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Enantio convergent biotransformation of *O*,*O*-dimethyl-4-oxoazetidin-2-ylphosphonate using fungal cells of *Penicillium minioluteum* and purified enzymes

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ABSTRACT

This report presents the bioconversion of *O*,*O*-dimethyl-4-oxoazetidin-2-ylphosphonate **1** performed in two ways: with the enzymatic system of *P. minioluteum* and with the application of purified enzymes: penicillinase and two proteases of different origin. Recorded NMR spectra allowed confirming the reaction progress and also postulating possible mechanism of conversion. The path of bioconversion was defined as enantio convergent process for both modes of applied biocatalysts. This means that kinetically driven resolution of racemic mixture of the substrate leads to the one enantiomer of the product. The bioconversion started from ester bond hydrolysis (equally in both enantiomers) with the conversion degree from 30% (whole-cell) to 35% (isolated enzymes) and with the production of optically pure monoester (compound **2**; 100% of *e.e*). For whole-cell bioprocess it was the initiative step for the enantioselective amide bond hydrolysis, what resulted in synthesis of desired product 3-amino-3-phosphonopropanoic acid **4**. However, the most effective enzymatic hydrolysis of ester bond performed with penicillinase from *Enterobacter cloacae* led only to the monoester product **2**.

1. Introduction

Bioconversions are an alternative to chemical tools, with great potential for the development of sustainable technologies for the production of chemicals and drugs. Their major advantages are diversity of the accessible enzymatic activities and complexity, what is relevant for the synthetic processes planning. Biocatalytic reactions are carried out under safe and mild conditions (room temperature, neutral pH and atmospheric pressure), biotransformation by-products are usually biodegradable and processes are environmentally friendly. Additionally, biological transformations have got a remarkable predominance because of their high enantioselectivity [1–4]. Organophosphorus compounds include a phosphorus atom covalently bound to a carbon. Phosphonates are very stable and resistant to thermo- or chemolysis and biochemical decomposition [5]. Among them, are aminophosphonic acids and their derivatives- analogues of amino acids with the carboxylic group replaced by phosphonic moiety. Such compounds are very stable and have variable biological activities [6,7]. They exhibit inhibitory activity, act as antibiotics, crop protection agents, herbicides, peptide mimics, and are also used in diagnostic medicine and applied in hydrometallurgy [5,8–11]. Also interesting group of biologically active molecules are compounds with β-lactam ring in their structure. They are tested as bacteriostatic or bactericidal pharmaceuticals and as inhibitors of β-lactamases-enzymes responsible for bacterial resistance to widely used β-lactam antibiotics [12].

The aim of this paper was to set the biocatalytic method of enantioselective hydrolysis of the phosphonolactam with the use of the whole-cell biocatalyst and isolated enzymes (penicillinase from *Enterobacter cloacae*, proteases from *Rhizopus* sp. and from *Bacillus licheniformis*). The activity of the enzymatic system of *P. minioluteum* and isolated enzymes were tested towards *O*,*O*-dimethyl-4-oxoazetidin-2-ylphosphonate **1**.

Penicillium minioluteum was chosen taking into account its hydrolytic activity towards phosphonic compounds, which has been described previously [17]. This allowed assuming, that ester or amide bond hydrolysis in phosphonalactam **1** can be performed. Experiments confirmed, that bioconversion with whole-cell biocatalyst was possible and turned out to be the multistep process, which starts from the ester bond hydrolysis. Studies proved that, the ester bond in substrate **1** is biohydrolyzed without any stereoselective discrimination but the compound is converted into optically pure product **2** (100% *e.e.*, Fig. 5). After ester bond hydrolysis is accomplished, amide bond hydrolysis is performed,

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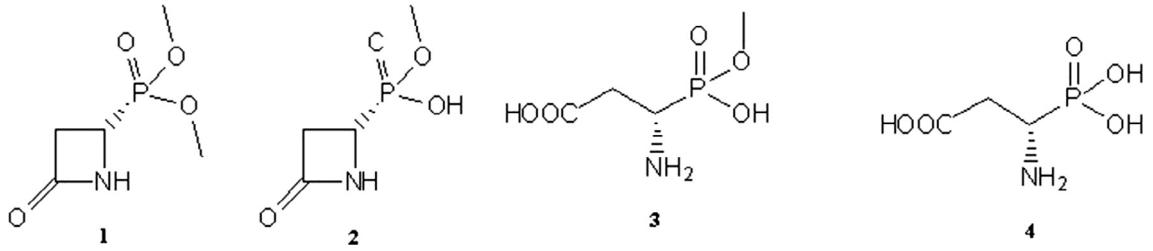

Fig. 1. Chemical structures of analysed compounds: *O,O*-dimethyl-4-oxoazetidin-2-ylphosphonate **1**, *O*-methyl-4-oxoazetidin-2-ylphosphonate **2**, 3-amino-3-hydroxymetoxyphosphonopropanoic acid **3**, 3-amino-3-phosphonopropanoic acid **4**.

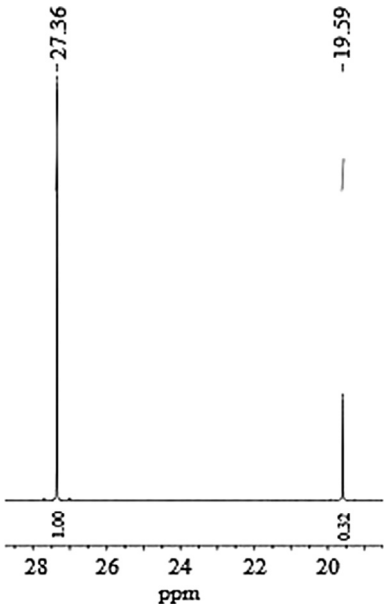

Fig. 2. 31P NMR spectrum of biotransformation of **1**-27.36 ppm; obtained product **2**-19.59 ppm.

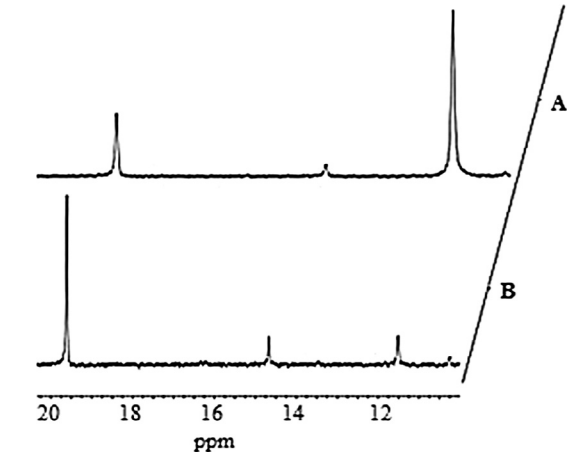

Fig. 4. 31P NMR spectra of products: **2**-19.61 ppm, **3**-14.60 ppm, **4**-11.46 ppm. A – extra addition of synthetic 4 (11.46 ppm.), B – spectrum after separation of *O,O*-dimethyl-4-oxoazetidin-2-ylphosphonate **1**.

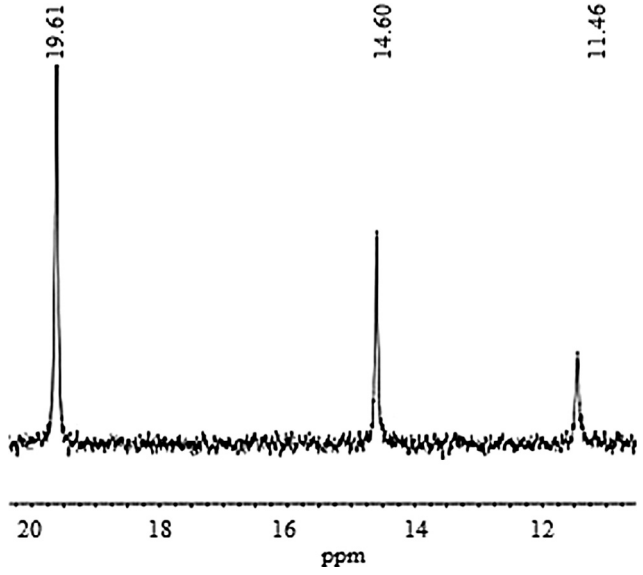

Fig. 3. 31P NMR spectrum of products obtained after storage a samples in D_2O after biotransformation of *O,O*-dimethyl-4-oxoazetidin-2-ylphosphonate **1**: **2**-19.61 ppm, **3**-14.60 ppm, **4**-11.46 ppm.

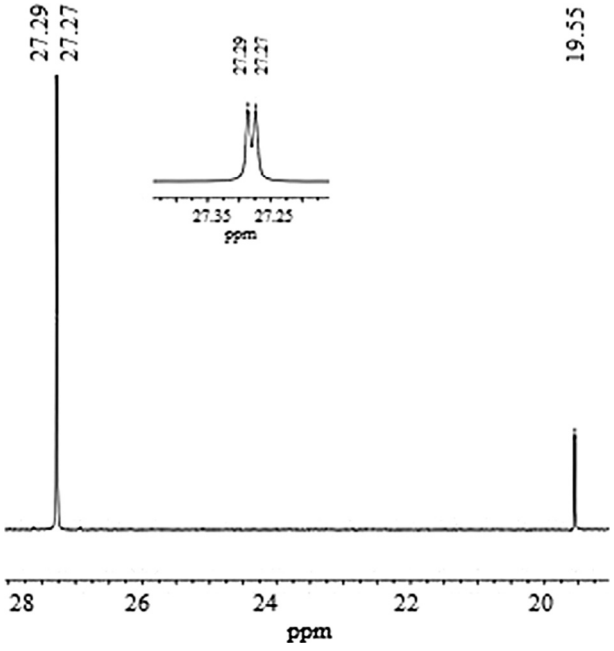

Fig. 5. 31P NMR spectrum after whole-cell biotransformation recorded with the addition of α-cyclodextrin: **1** – substrate (27.29 ppm, 27.27 ppm), **2** – *O*-methyl-4-oxoazetidin-2-ylphosphonate (19.55 ppm).

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