BioNanoScience (2018) 8:1045–1053
https://doi.org/10.1007/s12668-018-0563-y

Cloning of Phytase Genes from *Pantoea* Sp. 3.5.1 and *Bacillus ginsengihumi* M2.11 in *Pichia pastoris*

Daria S. Troshagina[1] · Aliya D. Suleimanova[1] · Daria L. Itkina[1] · Margarita R. Sharipova[1]

Published online: 27 September 2018

Abstract
Phytases (myo-inositol hexakisphosphate hydrolase) catalyze the hydrolysis of phytate to inorganic phosphate and less phosphorylated myo-inositol derivatives and are widely used as feed additives in animal nutrition. Nevertheless, nowadays, there is a constant search for new phytases and new expression systems for better production of these important enzymes. In this study, we report cloning of two novel bacterial phytases belonging to the different enzyme classes and having different properties in the methylotrophic yeast *Pichia pastoris*. Sequences of *agpP* and *phyC* genes, encoding histidine acid phytase from *Pantoea* sp. 3.5.1 and β-propeller phytase from *Bacillus ginsengihumi* M2.11, respectively, were optimized and chemically synthesized according to the *P. pastoris* codon usage bias. The optimized genes were cloned into the yeast vectors pPINK-HC and pPINK-LC under the control of the inducible promoter AOX1 and two different signal peptides—signal sequence of α-amylase gene from *Aspergillus niger* and presequence of inulinase gene from *Kluyveromyces maxianus*. PCR analysis, restriction analysis, and DNA sequencing confirmed correct integration of *agpP* and *phyC* genes into *P. pastoris* genome. As a result, recombinant strains of *P. pastoris* with codon-optimized bacterial phytase genes (*agpP* and *phyC)*, encoding histidine acid and β-propeller phytases, integrated into the genome under the alcohol oxidase promoter AOX1 and two different signal peptides, were obtained. Recombinant phytase AgpP was stably expressed and secreted into the culture medium of yeasts, whereas the expression of *phyC* gene was only confirmed at transcription level.

Keywords Histidine acid phytase · β-Propeller phytase · *Pichia pastoris* · Heterologous expression · Process optimization · Alcohol oxidase promoter

1 Introduction

The significant amount of phosphorus in cereals, legumes, and oilseeds is presented by phytates (myo-inositol hexakisphosphate) [1].

Phytases (myo-inositol hexakisphosphate hydrolase) catalyze the hydrolysis of phytate to inorganic phosphate and less phosphorylated myo-inositol derivatives [2, 3]. Phytases are widely used in animal nutrition to increase the digestibility of phytate phosphorus in monogastric animals, such as poultry, pig, and fish, besides reducing environmental pollution significantly [4, 5].

✉ Aliya D. Suleimanova
aliya.kzn@gmail.com

[1] Institute of Fundamental Medicine and Biology, Kazan Federal University, Kremlyovskaya Street, 18, 420008 Kazan, Russia

Moreover, phytate also acts as an antinutritional agent, since it forms insoluble chelates with proteins and valuable metal ions (calcium, copper, and zinc) making them unavailable for absorption in the human intestine [6]. The human small intestine has limited ability to hydrolyze phytates, resulting in adverse nutritional consequences with respect to metabolic cation imbalances [7]. Phytases have potential to be used as food additives in human nutrition to enhance the absorption of cations [8].

Furthermore, phytases are envisaged to serve as potential enzymes that can produce lower *myo*-inositol phosphates isomers of pharmaceutical importance [9]. For example, *Saccharomyces cerevisiae* phytase was successfully used to produce medically important compounds, as d-*myo*-inositol-1,2,6-triphosphate, d-*myo*-inositol-1,2,5- triphosphate, l-*myo*-inositol-1,3,4-triphosphate, and *myo*-inositol-1,2,3-triphosphate [10, 11].

Phytases are widely distributed among microorganisms, and also found in plants and animals. Phytases of the micromycetes of *Aspergillus* genus—*A. niger* [12], *A.*