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Expression of *Pantoea* sp. 3.5.1 AgpP Phytase in Three Expression Systems

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Abstract
Nowadays, microbial phytases have been widely used as feed additives that increase the bioavailability of phosphorus for monogastric animals. Still, there is an active search for new phytases, development of effective production systems, and study of their properties. In this study, we compared the biochemical characteristics of bacterial histidine acid phytase of *Pantoea* sp. 3.5.1 produced in three different expression systems: *Escherichia coli*, methylotrophic yeast *Pichia pastoris*, and yeast *Yarrowia lipolytica*. The maximum activity of the recombinant phytase AgpP-P expressed by *P. pastoris* occurred at pH 4.0, while the pH optimum of the recombinant AgpP-Y phytase expressed by *Y. lipolytica* is shifted towards more acidic pH 3.0. The recombinant AgpP-P enzyme is stable at pH values from 2.0 to 5.0, while the AgpP-Y remains active at pH values from 3.0 to 7.0; however, at pH above 8.0, the enzyme becomes inactive. The temperature optimum of recombinant AgpP-P phytase corresponded to 50 °C, while the temperature optimum for AgpP-Y was at 45 °C. The recombinant enzymes AgpP-P and AgpP-Y retained activity at temperatures from 4 to 70 °C and from 4 to 60 °C, respectively. Bivalent metal ions at a concentration of 1 mM had the same effect on the activity of recombinant phytases from *E. coli*, *P. pastoris*, and *Y. lipolytica*: Ca^{2+}, Mg^{2+}, and Mn^{2+} ions more than doubled the activity of enzymes, while Co^{2+} did not affect the activity of phytases, while ions Zn^{2+}, Cu^{2+}, and Fe^{2+} inhibited the activity of enzymes.

Keywords Histidine acid phytase · *Pichia pastoris* · *Yarrowia lipolytica* · *E.coli* · Heterologous expression

1 Introduction

The problem of a lack of available phosphorus in animal feed is due to the fact that a significant amount of phosphorus in feed grains is presented in the form of insoluble phytate complexes [1]. Phytic acid is the main form of phosphorus storage in plant seeds and is capable of forming complexes with metal ions. Therefore, phytate phosphorus becomes inaccessible for monogastric animals that are lacking the enzymes for phytate hydrolysis in their gastrointestinal tract. As a result, undigested phosphorus is excreted along with animal feces, causing environmental pollution and eutrophication of water reservoirs. Due to the low content of available phosphorus, inorganic phosphates are added to feed, which significantly increases their cost [2, 3].

Phytate hydrolysis is carried out by the enzymes, phytases, which are capable of stepwise phytate dephosphorylation with the release of inositol derivatives and free phosphates. Over the past 20 years, microbial phytases have attracted particular attention from scientists, entrepreneurs, biotechnologists, and ecologists [4]. Microbial producers are considered the most promising for the commercial production of phytases. Nowadays, microbial phytases have been widely used as feed additives that increase the bioavailability of phosphorus for monogastric animals and reduce its release into the environment. However, there is no perfect phytase that can meet all practical needs. Therefore, there is an active search for new phytases, development of effective production systems, and study of their properties [4].

The aim of this work is to compare the characteristics of bacterial histidine acid phytase of *Pantoea* sp. 3.5.1 produced in three different expression systems: *Escherichia coli*, methylotrophic yeast *Pichia pastoris*, and yeast *Yarrowia lipolytica*.

2 Materials and Methods

Histidine acid phytase of *Pantoea* sp. 3.5.1 was produced by the recombinant *E. coli* BL 21 plysS pET28a/agpP strain,

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