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Prediction of Geraniol Bond Mode in *Aspergillus niger* Linalool Dehydratase – Isomerase

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ABSTRACT

Geraniol is a very valuable aroma chemical and has wide use in fragrances and aroma compound. Geraniol biotransformation by *Aspergillus niger* has been studied. The main bioconversion products obtained from geraniol and liquid culture of *A. niger* are linalool and alpha-terpineol. Linalool plays a major role in anti-inflammatory, antibacterial and antioxidant activities. This study aims to know the interaction of geraniol in *Aspergillus niger* enzyme with docking molecular. Comparative modelling of *Aspergillus niger* enzyme was conducted by means of one of the crystal structures of Linalool Dehydratase – Idomerase (LDI) as a template. The best model of this comparative modelling was then used in docking molecular to investigate geraniol binding mode inactive site enzyme of *Aspergillus niger.* Inactive site enzyme of *Aspergillus niger,* geraniol is located with hydrophobic and hydrogen bonds: Amino acid – the amino acids are Asn 105, Arg 96, Lys 112 inactive site - OH with hydrogen bond, Arg 97 inactive site – CH3 with hydrophobic bond and Leu54 inactive site – CH3 with the hydrophobic bond. The distances among pharmacophore respectively are 3,603 A, 6,768 A and 7,345A. It has higher score (ΔG_{bind}: -3.4 kcal/mol) compared to linalool (ΔG_{bind}: -3.6 kcal/mol). Virtual tethering of linalool with LDI *Aspergillus niger* enzyme in amino acid Leu120 and Glu118 had been done. The pharmacophore are– OH and methyl C_8 group. The distances among pharmacophore respectively are 5,835 Å, 2,52 Å and 5,32 Å. Virtual tethering of LDI *Aspergillus niger* enzyme with geraniol has a higher score (ΔG_{bind}: -3.4 kcal/mol) compared to linalool (ΔG_{bind}: -3.6 kcal/mol). It shows that interaction between linalool and LDI *Aspergillus niger* enzyme is easier to occur than the interaction between geraniol and LDI *Aspergillus niger* enzyme, geraniol reaction to linalool that occurs is rearrangement reaction.

Key words: *Aspergillus niger*, Docking molecular, Geraniol, Linalool Dehydratase-Isomerase, Comparative modelling.

INTRODUCTION

Geraniol is a very valuable aroma chemical and has wide use in fragrances and aroma compound. There are three sources producing this chemical aroma. Palmarosa Oil *Cymbopogon martini* commonly known as Rosha' or Russa is the main source of geraniol (80 – 95%) and Jamrosa oil contains 80-89%. Other geraniol sources are *Cymbogopon winterianus* (Javanese lemongrass oil) containing 40-45% including citronellol. (Akbar N. Saxena B. K, 2009) Biotransformation of geraniol, nerol and citral by *Aspergillus niger* has been studied. Main bioconversion product obtained from geraniol and nerol with liquid culture *A. Niger* are linalool and alpha-terpineol. (Demyttenaere, Del Carmen Herrera and De Kimpe, 2000) Linalool plays a major role in anti-inflammatory activities. (Panin *et al.*, 2002) Antibacterial and antioxidant activities of α-terpineol, linalool, eucalyptol and α-pinene obtained from essential oil, against pathogenic forming bacteria and decomposers have also been determined. Bacterial activities of this compound are observed in vitro on four Gram-negative and three Gram-positive strains. *S. putrefaciens* is the most resistant bacteria of all tested components with the value of MIC 2% or higher, whereas *E. coli* O157: H7 is the most sensitive strain among the tested bacteria. Eucalyptol extends lag phase of *S. typhimurium, E coli*)157: H7 and *S. aureus* at 0.7, 0.6% and 1% concentrations respectively. (Zengin and Baysal, 2014)

Recognition of microbial biotransformation as an important manufacturing tool has increased in the chemical and pharmaceutical industries.

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Biotransformation can be clarified as a specific modification of certain compound to different products with structural similarity, by means of biological catalysis including microorganism. Biological catalysis can be described as an enzyme or whole microorganism. (Hegazy *et al.*, 2015)

Aspergillus niger is a famous fungus that has been used for various organic compound biotransformations. *A. niger* adds hydroxyl, carbonyl and other groups in a certain position or reduces double bonds to produce new valuable compounds. (Parshikov and Sutherland, 2014) *Aspergillus niger* is a broad aerobic fungus that has been used for various biotransformation. Monoterpene that is commonly used in the pharmaceutical product is containing two terpene molecules; isoprene units and derivatives. (Hu *et al.*, 2017)

Biotransformation of (±) –linalool with submerged *Aspergillus niger* culture particularly *A. niger* ATCC 9142 produces a mixture of linalool oxide cis and trans-furanoid (15-24%) and cis- and trans- pyranoid linalool oxide (5-9%). Biotransformation of (R) – (-) – linalool with the same strains produces almost pure linalool oxide trans-furanoid and trans-pyranoid (ee> 95). This conversion is purely biocatalytic since in acidified water (pH <3.5), almost 50% linalool is regained unchanged, the remaining is lost by evaporation. (Demyttenaere and Willemen, 1998) This *A. niger* strain can only convert (-) beta-pinene to alpha-terpineol (4%). (Toniazzo *et al.*, 2005)

A. niger produces several extracellular enzymes for significant industrial purposes, including amylase, protease, pectinase, lipase and chitin. (Raper KB, 1965) (Shubakov AA, 2002) *A. niger* also degrades cellulose and hemicelluloses (Raper KB, 1965) (Ademark P, Varga A, Medve J, Harjunpää V, Drakenberg T, Tjerneld F, 1998) and results in biodeterioration of lubricant derived from oil, (Yemashova NA, Murygina VP, Zhukov DV, Zakharyantz AA, Gladchenko MA, Appanna V, 2007) polyvinyl chloride and starch plastic/polyethylene. (Shah AA, Hasan F, Hameed A, 2008) The ability of *A. niger* culture to produce acid citrate and vitamin, oxalate, gluconate, fumarate and gallate including biotin, thiamin and riboflavin is widely used in the industry. (Raper KB, 1965) Because of its capability, *A. niger* has become the most often used fungi as a catalyst for biotransformation of different organic compounds. (Borges KB, Borges WdS, Durán-Patrón R, Pupo MT, Bonato PS, 2009) (Ward OP, Qin WM, Dhanjoon J, Ye J, 2006)

Castellaniella (ex Alcaligenes) defragans strain 65 mineralizes monoterpene without oxygen. Anaerobic soluble cell extract catalyzes geraniol isomerization into linalool and linalool dehydration into myrcene. Linalool dehydratase catalyzes two-reaction in vitro in both directions depending on thermodynamic driving force: water separation from linalool tertiary alcohol to suitable acyclic monoterpene myrcene and isomerization of primary geraniol alyl alcohol in linalool steinaloisomer. (Brodkorb *et al.*, 2010) Linalool dehydratase-isomerase of Castellaniella defragans 65 strain catalyzes in thermodynamic direction of β-myrcene hydration to linalool and then isomerization to geraniol, initial steps in anaerobic β-myrcene biodegradation. (Lüddeke and Harder, 2011)

This study aims to investigate geraniol interaction in the active site of *Aspergillus niger* enzyme with docking molecular; however, LDI crystal structure in *Aspergillus niger* which is not available is required.

METHODOLOGY

The main tool for comparative modelling is hardware in the form of ASUS brand laptop. In addition to such a tool, the software is also used: SWISS-MODEL (https://swissmodel.expassy.org). The tool for virtual tethering of geraniol with *Aspergillus niger* model enzyme is similar to the one that is used for comparative modelling of LDI enzyme. In addition to such a tool, software of OpenBabel version 2.3.2, Discovery Studio version 4.1, and Pyrex version 0.8 are also used.

The material for comparative modelling of this LDI enzyme is an amino acid sequence of *Aspergillus niger* downloaded from Uniprot site (https://www.uniprot.org/) (amino acid sequence: A0A117DZX7) and complex crystal structure of Linalool Dehydrotase Isomerase enzyme with resolution 2.10 Å downloaded from RSCB site (https://www.rcsb.org/) (crystal structural code: 5i3t). Material for virtual tethering of geraniol and LDI *Aspergillus niger* is *Aspergillus niger* enzyme structure, the output of above comparative modelling and substrate structure (geraniol). Geraniol structure is downloaded from Pubchem site (https://pubchem.ncbi.nlm.nih.gov) (Code CID-637566)

Comparative Modeling Procedure of LDI Enzyme

At first, the alignment of the amino acid sequence of *Aspergillus niger* enzyme to the amino acid sequence of each chain from LDI enzyme crystal structure with Swiss-Model software is conducted automatically. (https://sissmodel. expasy.org/interactive/14Mw28/templates/).

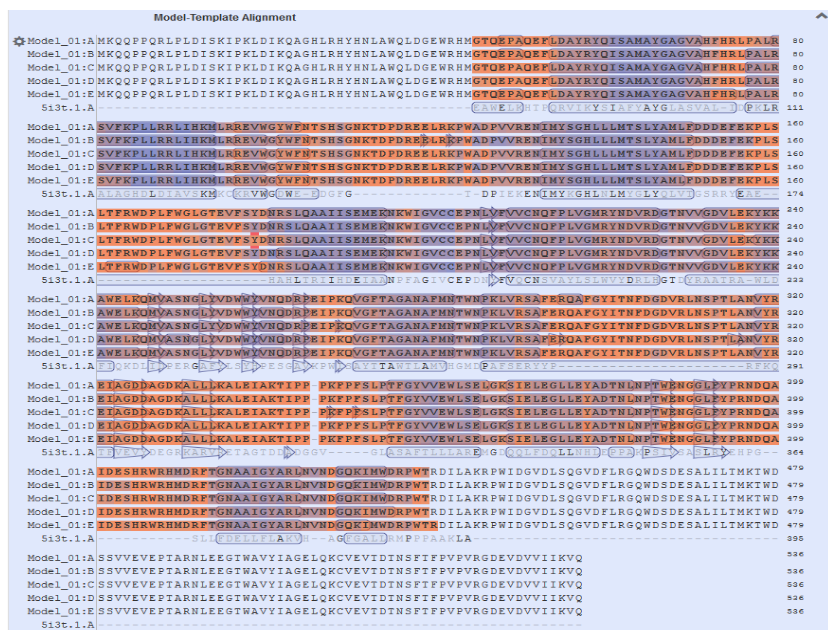


Figure 1. Alignment of Amino Acid sequence of *Aspergillus niger* Enzyme (Query: > 400 Amino Acid)

Comparative modelling of *Aspergillus niger* enzyme was then carried out based on the LDI enzyme chain.

Virtual Tethering Procedure of Geraniol and LDI *Aspergillus niger*

Geraniol structure and LDI *Aspergillus niger* enzyme are converted into GDP with Open Babel software and then manually checked with Discovery Studio software.

Virtual tethering of geraniol in *Aspergillus niger* LDI enzyme is conducted automatically with Pyrex software with the original parameter (default). This virtual tethering produces 8 poses of geraniol tethering with LDI *A. niger* enzyme as well as their tethering energy. The pose of geraniol virtual tethering with LDI *A. niger* enzyme with the most negative tethering energy value is then analyzed by the interaction pattern with LDI enzyme with Discovery studio software. From the analysis, geraniol virtual interaction with LDI *Aspergillus niger* enzyme is obtained.

RESULT AND DISCUSSION

Comparative Modeling of *Aspergillus niger* Enzyme

Crystal structure of LDI enzyme is selected as a reference for comparative modelling of *Aspergillus niger* with respect to Sharper resolution: ≤ 3Å, the most coverage among another closing to 1 and higher similarity.

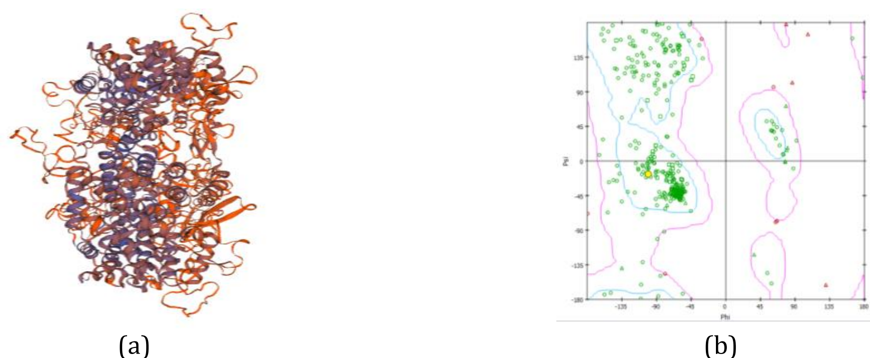
Crystal structure of LDI enzyme consists of 5 strains (A-E). In Figure 1, it is seen that more than 80% *Aspergillus niger* enzyme amino acid can line with acid – the same amino acid (conserved) in LDI enzyme. The high similarity between the amino acid sequence of *Aspergillus niger* enzyme and amino acid sequence of the LDI enzyme enables to be obtained the relatively accurate *Aspergillus niger* enzyme model.

The alignment result of *Aspergillus niger* enzyme model that has been optimized for LDI enzyme is shown in the following (Figure 2).

From Figure 2 it can be seen that the amino acids in the active site, namely Asn 105, Arg 96, Lys 112 and Leu 54 are not in the disallow region. And only 5% are in the disallow region.

Virtual Tethering of Geraniol and *Aspergillus niger* Enzyme

Through analysis of virtual tethering poses, several amino acids of *Aspergillus niger* enzyme interacting with geraniol are found: Asn105, Lys112, Arg96, Leu54, Arg97. The pharmacophore is OH, methyl C_3 and methyl C_8 group. The distances among pharmacophore respectively are 3,603 Å, 6,768 Å and 7,345 Å. Leu54 enzyme holds geraniol tip so that it does not shift far and stable. If geraniol is held steady in enzyme active site (restrained), geraniol has a chance to react. Catalytic amino acids such as Asn105, Lys112, Arg96 assist the reaction process because they



(a) (b)

Figure 2. (a) Model alignment of *Aspergillus niger* Enzyme (red) against LDI enzyme (green); (b) Ramachandran Map of *Aspergillus niger* Enzyme Model

Figure 3. Virtual Attachment Pose of *Aspergillus niger* Enzyme and Geraniol

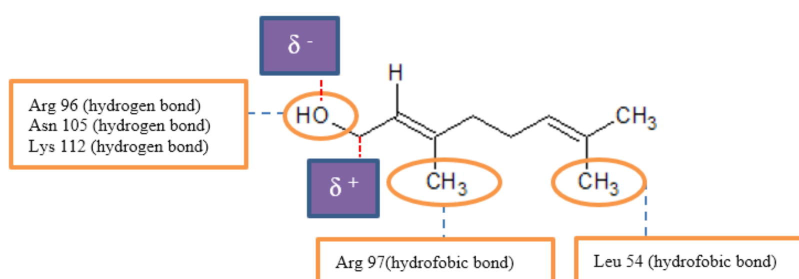


Figure 4. Prediction of geraniol and LDI *Aspergillus niger* bond

interact with OH (hydrogen bond) group. The interaction that occurs is an attractive trade-off bond, not a covalent bond. From geraniol virtual tethering in *Aspergillus niger* enzyme model, geraniol pose with the most negative value of ΔG_{bind}:-3.4 Kcal/mol) (Figure 3). In such pose: OH group faces Asn 105, Arg 96, Lys 112 enzymes with hydrogen bond, Methyl faces Leu54 enzyme with the hydrophobic bond. The double bonds face Arg 97 enzyme with a hydrophobic bond (Figure 4). Thus, the geraniol obtained inaccurate virtual tethering pose.

The possibility of the mechanism of geraniol change reaction to linalool is rearrangement reaction. The orientation of such OH group supports geraniol rearrangement reaction to linalool through the mechanism of rearrangement action. (Figure 5). OH is the good leaving group. OH is drawn by 3 amino acids at once so that OH is easy to escape and then a rearrangement reaction takes place. This is supported by the result of a study reported by Cori *et al.,* (Cori *et al.*, 1986)

In common synthesis, the acid ambience is required as H^+ donor to protonize OH. OH captures

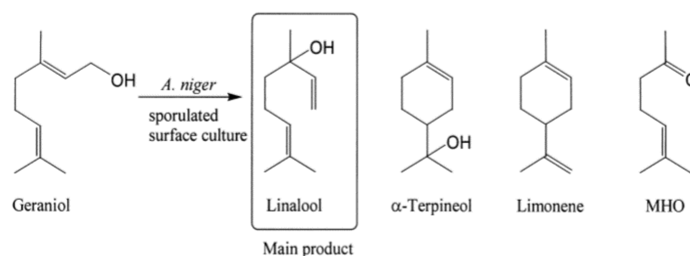


Figure 5. Biotransformation of Geraniol with *Aspergillus niger* (Demyttenaere *et al.*, 2000)

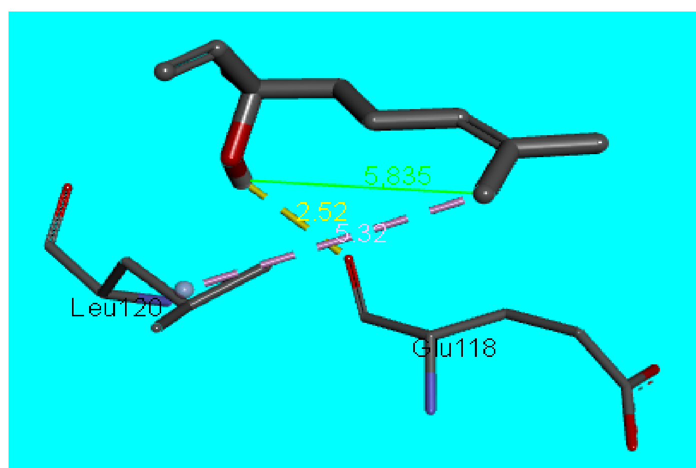


Figure 6. Virtual tethering pose of *Aspergillus niger* enzyme and Linalool

Figure 7. Interaction between Linalool and Geraniol in LDI *Aspergillus niger* LDI

captures H^{+} tp OH_2^{+}. Simultaneously OH is released, the double bond is displaced, OH occupies position C in the previous double bond. Takeo *et al.,* (1991) reported the transformation rate from geraniol to linalool depending on pH and transformation rate is proportional to the concentration of hydrogen (H:) in shochu model solution. (Cori *et al.*, 1986) (Takeo Ohta, Yuzo Morimitsu, Yoshihiro Sameshima, 1991)

Virtual tethering of linalool with LDI *Aspergillus niger* enzyme in amino acids Leu120 and Glu 118. The pharmacophore is OH and methyl, C_8 group. The distances among pharmacy respectively are 5,835 Å, 2,52 Å and 5,32 Å.

Virtual tethering of LDI *Aspergillus niger* with linalool has the score of ΔG_{bind}: -3,6 Kcal/mol (higher than geraniol: ΔG_{bind}: -3,4 Kcal/mol). This shows that interaction between linalool and LDI *Aspergillus niger* enzyme is easier than the interaction between geraniol and LDI *Aspergillus niger* enzyme since it releases higher energy which is similar to what was reported by Brodkorb D *et al.,* (2010). (17)

Figure 7 above shows that the interaction between geraniol and linalool with LDI *Aspergillus niger* LDI occurs in different positions.

CONCLUSION

Virtual tethering of LDI *Aspergillus niger* enzyme with geraniol has a higher score (ΔG_{bind}: -3,4 Kcal/mol) compared to linalool (ΔG_{bind}: -3,6 Kcal/mol). This shows that interaction between linalool and LDI *Aspergillus niger* enzyme is easier to occur compared to the interaction between geraniol and LDI *Aspergillus niger* enzyme since it releases higher energy. The occurring reaction geraniol to linalool is rearrangement reaction.

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