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# MICROBIOLOGIA BALKANICA 2011

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### *8th CONGRESS OF SERBIAN MICROBIOLOGISTS*

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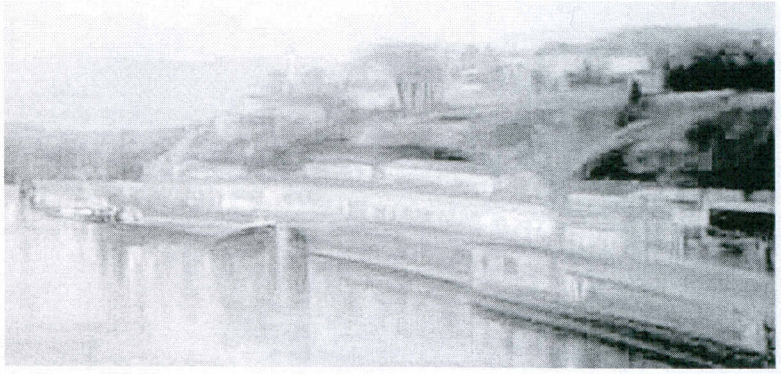

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# *Pseudomonas* lipase catalyzed synthesis of ergosterol oleate

Lidija Izrael-Živković[1], Gordana Gojgić-Cvijović[2], Maja Natić[3], Miroslav M. Vrvić[3] Ivanka Karadžić[1]

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**Introduction**

Microbial enzymes, particularly those produced by extremophiles, are very often stable and active in organic solvents. Lipases are among the most promising and important biocatalysts for carrying out reactions in both, aqueous and nonaqueous, media.

**Aim**

In the present study, the potential of bacterial lipase isolated from *Pseudomonas aeruginosa* NCAIM (P) B 001380 to catalyze the esterification of ergosterol with oleic acid in the simple process, was evaluated. The prepared ergosterol oleate was purified by column chromatography and identification was conducted by NMR.

**Material and methods**

*P. aeruginosa* was cultivated and crude lipase was produced as described by *Karadzic et al., 2006.* Reaction of esterification was carried out in 20 ml hermetically seald vials. Reaction mixture was consisted of 4 mmol of acid and 100 mg of ergosterol in 6 ml of n-hexane with 100 mg of crude lypase. Reaction was performed at 40°C with rotational shaking (250 cycles/min). Purified ergosterol oleate was analyzed: $^{1}H$, $^{13}C$ and DEPT$^{13}C$ NMR spectra were measured in $CDCl_3$ as solvent, with Gemini 200 NMR spectrometer. Quantitative analysis of the desired product was done using Camag TLC Scanner 3.

**Results**

Detection and identification of synthesized ergosterol oleate was done by TLC on silica gel. The product was purified on silica gel 100 (0.063 – 0.200) 60x1cm column, eluted with n-hexane:ethyl-acetate (6:4). Fractions were collected and then detected by TLC. Degree of esterification was 64 % after 5 days of incubation.

Product identification $^{13}C$ NMR spectral data (50 MHz, $CDCl_3$) of ergosterol oleat was: $\delta$ = 11.96 ($CH_3$-18), 14.06 ($CH_3$-51), 16.17($CH_3$-19), 17.55($CH_3$-29), 19.59($CH_3$-30), 19.90($CH_3$-31), 21.05 ($CH_3$-21), 22.63 ($CH_2$), 22.94 ($CH_2$), 24.64 ($CH_2$), 27.15 ($CH_2$), 28.24 ($CH_2$), 29.10 ($CH_2$), 29.48 ($CH_2$), 29.63 ($CH_2$),29.72 ($CH_2$), 31.74 ($CH_2$), 31.86 ($CH_2$), 33.03 (CH-28), 34.03(CH-24) 36.94 (C-10), 38.29(CH2-1), 39.04 (CH2-12), 40.40 (CH2-4), 40.53(CH-20), 42.77 (C-13), 46.17 (CH-9), 54.50 (CH-17), 55.68 (CH-14), 70.44 (CH-3), 116.31 (CH-7), 119.62 (CH-6), 129.71 (C-43), 129.98 (C-42), 131.95 (CH-23), 135.55 (CH-22), 139.67 (C-8), 141.25 (C-5), 179.72 (C=O-33).

**Conclusion**

Our data show that sterols can be efficiently converted into esters by lipase. Further investigations such as optimization of synthesis and synthesis of other steroids, is underway.

**Refrence**

Karadžić I., Masui A., Izrael Živković L., Fujiwara N., *J. Biosci. Bioeng.*, 2006; 102: 82-89

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[3]Faculty of Chemistry, University of Belgrade,

## Introduction

Microbial enzymes, particularly those produced by extremophiles, are very often stable and active in organic solvents. Lipases are among the most promising and important biocatalysts for carrying out reactions in both, aqueous and nonaqueous, media. Under natural conditions, lipase catalyzes the hydrolysis of ester bonds at the interface between an insoluble substrate phase and the aqueous phase in which the enzyme is dissolved. In reduced water conditions, lipases catalyze the reverse reaction - esterification. So their potential for the hydrolysis and synthesis made lipases used for production of esters of commercial interest. In the last decade interest for enzyme-catalysed production of esters has increased. One of the advantages of enzyme-catalysed process (besides the energy saving) are mild conditions which allow transformations of sensitive supstrates.

## Material and methods

*P. aeruginosa* was cultivated at 30°C for 20h in the Luria-Bertani medium (0.5% NaCl, 0.5% yeast extract, and 1% tryptone) with vigorous shaking 250 at cycles/min. An actively growing culture was dispensed into erlenmaer flasks (1%), and fermentation was carried out in the same medium at 30°C for 72h. After 72h, the culture filtrate obtained by centrifugation at 7500 rpm for 15min collected and liophilized. This sample was used as crude lipase. Reaction of esterifitcation was carried out in 20ml hermetically seald vials. Reaction mixture was consist of 4mmol of acide and 100mg of ergosterol in 6ml of n-hexane with 100mg of crude lypase. Reaction was performed at 40°C with rotational shaking (250 cycles/min). Purified ergosterol oleate was analyzed: $^{1}H$, $^{13}C$ and DEPT$^{13}C$ NMR spectra were measured in CDCl3 as solvent, with Gemini 200 NMR spectrometer. Quantitative analysis of the desired product was done using Camag TLC scanner 3.

## Aim

In the present study, the potential of bacterial lipase isolated from *Pseudomonas aeruginosa* NCAIM (P) B 001380 to catalyzed the esterification of ergosterol with oleic acid in the simple process, was evaluated. The prepared ergosterol oleate was purified by column chromatography and identification was conducted by NMR.

## Results and discussion

Stability and activity of crude enzyme preparation in different organic solvents has been studed previously, and according to this, condition for reaction of esterification were chosen. Reaction of esterification of ergosterol and oleic acid catalyzed by lipase was carried out in n-hexane at 40°C. Preliminary detection of synthesized ergosterol oleate was done by TLC on silica gel. After 24, 48, 72, 96 and 120h product formation was checked by TLC and the total formation of ergosterol oleat was quantified by TLC scanner. Conditions for esterification were: 4 mmol of oleic acide and 100 mg of ergosterol in 6 ml of n-hexane with 100 mg of crude lypase, at 40°C with rotational shaking (250 cycles/min). Compared to two other microbial lipases, our lipase showed similar yield formation after 24h (8%). Lipases from *Candida antartica* B and *Candida rugosa* had higher degree of esterification after 24h (16.1% and 38.5%). Degree of esterification with our lipase was 64% after 5 days of incubation. These results were obtained without optimization of the conditions.

The product was purified on silica gel 100 (0.063 – 0.200) 60x1cm column, eluted with n-hexane:ethyl-acetate (6:4). Fractions were colected and then detected by TLC. After purification ergosterol oleat was analyzed.

Product identification 13C NMR spectral data (50 MHz, $CDCl_3$) of ergosterol oleat was: δ = 11.96 ($CH_3$-18), 14.06 ($CH_3$-51), 16.17 ($CH_3$-19), 17.55 ($CH_3$-29), 19.59 ($CH_3$-30), 19.90 ($CH_3$-31), 21.05 ($CH_3$-21), 22.63 ($CH_2$), 22.94 ($CH_2$), 24.64 ($CH_2$), 27.15 ($CH_2$), 28.24 ($CH_2$), 29.10 ($CH_2$), 29.48 ($CH_2$), 29.63 ($CH_2$), 29.72 ($CH_2$), 31.74 ($CH_2$), 31.86 ($CH_2$), 33.03 (CH-28), 34.03(CH-24) 36.94 (C-10), 38.29 ($CH_2$-1), 39.04 ($CH_2$-12), 40.40 ($CH_2$-4), 40.53(CH-20), 42.77 (C-13), 46.17 (CH-9), 54.50 (CH-17), 55.68 (CH-14), 70.44 (CH-3), 116.31 (CH-7), 119.62 (CH-6), 129.71 (C-43), 129.98 (C-42), 131.95 (CH-23), 135.55 (CH-22), 139.67 (C-8), 141.25 (C-5), 179.72 (C=O-33).

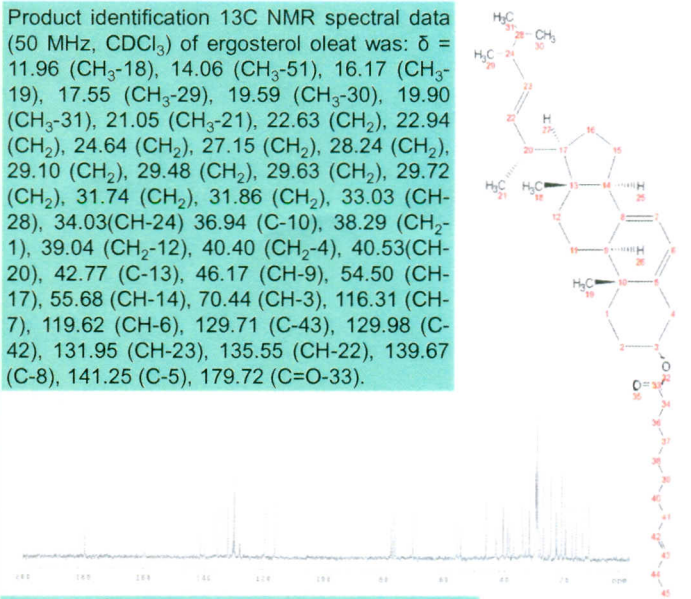

Figure 2. $^{13}C$ NMR spectrum of ergosterol oleat

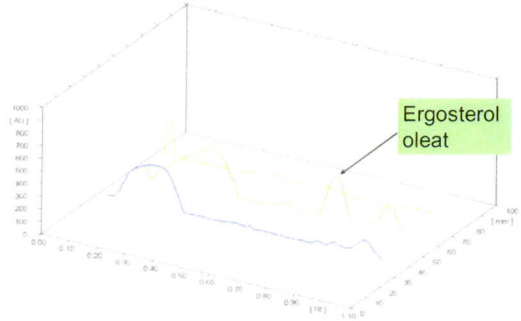


Figure 1. Chromatogram of: **oleic acid**, **ergosterol** and lipase sythesized **ergosterol oleat**.

## Conclusion

Our data show that ergosterol can be efficiently converted into ester by lipase. Further investigations such as optimization of synthesis: influence of temperature, effect of substrate molar ratio, effect of molecular sives and synthesis of other steroides, is underway.

## Acknowledgment

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## Refrence

Karadžić I.,et al., *J. Biosci. Bioeng.*, 2006; 102: 82-89
Villeneuve P. et al., Enzyme Microb. Technol., 2005; 37:150-155