from nerol and citral by sporulated surface culture [15]. Using a surface culture of the organism and adding a methanolic solution of the terpene it was found that geraniol converted to linalool and partially oxidized to citral [16].

Our study deals with biotransformation of (–)–menthol by sporulated surface cultures of *Penicillium* sp. strain gave results in higher yields [Herba Polonica 2009, in press].

MATERIALS AND METHODS

Microorganisms

A strain of *Mucor ramannianus was* isolated from the soil of our laboratories in Tehran prefecture and was identified according to its physiological and morphological characteristics. *Mucor ramannianus (*PTCC5125) was identified according to its Persian Type Culture Collection, Iranian Research Organization for Science & Technology, Tehran, Iran.

Growth medium and conditions

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

The solid agars medium was inoculated with spores of *Mucor ramannianus.* First germination of the spores and mycelial growth took place, and then the growth medium was stored at room temperature. After 1 week, the surfaces of Petri dishes were covered with spores and biotransformation reaction had started.

Experiments with spore suspension

In both experiments, spores recovered from 1-week-old surface cultures of *Mucor ramannianus were grown* in Petri dishes on SDA. This was done by adding 10 ml of a sterile Tween 80, solution 0.2%, in distilled water to each culture, accumulating the spores in the suspension. A total spore suspension of 50 ml was obtained and shaken in a 250 ml conical flask [17].

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

The GC/MS analysis of the sample

The analysis was performed using Hewlett-Packard 6890 with a DB-5 capillary column (30 m $\times$ 0.25 mm; film thickness 0.25 μm) programmed as follows: 60°C for 5 min. and 220°C at a rate of 4°C/min. The flow rate of helium as a carrier gas (2 mL/min) with MS was taken at 70 eV. The retention indices of C_9-C_{28} n-alkanes, computer matching the Wiley275 Library, as well as by comparison of their mass spectrums with those of authentic samples or with data are already available in the literature [18-20].

RESULTS AND DISCUSSION

In this experiment, the biotransformation of (–)–menthol by sporulated surface cultures of *Mucor ramannianus (*PTCC5074), grown on some medium culture flasks was monitored similarly for the two, 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 Experimental section). It was noticed that after 7 days, the cultures with 0.05 (–)–menthol were fully grown and sporulation had occurred. The cultures with 0.1% (–)–menthol covered only part of the surface. The suspension was extracted with Et_2O three consecutive times and directly analyzed by GC and GC/MS.

In these analyses, various chemicals were obtained. The main products obtained in the bioconversion of *Mucor ramannianus of* (–)–menthol were *p*-menthan-8-ol (40.1%), *trans*-menth-2-ene-1-ol (18.9%), sabinene (11.6%), *p*-menthane-3,8-diol (9.0%), isomenthol (7.6%) and 1,8-cineole (6.4%), respectively. From the data in the Scheme 1, 2 and 3 it was concluded that (–)–menthol has been converted much more than main products. The synthesis of all compounds from (–)–menthol has been shown. The mass spectra of 8 main peaks are as follows:

trans-p-menthan-8-ol :
156[M $^{+}$]: 59(100),58(20),56(10),41(10),43(5),80(7),60(4),30(5).

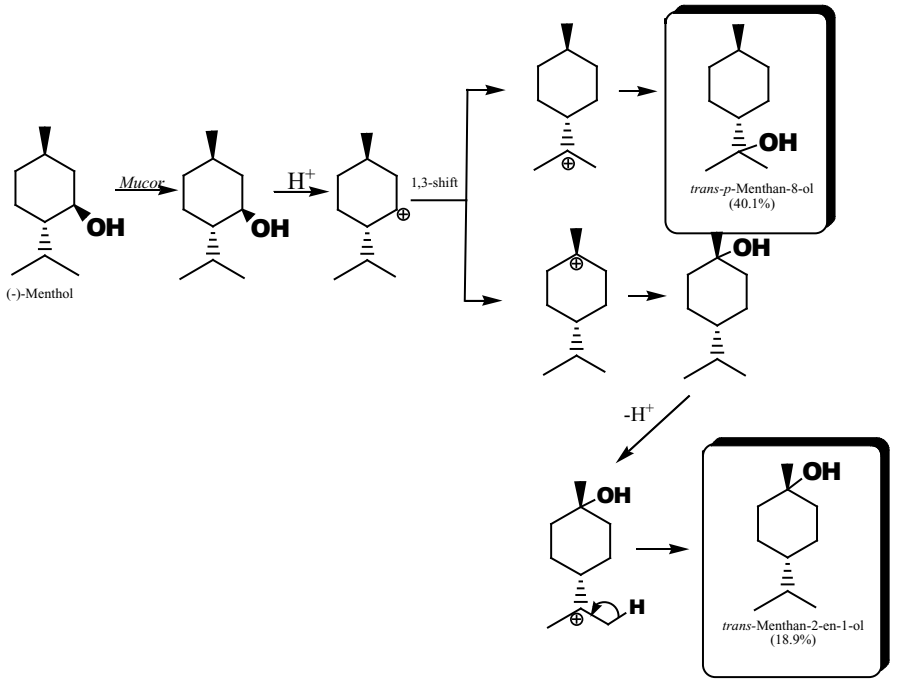
trans-menth-2-ene-1-ol:
156[M $^{+}$]: 71(100),43(60),98(51),113(27),41(20),98(20),56(15),81(10).

sabinene:
136[M $^{+}$]: 93(100),77(24),91(20),79(17),41(20),137(10),94(10),69(10).

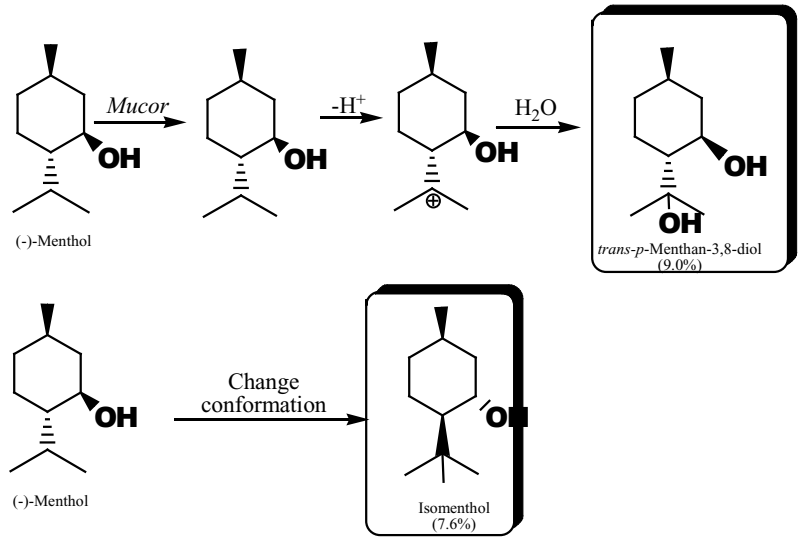
p-menthane-3,8-diol:
172[M $^{+}$]: 81(100),59(73),96(44),43(26),54(22),67(15),139(13),154(10).

isomenthol:
156[M $^{+}$]: 71(100),81(56),95(58),41(43),55(36),43(34),70(30),56(20).

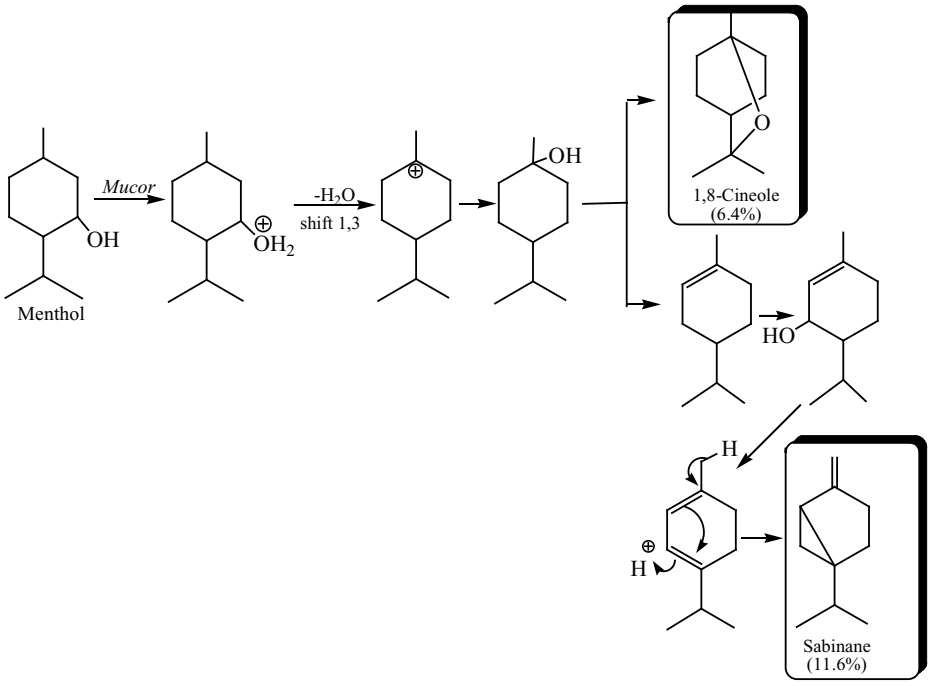
1,8-cineole:
154[M $^{+}$]: 43(100),41(30),81(30),71(23),40(20),27(15),55(13),69(25).



Scheme 1. Biosynthesis of (–)–Menthol to *trans-p*-Menthan-8-ol and *trans*-Menthan-2-en-1-ol by *Mucor*



Scheme 2. Biosynthesis of (–)–Menthol to *trans-p*-Menthan-3,8-diol and Isomenthol by *Mucor*



Scheme 3. Biosynthesis of Menthol to sabinene and 1,8-cineole by *Mucor*

CONCLUSION

1. In this experiment, the biotransformation of (–)–menthol by *Mucor ramannianus* was studied. It was carried out with sporulated surface cultures of *Mucor ramannianus*.
2. The main components of the essential oil were (–)–menthol were *trans-p*-menthan-8-ol, *trans*-menth-2-en-1-ol, sabinane, *p*-menthane-3,8-diol, isomenthol, and 1,8-cineole, also resulting in higher yields.
3. The pathways involved in the biotransformation of (–)–menthol by all main products are also discussed.
4. Main products (93.6%) found *Mucor ramannianus* as a biocatalyst and biotransformation of (–)–menthol was investigated.

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BIOTRANSFORMACJA (–)–MENTOLU ZA POMOCĄ ZARODNIKÓW *MUCOR RAMANNIANUS* ORAZ BADANIA SZLAKÓW METABOLICZNYCH

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S t r e s z c z e n i e

Metabolizm biotransformacji (–)–mentolu badano przy użyciu grzyba pleśniowego *Mucor ramannianus*. Doświadczenie przeprowadzono na podłożu sprzyjającym zarodnikowaniu *Mucor ramannianus*. Podstawowymi produktami metabolizmu otrzymanymi z (–)–mentolu były: *trans-p-*

mentan-8-ol, *trans*-ment-2-en-1-ol, sabinan, *p*-mentan-3,8-diol, izomentol, i 1,8-cineol. Badania prowadzono na stałym podłożu Sabouraud Dextrose Agar (SDA) w płytkach Petriego. Efekty biotransformacji (–)–mentolu pod wpływem zarodnikującego szczepu *M. ramannianus* oceniano po tygodniu inkubacji. W trakcie biotransformacji (–)–mentolu powstało 6 składników, które stanowiły 93,6% całego substratu. Ponadto przedyskutowano drogę biotransformacji (–)–mentolu w następstwie rozwijającego się szczepu grzyba *M. ramannianus* do dwóch głównych produktów.

Słowa kluczowe: *biotransformacja, biokonwersja, Mucor ramannianus, grzyby, mentol*