Microbial Cell Factories

Poster Presentation

Cloning and overexpression of a yeast phytase gene in *Pichia pastoris*

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from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):P31 doi:10.1186/1475-2859-5-S1-P31

Background

Phytate (*myo*-inositol hexakisphosphate) represents the major storage form of phosphorus in cereal grains, legumes, pollens and oilseeds. However, the bioavailability of phosphorus-phytate in plant derived feedstuffs is limited because monogastric animals lack intestinal phytase at the level needed to hydrolyse phytate and release the necessary inorganic phosphorus [1]. One solution currently employed is amendment of animal rations with phytase [2]. Phytases (*myo*-inositol hexakisphosphate 3- and 6-phosphohydrolase) catalyse the hydrolytic degradation of phytate yielding lower inositol phosphates ($InsP_5$ to $InsP_1$), *myo*-inositol and inorganic phosphate. Phytases are produced by a wide range of organisms : plant, animal and especially microorganisms.

Results

Our aim was to overexpress a new yeast phytase gene in the methylotrophic yeast *Pichia pastoris*. The ORF coding this new yeast phytase was isolated from *Debaryomyces castellii*. The deduced 461-amino-acid protein sequence, corresponding to a 51.2 kDa molecular mass, contained the consensus motif (RHGXRXP) which is conserved among phytases. This protein shared 21 to 69 % sequence similarities with various phytases of yeast or fungal origin. Heterologous expression of this phytase in the methylotrophic yeast *P. pastoris* was tested both under the *P. pastoris* inducible alcohol oxidase (AOX1) promoter and the constitutive glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter in fermentor. In both cases, the α-factor signal sequence was utilised, resulting in secretion of phytase into the culture medium. Maximum production level obtained was 107 U/mL, i.e. 1340 U/g DCW with the AOX1 expression system and 16.5 U/mL i.e. 300 U/g DCW with the GAP one.

Conclusion

These productions corresponded to an overexpression by 100 and 10 times respectively compared to the wild production. The biochemical characteristics of the recombinant phytase are identical to those of the native one. Thus, the new yeast phytase gene of *Debaryomyces castellii* has been successfully expressed in *P. pastoris*.

Acknowledgements

The financial support by ADISSEO and ANRT (Agence Nationale de la Recherche Technologique) to M. R., in the form of doctoral grant, is gratefully acknowledged.

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