

**Ензиматичний синтез  
(R)-манделонітрилу  
з використанням перехресно-  
зв'язаних ензимових агрегатів  
*Prunus cerasifera*  
гідроксинітрил ліази  
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Гідроксинітрил ліази (HNL) – група нестійких ферментів, які каталізують оборотне розщеплення  $\alpha$ -гідроксинітрилу і використовуються для виготовлення енантіочистих ціангідринів з альдегідів або кетону і синильної кислоти (HCN) [1]. Хіральні ціангідрини виконують важливу роль синтетичного допоміжного засобу для багатьох промислових продуктів оскільки обидві функціональні групи ціангідринів, гідроксильна і ціанідна частка приєднана до того ж вуглецю і може бути легко перетворена в багато інших хіральних продуктів, наприклад,  $\alpha$ -гідроксиальдегід і кетони,  $\beta$ -аміноалкоголі,  $\alpha$ -фторціаніди тощо [2,3]. Для того, щоб покращити стійкість HNL вивчалася їх фіксація до опор таких, як целіт [4], целюлоза [4, 5-7] чи нітроцелюлоза [6,7].

У цій статті описано дослідження, у якому вперше поперечно-зв'язані ензимові агрегати (CLEA) гідроксинітрил ліази *Prunus cerasifera* (PcHNL-CLEA) були утворені з насіння *Prunus cerasifera*. Проаналізовано вплив pH і температури на ліазу PcHNL-CLEA. Оптимальний pH і температура для активності ліази PcHNL-CLEA склали відповідно 5,5 і 25°C. Ми визначили  $K_m$  і  $V_{max}$  PcHNL-CLEA відповідно як 4,0 мМ і 22 Од/г PcHNL-CLEA. Досліджувалася теплова стабільність PcHNL-CLEA при 25 і 50°C. PcHNL-CLEA утримали 72% початкової активності коли зберігалися при 25°C і 54% початкової активності, коли зберігалися в 50°C під кінець 16 годинного періоду. PcHNL-CLEA також використано для синтезу енантіочистих ціангідрин з бензалдегіду і ціаніду калію. Вихід і енантіочистота (R)-манделонітрилу склали відповідно 100 і 94%.

У висновку наведено характеристики PcHNL-CLEA і описано здатність PcHNL-CLEA до синтезу (R) - манделонітрилу.

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**Enzymatic Synthesis of (R)-  
Mandelonitrile by Using Cross  
Linked Enzyme Aggregates  
of *Prunus cerasifera*  
Hydroxynitrile Lyase**

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*In this study, cross-linked enzyme aggregates (CLEA) of *Prunus cerasifera* hydroxynitrile lyase (PcHNL-CLEA) were prepared from seeds of *Prunus cerasifera* and characterized for the first time. The influence of pH and temperature on the PcHNL-CLEA lyase activity was investigated. The optimal pH and temperature for lyase activity of PcHNL-CLEA were 5.5 and 25°C, respectively. We determined  $K_m$  and  $V_{max}$  of PcHNL-CLEA as 4.0 mM and 22 U/g PcHNL-CLEA, respectively. The thermal stability of PcHNL-CLEA at 25 and 50°C was studied. PcHNL-CLEA retained 72% of its initial activity when incubated at 25°C and 54% of initial activity when stored at 50°C at the end of 16 hours. The PcHNL-CLEA was also used for the enantiopure mandelonitrile synthesis from benzaldehyde and potassium cyanide. The yield and enantiopurity of (R)-mandelonitrile were obtained as 100 and 94% respectively.*

**Keywords** – hydroxynitrile lyase, cross linked enzyme aggregates, mandelonitrile, enantiopurity, immobilization

## Introduction

(R)-Mandelonitrile, produced by hydrolysis of cyanogenic glucosides that occur in members of the family *Rosaceae*, is reversibly dissociated into benzaldehyde and HCN by the enzyme called mandelonitrile lyase or hydroxynitrile lyase (EC 4.1.2.10) (Hydroxynitrile lyases (HNLs) are important defense enzymes that protect plant cells from herbivores or microbial attack. Plants can release hydrocyanic acid (HCN) to protect themselves against predators. This ability of living organisms to produce HCN is known as cyanogenesis. The cyanogenesis phenomenon is widely observed in higher plants and is also reported in taxonomically diverse group of organisms like bacteria, fungi, lichen, millipedes, arthropods and insects. This phenomenon involves enantioselective cleavage of cyanohydrin ( $\alpha$ -hydroxynitrile, alcohol containing a cyano and a hydroxyl group attached to the same carbon atom) from cyanogenic glycosides (cyanogenic precursors) by action of an HNL into their corresponding aldehydes or ketones and HCN. The reverse reaction of HNL, synthesis of chiral cyanohydrins, has attracted the attention of scientists and industry. In synthetic chemistry the reverse of the natural reaction, namely the addition of (hydro)cyanide to carbonyl groups, is exploited for synthesis of optically pure cyanohydrins. Chiral cyanohydrins have

attracted much attention as components or precursors of numerous pharmaceuticals and agrochemicals such as  $\alpha$ -hydroxy acids,  $\alpha$ -hydroxy ketones and  $\beta$ -amino alcohols [8].

CLEA was prepared based on the reported procedure [9]. The lyase activity and carboligation activity of PcHNL-CLEA was measured according to Tükel et al. [10]. The yield and enantiomeric excess (ee) of synthesized (R)-mandelonitrile was analyzed by HPLC equipped with Nucleocel Delta S chiral column (4.6x250 mm) at 220 nm.

The influence of pH and temperature on the lyase activity of PcHNL-CLEA was investigated. The optimal pH and temperature for lyase activity of PcHNL-CLEA were 5.5 and 25°C, respectively. Kinetic parameters of PcHNL-CLEA were characterized at predetermined conditions for lyase activity.  $K_m$  and  $V_{max}$  values were determined as 4.0 mM and 22 U/mg prot., respectively. In our previous study,  $K_m$  values were found as 2.23 mM for free *Prunus pseudoarmeniaca* HNL, 1.60 and 1.03 mM for immobilized *Prunus pseudoarmeniaca* HNLs onto Eupergit C and Eupergit C 250 L, respectively [10]. In the previous studies, the  $K_m$  values of different HNLs were reported as 0.172 mM for *Prunus serotina* HNL, 0.290 mM for *Prunus dilcus* HNL, 0.790 mM for *Sorghum bicolor* HNL and 0.093 mM for *Prunus lyonii* HNL towards mandelonitrile [11, 12].

The thermal stability of PcHNL-CLEA at 25 and 50°C was studied. PcHNL-CLEA retained 72% of its initial activity when incubated at 25°C and 54% of initial activity when incubated at 50°C at the end of 16 hours.

The carboligation activities of PcHNL-CLEA was evaluated by the synthesis of (R)-Mandelonitrile ((R)-MN) from benzaldehyde and HCN. The yield and ee of (R)-MN were determined as 100% and 94%, respectively. In the literature, the yield and ee were reported as 65-100% and 94-99% for (R)-MN, respectively [13,14].

## Conclusion

In this study, the optimal pH and temperature of PcHNL-CLEA were 5.5 and 25°C, respectively.  $K_m$  and  $V_{max}$  values of PcHNL-CLEA were determined as 4.0 mM and 22 U/mg prot., respectively towards mandelonitrile. PcHNL-CLEA showed great ability in (R)-MN synthesis and (R)-MN were obtained with 100% yield and 94% ee.

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