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Biotransformation of 1, 8-Cineole by *Penicillium citrinum* Isolated from Soil Sample in Jordan

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Abstract: Biological transformation of cheap and available compounds can be achieved by many microorganisms. Cineole (1, 8- cineole) forms more than 50% of the essential oil extracted from some medicinal plants in Jordan. This study was aimed to investigate the ability of locally isolated *Penicillium citrinum* to biotransform 1, 8- cineole to valuable products. Cineole was added to a five days old *P. citrinum* culture to get 0.1 % final concentration and incubated for two weeks with shaking. The culture was extracted with diethyl ether the extract was subjected to gas chromatography - mass spectrometric (GC-MS) analysis. The result showed that two new products were identified as Exo-2-Hydroxycineole (1, 3, 3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol) and hydroxy-1, 8-cineole (cis-1, 3, 3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-ol) using mass spectral comparison database libraries.

Key words: Biotransformation · 1 · 8-Cineole · *Penicillium citrinum* · Exo-2-Hydroxycineole

INTRODUCTION

Cineole (1, 3, 3-trimethyl-oxabicyclo [2.2.2] octane) also known as eucalyptol or 1, 8 cineole is a monoterpene that presents in the essential oils of different aromatic medicinal plants species belongs to *Lamiaceae* family in Jordan such as: *Salvia officinalis* (60%) [1] and *Rosmarinus officinalis* L. (31 %) [2]. It contributes in the biological activity of these essential oils [3].

The transformation of the cheap and available raw material such as 1, 8- cineole can be achieved by chemical [4] and biological process that known as biotransformation [5]. The advantages of biotransformation over chemical transformation include production of pure naturally labeled products that may be safely used in food industry [6]. Biotransformation provides important synthetic way for the production of valuable natural compounds that cannot be produced using economically available procedures [7].

Cineole biotransformation by different microorganisms leads to the production of different products. The result of García *et al.* [5] study showed that 2-exo-hydroxy-1, 8-cineole is form 51% of the biotransformation product when *Aspergillus terreus* was used for biooxidation of 1, 8-cineole. While, transformed 1, 8-cineole was converted to hydroxyl-cineole when *Pseudomonas flava* was used in the biotransformation process[8]. Among many species that have the ability to biotransform naturally occurring compounds fungi are in the point of view to be used in biotransformation process due to their versatile metabolic pathways [9].

In this work the biotransformation of 1, 8- cineole using a locally *Penicillium citrinum* isolated from soil from beneath *R. officinalis* was presented.

MATERIALS AND METHODS

Microorganisms: *P. citrinum* was isolated from the soil where *R. officinalis* was cultivated. Serial dilutions soil sample were made and 1 ml of each dilution (10^{-1} to 10^{-5}) was transferred to Potato Dextrose Agar (PDA) plates containing 1% streptomycin. The plates were incubated at 28°C in the dark. The plates were observed for five days. The isolated strain was identified according to morphological features using macroscopic colony morphology and microscopic examination of conidia. Extraction of genomic DNA from fungus was conducted using DNeasy Plant Mini Kit (Supplied by QIAGEN). The DNA was purified using the QIA quick PCR purification kit and amplified by PCR using internal transcribed spacer ITS1F and ITS4 primers. The PCR products were visualized after electrophoresis and sent for sequencing

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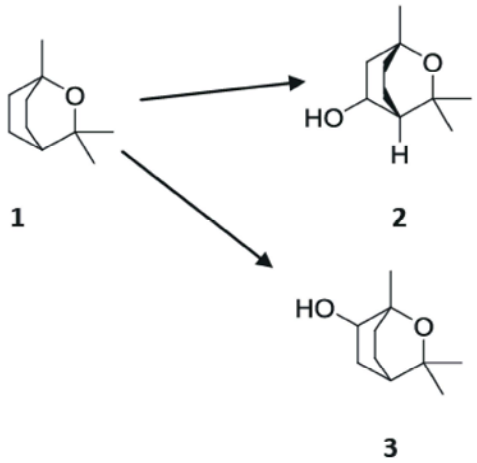
at (Princess Haya Biotechnology Center – Jordan University of Science and Technology). The obtained sequences were compared with the other related sequences in Gene Bank (NCBI- BLAST) [10].

Biotransformation of 1, 8 Cineole: The biotransformation experiment was conducted using spore suspension and active culture method according to the method of Esmaeili and Tavassoli [11] with some modification. The Spores suspension was recovered from 1-week-old surface cultures of *P. citrinum* grown in PDA slants. Spore suspension was prepared by adding 25 ml of 1 % sterile Tween 80 solution in distilled water. A 250 ml Erlenmeyer flask containing 100 ml potato dextrose broth (PDB) was inoculated with a 5 ml of spore suspension of *P. citrinum* (5×10^6 spore / ml) and incubated at 25°C for five days on a shaker at 150 rpm. After that 1 ml of a solution of 5 % 1, 8-cineole in absolute ethanol was added. After 14 days this culture was extracted with 3 × 50 ml diethyl ether and the products were analyzed by gas chromatography – Mass spectrophotometry (GC-MS).

Analysis of Samples Using Gas Chromatography-Mass Spectrometry (GC-MS): The percentages and ratio of produced compounds were determined by using Gas Chromatography Mass Spectrometry (GCMS). Varian chrompack CP-3800 GC/MS/MS-200 equipped with split/splitless injector and DB-5 GC column (5% diphenyl 95% dimethyl polysiloxane), (30 m × 0.25 mm ID, 0.25 µm film thickness) was used. The injector temperature was set at 250 C° with a split ratio of 1:10. Detector and transfer-line temperatures were 160 °C and 230 °C respectively. A linear temperature program was used to separate the different oil components [12].

RESULTS

The biotransformation of 1, 8-cineole by *P. citrinum* Isolated from soil sample was assessed and the result (Scheme 1) showed that two main products were identified using GC- MS. The chromatograph showed the presence of two new peaks for the extracted compound from the culture comparing the only one peak when for 1, 8- cineole chromatograph. These two derivatives of 1, 8- cineole 1 were identified as 3- hydroxy-1, 8-cineole (cis-1, 3, 3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-ol) 2 and Exo-2-Hydroxycineole (1, 3, 3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol) 3 using mass spectral comparison database libraries.



Scheme 1: Products result from biotransformation of 1, 8-cineole (1): 3- hydroxy-1, 8-cineole (2) and Exo-2-Hydroxycineole (3)

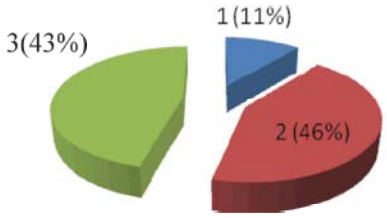


Fig. 1: Ratio of biotransformation products (%)

Fig. 1 shows the ratio of identified compound after the biotransformation process. The result showed that 89% of 1 was biologically transformed by *P. citrinum*. The ratios of produced derivatives 2 and 3 were 46 and 43% respectively while 11% of the starting compound 1 remained untransformed.

DISCUSSION

The isolated fungus was identified as *P. citrinum* using morphological characteristic and rDNA sequencing. The analysis of internal transcribed spacer (ITS) rDNA sequence showed the 97 % identity between the isolated fungi and *P. citrinum*.

The biotransformation of naturally occurring molecule is a useful process that leads to the generation of valuable compound. Cineole is one of the naturally occurring cheap compounds that identified and isolated from the essential oils of different aromatic medicinal plants. Many studies were conducted to investigate the biotransformation of cineole using different types of bacteria and fungi. Different types of bacteria that have the ability to transform 1, 8- cineole have been reported in

literature such as: *Pseudomonas flava* [8] *Bacillus cereus*[13] *Novosphingobium subterraneum* [14] and *Rhodococcus* sp.[15]. On other hand, different fungal species were investigated for their ability to transform cineole, these fungi include *Mucor ramannianus* and *Aspergillus niger* [5, 14] *Penicillium purpurogenum* [15].

The result of this study revealed that *P. citrinum* convert 1 to two derivatives that were identified as 2 and 3. These hydroxlated compounds are generated by introducing oxygen in non- activated carbon of 1 that catalyzed by members of cytochromes P450 which is responsible for the hydroxylation of 1 by *B. cereus* [13] and *Citrobacter baraakii* [16]. Rasmusen *et al.* [17] reported that *Penicillium* sp. transform of 1 to only the 3-oxygenated derivatives.

Previous studies reported the bioconversion of 1 in several pathways, the result of Carman *et al.* [8] showed that *P. flava* transforms 1 to Hydroxy-cineole. *Bacillus cereus* catalyses the transformation of 1 by cytochrome P450 monooxygenase to either 2R-endo- or 2R-exo-hydroxy-1, 8-cineole [13]. Hawkes *et al.* [16] reported that P450 monooxygenase isolated from *C. braakii* yielded 2-endo-hydroxy-1, 8-cineole only from 1, 8- cineole. García *et al.* [5] used *A. niger* in the biooxidation process of 1, the result revealed that following four derivatives were identified:2-exo-hydroxy-1, 8-cineole, 2-endohydroxy-1, 8-cineole, 3-exo-hydroxy-1, 8-cineole and 3-endo-hydroxy-1, 8-cineole.

P. citrinum was isolated from soil samples collected from *R. officinalis* cultivation site. Hudaib *et al.* [2] reported that cineole forms 31% of *R. officinalis* essential oil.

CONCLUSIONS

This study reported the ability of *P. citrinum* to transform 1, 8-cineole into two hydroxylated derivatives: Exo-2-Hydroxycineole (1, 3, 3-Trimethyl-2-oxabicyclo[2.2.2] octan-6-ol) and 3- hydroxy-1, 8-cineole (cis-1, 3, 3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-ol).

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REFERENCES

1. Abu Darwish, M., E. AL-Ramamneh, I. Salamon, Z. Abu-Dieyeh, M. Al Nawaiseh, T. Albdour, 2013. Determination of Essential Oil Bioactive Components and Rosmarinic Acid of *Salvia officinalis* Cultivated under Different Intra-row Spacing Notulae Scientia Biologicae; Cluj-Napoca, 5(2): 198.
2. Hudaib, M.M., K. Tawaha, H.S. Hudaib and A.H. Battah, 2015. Chemical Composition of Volatile Oil from the Aerial Parts of *Rosmarinus officinalis* L. Grown in Jordan, Journal of Essential Oil Bearing Plants, 18(5): 1282-1286. DOI: 10.1080/0972060X.2014.895188.
3. Radulescu, V., S. Chiliment and E. Oprea, 2004. Capillary gas chromatography-mass spectrometry of volatile and semi-volatile compounds of Salvia officinalis. Journal of Chrom., 1027(1-2): 121-126. https://doi.org/10.1016/j.chroma.2003.11.046.
4. Carman, R.M., W.T. Robinson and C.J. Wallis, 2005. The 3-hydroxycineoles, Aust. J. Chem., 58: 785-791.
5. García, C., P. Rodríguez, E. Días, H. Heinzen and P. Menéndez, 2009. Biooxidation of 1, 8-cineole by Aspergillus terreus. Journal of Molecular Catalysis B: Enzymatic, 59: 173-176. https://doi.org/10.1016/j.molcatb.2009.02.013.
6. Menéndez, M.P., C. García and H. Heinzen, 2005. in: A.F. Barrero (Ed.), Biotransformacinde terpenos, con especial referencia amonoterpenos y los aspectosmetodolgicosde la biotransformacin con microorganismos, Programa CYTED, Granada, Espa˜na, pp: 149-172.
7. Ohlo, V.G., 1994. Scent and fragrances. The fascination of odors and their chemical perspectives. Springer, Berlin.
8. Carman, R.M., I.C. Macrae and M.V. Perkins, 1986. Theoxidationof1, 8- cineole by Pseudomonas flava. Aust. J. Chem., 39: 1739-1746. doi:10.1071/CH9861739
9. Onken, J. and R.G. Berger, 1999. Effects of R-(+)-limonene on submerged cultures of the terpene transforming basidiomycete Pleurotus sapidus. J Biotechnol., 69: 163-168. doi:10.1016/S0168-1656(99)00040-1.
10. Javadi, M.A., M.A.T. Ghanbary and Z.Tazick, 2012. Isolation and Molecular Identification of Soil Inhabitant Penicillia. Annals of Bio. Res., 3: 5758-5761.

11. Esmaeili, A. and A. Tavassoli, 2015. Microbial transformation of citral by Penicillium sp. Acta Biochim Pol., 57: 265-268.
12. Alsohaili S.A. and A.T. Al-fawwaz, 2014. Composition and antimicrobial activity of *Achillea fragrantissima* essential oils using food model media. European Scientific Journal, 10(30): 156-165.
13. Liu, W.G. and J.P.N. Rosazza, 1990. Stereospecific hydroxylation of 1, 8-cineole using a microbial biocatalyst. Tetrahedron Lett., 31: 2833-2836. doi: 10.1016/0040-4039(90)80160-N.
14. Ramos, A.D., J.B. Ribeiro, J.L.B. Ferreira, R.O.M. Alves de Souza and A.C.F. Amaral, 2015. Hydroxylation of 1, 8-cineole by *Mucor ramannianus* and *Aspergillus niger*. Brazilian Journal of Microbiology, 46(1): 261-264. DOI: http://dx.doi.org/10.1590/S1517-838246120131020.
15. Williams, D.R., P.W. Trudgill and D.G. Taylor, 1989. Metabolism of 1, 8-Cineole by a Rhodococcus Species: Ring Cleavage Reactions. Journal of General Microbiology, 135: 1957-1967.
16. Hawkes, D.B., G.W. Adams, A.L. Burlingame, P.R.O. De Montellano and J.J. De Voss, 2002. Cytochrome P450cin (CYP176A), isolation, expression and characterization. J. Biol. Chem., 277: 27725-27732. doi: 10.1074/jbc.M203382200.
17. Rasmussen, J.M., K.A. Henderson, M.J. Straffon, G.J. Dumsday, J. Coulton and M.Z achariou, 2005. Two new biocatalysts for improved biological oxidation of 1, 8-cineole. Australian Journal of Chemistry, 58(12): 912-916.