

Microbial transformation of α -naphthol by *Aspergillus niger* – PTCC 5011

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S u m m a r y

The metabolism of α -naphthol by *Aspergillus niger* PTCC 5011, a fungi isolated in Ardebil, Iran, from industrial wastewater, was studied. *A. niger* metabolized approximately 80% of α -naphthol within 5 days. The identification and quantification of degradation products using GC-MS demonstrated that approximately 41% of the parent compound was converted into 1-ethyl-2-methyl benzene, 7.43% was converted into acetonephthone, 5.55% was transformed into 4-hydroxy-1-naphthyl sulfate, 3% into 1,4-naphthoquinone, and about 6.68% into 2-phenyl-1,2,3-tetrahydro-1-naphthol. These results support a role for *A. niger* in affecting the environmental fat of pollutants in ecosystems.

Key words: biotransformation, bioconversion, *Aspergillus niger*, α -naphthol, fungi

INTRODUCTION

The polycyclic aromatic hydrocarbon naphthalene is used in the production of phthalate plasticizers and resins, azo dyes, dispersants, and tanning agents in the rubber and leather industries [1]. Naphthol is a common bicyclic aromatic often released into the environment [2]. Naphthol is often liberated into the environment as a result of the oxidation of naphthalene by certain fungi and bacteria [2, 5, 6] and is particularly important both as a synthetic precursor of the

insecticide Sevin (1-naphthyl-N-methylcarbamate) and as a degradation product of this compound via chemical and biological processes [2, 5, 6, 8]. It has toxic effects in aquatic ecosystems, especially on marine invertebrates [15].

Due to its toxicity to marine life [8] and human beings [18, 21], industrial wastewater containing α -naphthol must be treated before it is discharged into or re-used in the environment [16]. Fungi play an important role in the metabolism of many chemicals, including aromatic hydrocarbons, in both aquatic and terrestrial environments [7, 14, 19, 21]. There are studies attesting to the ability of strains of different bacteria, e.g., *Brevibacterium* spp. HL 4, *Pseudomonas* spp. DLC-P11, *Arthrobacter sulphureus* RKJ4, *Acidovorax delafieldii* P4-1 [17], *Halosphaeria mediose-tigera*, *Culcitalna achraspora*, *Humicola alopallonella*, *Brevibacterium* spp., *Flavobacterium* spp., *Serratia marina*, *Spirillum* spp., *Candida parapsilosis*, *Rhodotorula glutinis*, *Trichosporon ferments*, *Aspergillus fumigatus* [22], *Micrococcus* spp. [18], *Heliscus lugdunensis* [7], and *Rhodococcus* spp. [2], to disintegrate α -naphthol and reports about isolation and examination of these fungal strains with respect to their α -naphthol tolerance. However, we are not aware of studies describing biotransformation of α -naphthol by *A. niger* PTCC 5011. This paper describes the ability of *A. niger* PTCC 5011 to transform this compound and the identification of pathways involved in the biotransformation of the resultant metabolites. In a previous study, microbial transformation of some monoterpenes (citral, geraniol, nerol, menthol, and myrcene) by various fungi, including *Aspergillus niger*, *Pseudomonas* sp., and *Penicillium* sp., produced a number of compounds, such as 1,8-cineole, 2,6-dimethyl-octane, α -pinene, α -terpineol, cis-p-menthan-7-ol, dihydrolinalool, γ -terpinene linalool, limonene, *p*-cymene, *p*-menth-1-ene, sabinene, and *trans-p*-menthan-1-ol [24-31].

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade (gradient grade in the case of chromatography solvents). α -Naphthol was purchased from Panreac Química S.A.U. (PS) of Barcelona, Spain (>99% purity). Tween 80 was provided by the Department of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran. Czapek-Dox broth (Czapek's medium) was purchased from Difco (Detroit, Michigan, USA). All other chemicals were purchased from Merck (Darmstadt, Germany).

Microorganism

A strain of *Aspergillus niger* was isolated from the wastewater of an industrial plant in Ardebil, and was identified according to its physiological and morphological

characteristics. *Aspergillus niger* (PTCC 5011) was identified according to the Persian Type Culture Collection, Iranian Research Organization for Science & Technology, Tehran, Iran. The fungus mycelia was stored on agar slants at 4°C.

Culture condition

Spores and mycelia (2×10^8 conidia/ml medium) were aseptically inoculated into 250 ml Erlenmeyer flasks containing 100 ml of Czapek-Dox broth. The Czapek's medium (in g L⁻¹ distilled water) consisted of bacto saccharose, 30.0; sodium nitrate (NaNO₃), 3.0; dipotassium phosphate (K₂HPO₄), 1.0; magnesium sulfate (MgSO₄), 0.5; potassium chloride (KCl), 0.5; and ferrous sulfate (FeSO₄·7H₂O), 0.01. The flasks were incubated for 48 h at 30°C on a rotary shaker at 150 rpm in the dark.

ANALYTICAL METHODS AND DEGRADATION EXPERIMENTS

Isolation, detection, and identification of metabolite

To identify α -naphthol and metabolites, the fungus was cultivated in 250 ml Erlenmeyer flasks containing 100 ml of Czapek-Dox medium and inoculated with 2×10^8 conidia/ml medium. The α -naphthol (7.5 mg) was dissolved in 375 μ l of ethyl acetate, with Tween 80 (400 mg L⁻¹) added to one flask after 24 h of incubation in darkness. Five days after the addition of α -naphthol, the suspension was extracted three times, consecutively, with ethyl acetate. The organic extracts were combined after the separation of organic and water phases. Afterwards, the organic extracts were concentrated at 50°C by means of a rotary evaporator to about 5 ml [16]. Residues (5 ml) were examined for α -naphthol and acetate-extractable transformation products by GC and GC-MS. Abiotic controls (without microorganism) were always included.

Analysis of the sample with GC-MS

The samples were analyzed using a Hewlett-Packard 6890 with a DB-5 capillary column (30 m \times 0.25 mm; film thickness 0.25 μ m) was programmed as follows: 60°C for 5 min and 220°C at a rate of 4°C/min. The flow rate to helium as a carrier gas with (2 ml/min) MS was taken at 70 eV.

RESULTS

Biotransformation of α -naphthol by *A. niger*

In this experiment, the biotransformation of α -naphthol by *A. niger* grown on Czapek' s medium for only 5 days was performed. After incubation, Czapek' s culture was extracted (see analytical methods and degradation experiments section). The suspension was extracted with ethyl acetate three consecutive times and directly analyzed by GC-MS (fig. 1). In these analyses, various chemicals were obtained (tab. 1). The main products obtained in the bioconversion of *A. niger* of α -naphthol were 1-ethyl-2-methyl benzene (41%) and acetonephthone (7.43%), respectively.

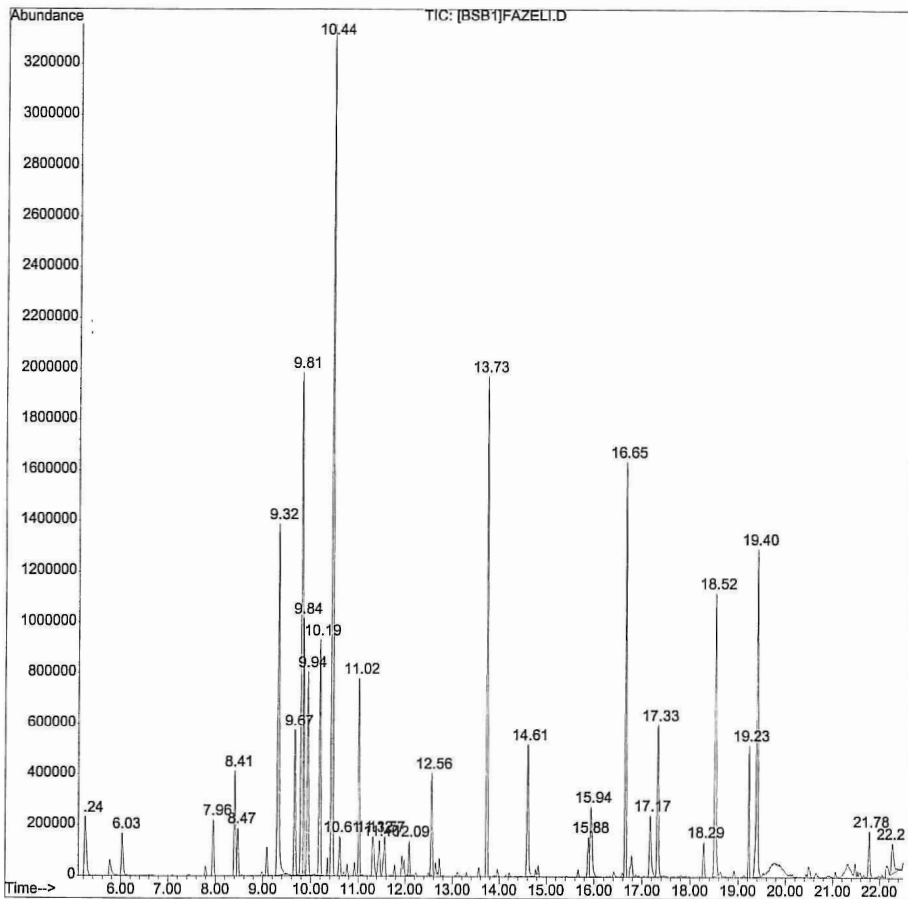


Figure 1.
Mass spectrum of biotransformation of α -naphthol by *A. niger*

Table 1.

Mass spectral data, retention times, and proposed identities of metabolites derived from α -naphthol after 5 days of incubation

| Proposed compound | Retention time (min) | Mass spectral ions, m/z (% of relative intensity) |
|--------------------------------------|----------------------|---|
| 1-Ethyl-2-methyl benzene | 10.45 | 105(100), 120(53.58), 119(13.51), 77(10.63), 91(9.27) |
| Acetonaphthone | 13.73 | 57.1(100), 43.1(72.28), 71.1(59.42), 85.1(35.96), 41.1(34.23) |
| 1,4-Naphthoquinone | 17.33 | 158(100), 102(45.43), 104(43.56), 130(33.49), 76(33.46) |
| 4-Hydroxy-1-naphthyl sulfate | 18.52 | 144(100), 115(80.27), 116(37.31), 145(10.85), 89(8.79) |
| 2-Phenyl-1,2,3-tetrahydro-1-naphthol | 19.40 | 105(100), 134(86.38), 77(31.34), 133(27.28), 120(23.89) |

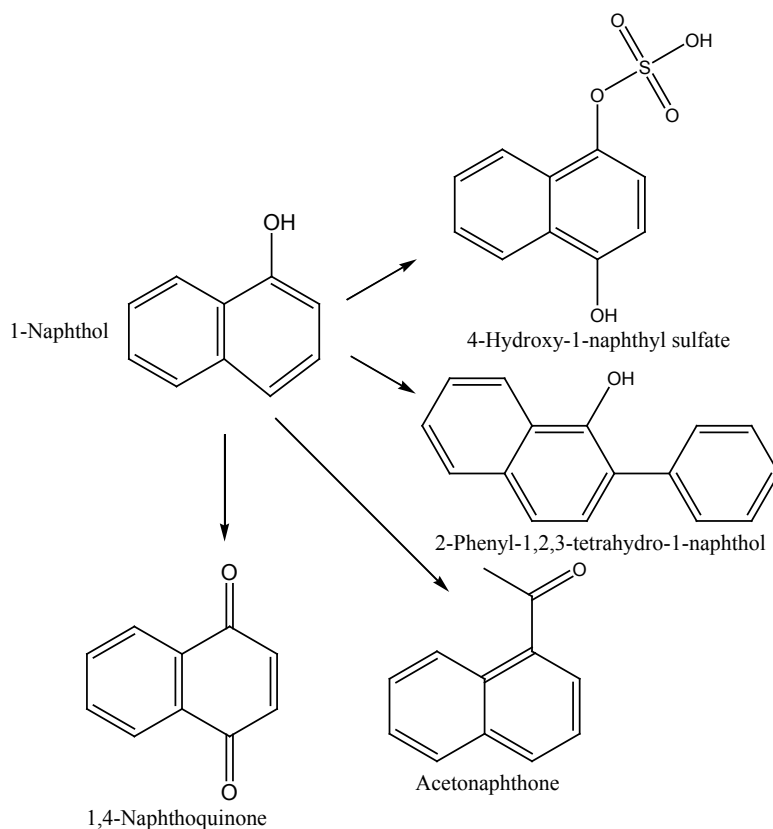


Figure 2.

Transformation of 1-naphthol by *A. niger*: 4-hydroxy-1-naphthylsulfate, 2-phenyl-1,2,3-tetrahydro-1-naphthol, 1,4-naphthoquinone and acetonaphthone were deduced from mass spectral data of 1-naphthol transformation products found in *A. niger* (tab. 1)

In a previous study of biotransformation of menthol by sporulated surface cultures of *A. niger* and *Penicillium* spp. the main bioconversion product obtained from menthol of *A. niger* was *cis-p*-menthan-7-ol, and the main products obtained by sporulated surface cultures of *Penicillium* spp. were limonene, *p*-cymene, and γ -terpinene [22]. Leuenberger reported that product yields could be effectively increased by solubilizing/emulsifying immiscible substrates [17]. However, careful selection of the nature and concentration of the solvent is necessary, because many miscible solvents are cytotoxic at lower concentrations [17]. Comparing the abovementioned investigation with the present study showed oxygenated monoterpenes to be the main compounds, with more monoterpenes yielded in the biotransformation. Figure 2 shows that transformation of α -naphthol by *A. niger* PTCC 5011 produces more sulphation products.

DISCUSSION

In previous research on biotransformation of α -naphthol by a strictly aquatic fungus studied the biotransformation of the environmental pollutant metabolite 1-naphthol has been studied. *H. lugdunensis* metabolized approximately 74% of 1-naphthol within 5 days [4]. The current research addressed the metabolism of α -naphthol by *A. niger* PTCC 5011, a coelomycete isolated in Ardebil, Iran, from industrial wastewater.

The study of microbial transformation of one monoterpene by sporulated surface cultures of *A. niger* and *Penicillium* sp. produced *cis-p*-menthan-7-ol from *A. niger*; the main products obtained were limonene, *p*-cymene, and γ -terpinene [11]. The two main products of microbial transformation of citral were similar to those obtained in former work. The main bioconversion products of (-)-menthol by *Mucor ramannianus* using the sporulated surface cultures method were *trans-p*-menthan-8-ol, *trans*-menth-2-en-1-ol, sabinane, *p*-menthane-3,8-diol, isomenthol, and 1,8-cineole [13].

The experimental work (the two latest articles) suggested that microbial transformation of monoterpenes with different genera: *Penicillium* and *Aspergillus* caused an oxidation reaction and resulted in a more stable product. Although, bioconversion by using *A. niger* PTCC 5011 showed that it was possible to obtain two or three main products of high percentage and selectivity.

CONCLUSION

In conclusion, reported literature and this research show bioconversion of α -naphthol by *A. niger* have been similarly effective for biotransformation. Oxidation and sulphation are two common characteristics in this investigation of bioconversion products.

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TRANSFORMACJA MIKROBIOLOGICZNA α -NAFTOLU PRZEZ *ASPERGILLUS NIGER* – PTCC 5011

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Streszczenie

Badano przetwarzanie α -naftolu za pomocą *Aspergillus niger* PTCC 5011, grzyba wyizolowanego ze ścieków przemysłowych pochodzących z Ardebil w Iranie. Kropidlak czarny metabolizował około 80% α -naftolu w ciągu pięciu dni. Identyfikacja i ocena jakościowa produktów degradacji za pomocą GC/MS wykazała, że około 41% związku macierzystego zostało przetworzone na 1-etyl-2-metylobenzen, 7,43% na acetonafeton, 5,55% zostało przekształcone w 4-hydrokso-1-naftylosulfat, 3% w naftochinon i około 6,68% w 2-fenyl-1,2,3-tetrahydro-1-naftol. Takie wyniki mogą pomóc znaleźć zastosowanie *A. niger* do regulacji wpływu zanieczyszczeń typu tłuszczowego, które istnieją w ekosystemach.

Słowa kluczowe: biotransformacja, biokonwersja, *Aspergillus niger*, α -naftol, grzyby