

A thermostable phytase from *Bacillus* sp. MD2: Cloning, expression and high-level production in *Escherichia coli*

Tran T.T., Mamo G., Mattiasson B., Hatti-Kaul R.

Department of Biotechnology, Lund University, Box 124, Lund 221 00, Sweden; Biotechnology and Microbiology Department, Hanoi National University of Education, 136 Xuan Thuy Street, Hanoi, Viet Nam

Abstract: Phytase is used as a feed additive for degradation of antinutritional phytate, and the enzyme is desired to be highly thermostable for it to withstand feed formulation conditions. A *Bacillus* sp. MD2 showing phytase activity was isolated, and the phytase encoding gene was cloned and expressed in *Escherichia coli*. The recombinant phytase exhibited high stability at temperatures up to 100°C. A higher enzyme activity was obtained when the gene expression was done in the presence of calcium chloride. Production of the enzyme by batch- and fed-batch cultivation in a bioreactor was studied. In batch cultivation, maintaining dissolved oxygen at 20-30% saturation and depleting inorganic phosphate below 1 mM prior to induction by IPTG resulted in over 10 U/ml phytase activity. For fed-batch cultivation, glucose concentration was maintained at 2-3 g/l, and the phytase expression was increased to 327 U/ml. Induction using lactose during fed-batch cultivation showed a lag phase of 4 h prior to an increase in the phytase activity to 71 U/ml during the same period as IPTG-induced production. Up to 90% of the total amount of expressed phytase leaked out from the *E. coli* cells in both IPTG- and lactose-induced fed-batch cultivations.

© 2009 Society for Industrial Microbiology.

Author Keywords: Alkaline phytase; *Bacillus* sp.; Fed-batch cultivation; Protein secretion

Index Keywords: calcium chloride; glucose; oxygen; phosphate; phytase; article; *Bacillus*; bioreactor; enzyme activity; enzyme isolation; enzyme synthesis; *Escherichia coli*; fed batch culture; gene expression; molecular cloning; nonhuman; nucleotide sequence; protein expression; protein secretion; temperature sensitivity; thermostability; 6-Phytase; *Bacillus*; Bacterial Proteins; Bioreactors; Calcium Chloride; Cloning, Molecular; Culture Media; Enzyme Activators; Enzyme Stability; *Escherichia coli*; Gene Expression; Hot Temperature; Isopropyl Thiogalactoside; Lactose; Protein Stability; Recombinant Proteins; Transcriptional Activation; *Bacillus* sp.; *Escherichia coli*

Year: 2010

Source title: Journal of Industrial Microbiology and Biotechnology

Volume: 37

Issue: 3

Page : 279-287

Link: Scopus Link

Molecular Sequence Numbers: GENBANK: GU143090

Chemicals/CAS: calcium chloride, 10043-52-4; glucose, 50-99-7, 84778-64-3; oxygen, 7782-44-7; phosphate, 14066-19-4, 14265-44-2; phytase, 37288-11-2, 37341-58-5, 9001-89-2; 6-Phytase, 3.1.3.26; Bacterial Proteins; Calcium Chloride, 10043-52-4; Culture Media; Enzyme Activators; Isopropyl

Thiogalactoside, 367-93-1; Lactose, 63-42-3; Recombinant Proteins

Correspondence Address: Hatti-Kaul, R.; Department of Biotechnology, Lund University, Box 124, Lund 221 00, Sweden; email: Rajni.Hatti-Kaul@biotek.lu.se

ISSN: 13675435

CODEN: JIMBF

DOI: 10.1007/s10295-009-0671-3

PubMed ID: 19997958

Language of Original Document: English

Abbreviated Source Title: Journal of Industrial Microbiology and Biotechnology

Document Type: Article

Source: Scopus

Authors with affiliations:

- Tran, T.T., Department of Biotechnology, Lund University, Box 124, Lund 221 00, Sweden, Biotechnology and Microbiology Department, Hanoi National University of Education, 136 Xuan Thuy Street, Hanoi, Viet Nam
- Mamo, G., Department of Biotechnology, Lund University, Box 124, Lund 221 00, Sweden
- Mattiasson, B., Department of Biotechnology, Lund University, Box 124, Lund 221 00, Sweden
- Hatti-Kaul, R., Department of Biotechnology, Lund University, Box 124, Lund 221 00, Sweden

References:

- Kesson, M., Hagander, P., Axelsson, J.P., Avoiding acetate accumulation in *Escherichia coli* cultures using feedback control of glucose feeding (2001) *Biotechnology and Bioengineering*, 73 (3), pp. 223-230. , DOI 10.1002/bit.1054
- Cheryan, M., Phytic acid interaction in food systems (1980) *CRC Crit Rev Food Sci Nutr*, 13, pp. 297-335. , 10.1080/10408398009527293 1:CAS:528:DyaL3MXivV2juw%3D%3D
- Choi, Y.M., Suh, H.J., Kim, J.M., Purification and properties of extracellular phytase from *Bacillus* sp. KHU-10 (2001) *Journal of Protein Chemistry*, 20 (4), pp. 287-292. , DOI 10.1023/A:1010945416862
- Fu, S., Sun, J., Qian, L., Li, Z., *Bacillus* phytases: Present scenario and future perspectives (2008) *Appl Biochem Biotechnol*, 151, pp. 1-8. , 10.1007/s12010-008-8158-7 1:CAS:528:DC%2BD1cXht1Snt7zF 18785018
- Golovan, S.P., Meidinger, R.G., Ajakaiye, A., Cottrill, M., Wiederkehr, M.Z., Barney, D.J., Plante, C., Forsberg, C.W., Pigs expressing salivary phytase produce low-phosphorus manure (2001) *Nature Biotechnology*, 19 (8), pp. 741-745. , DOI 10.1038/90788
- Gombert, A.K., Kilikian, B.V., Recombinant gene expression in *Escherichia coli* cultivation using lactose as inducer (1998) *Journal of Biotechnology*, 60 (1-2), pp. 47-54. , DOI 10.1016/S0168-1656(97)00185-5, PII S0168165697001855
- Gulati, H.K., Chadha, B.S., Saini, H.S., Production and characterization of thermostable alkaline phytase from *Bacillus laevolacticus* isolated from rhizosphere soil (2007) *J Indust Microbiol Biotechnol*, 34, pp. 91-98. , 1:CAS:528:DC%2BD28Xht1Shs7fP
- Hara, A., Ebina, S., Kondo, A., Funagua, T., A new type of phytase from *Typha latifolia* L (1985) *Agric Biol Chem*, 49, pp. 3539-3544. , 1:CAS:528:DyaL28XkslKjsA%3D%3D
- Holm, T., Arvidson, S., Lindholm, B., Pavlu, B., Enzyme laboratory-scale production (1970) *Process Biochem*, 5, pp. 62-66
- Horn, U., Strittmatter, W., Krebber, A., Knupfer, U., Kujau, M., Wenderoth, R., Muller, K., Riesenber, D., High volumetric yields of functional dimeric miniantibodies in *Escherichia coli*, using an optimized expression vector and high-cell-density fermentation under non-limited growth conditions (1996) *Applied Microbiology and Biotechnology*, 46 (5-6), pp. 524-532. , DOI 10.1007/s002530050855

- Huber, R.E., Kurz, G., Wallenfels, K., A quantitation of the factors which affect the hydrolase and transgalactosidase activities of -galactosidase (*E. coli*) on lactose (1976) *Biochem*, 15, pp. 1994-2001. , 10.1021/bi00654a029 1:CAS:528:DyaE28XktFKhs70%3D
- Kerovuo, J., Lauraeus, M., Nurminen, P., Kalkkinen, N., Apajalahti, J., Isolation, characterization, molecular gene cloning and sequencing of novel phytase from *Bacillus subtilis* (1998) *Appl Environ Microbiol*, 64, pp. 2079-2085. , 1:CAS:528:DyaK1cXjslWhsLo%3D 9603817
- Kerovuo, J., Von Weymarn, N., Povelainen, M., Auer, S., Miasnikov, A., A new efficient expression system for *Bacillus* and its application to production of recombinant phytase (2000) *Biotech Lett*, 22, pp. 1311-1317. , 10.1023/A:1005694731039 1:CAS:528:DC%2BD3cXnt1aqt70%3D
- Kim, Y.-O., Kim, H.-K., Bae, K.-S., Yu, J.-H., Oh, T.-K., Purification and properties of a thermostable phytase from *Bacillus* sp. DS11 (1998) *Enzyme and Microbial Technology*, 22 (1), pp. 2-7. , DOI 10.1016/S0141-0229(97)00096-3, PII S0141022997000963
- Kim, Y.O., Kim, H.K., Lee, J.K., Yu, J.H., Oh, T.K., Cloning of the thermostable phytase gene (phy) from *Bacillus* DS11 and its over expression in *Escherichia coli* (1998) *FEMS Microbiol Lett*, 162, pp. 185-191. , 10.1111/j.1574-6968.1998.tb12997.x 1:CAS:528:DyaK1cXisFalt7o%3D 9595681
- Kim, D.-H., Oh, B.-C., Choi, W.-C., Lee, J.-K., Oh, T.-K., Enzymatic evaluation of *Bacillus amyloliquefaciens* phytase as a feed additive (1999) *Biotechnology Letters*, 21 (11), pp. 925-927. , DOI 10.1023/A:1005602717835
- Kim, Y.O., Lee, J.K., Oh, B.C., Oh, T.K., High-level expression of a recombinant thermostable phytase in *Bacillus subtilis* (1999) *Biosci Biotechnol Biochem*, 63, pp. 2205-2207. , 10.1271/bbb.63.2205 1:CAS:528:DC%2BD3cXlsFKktQ%3D%3D
- Kleist, S., Miksch, G., Hitzmann, B., Arndt, M., Friehs, K., Flaschel, E., Optimization of the extracellular production of a bacterial phytase with *Escherichia coli* by using different fed-batch fermentation strategies (2003) *Appl Microbiol Biotechnol*, 61, pp. 456-462. , 1:CAS:528:DC%2BD3sXjvFCl7w%3D 12764560
- Lassen, S.F., Breinholt, J., Ostergaard, P.R., Brugger, R., Bischoff, A., Wyss, M., Fuglsang, C.C., Expression, gene cloning, and characterization of five novel phytase from four Basidiomycete fungi: *Peniophora lycii*, *Agrocybe pediades* a *Ceriporia* sp., and *Trametes pubescens* (2001) *Appl Environ Microbiol*, 67, pp. 4701-4707. , 10.1128/AEM.67.10.4701-4707.2001 1:CAS:528:DC%2BD3MXns1Witrs%3D 11571175
- Lopez, H.W., Leenhardt, F., Coudray, C., Remesy, C., Minerals and phytic acid interactions: Is it a real problem for human nutrition? (2002) *International Journal of Food Science and Technology*, 37 (7), pp. 727-739. , DOI 10.1046/j.1365-2621.2002.00618.x
- Maddaiah, V.T., Kurnick, A.A., Reid, B.L., Phytic acid studies (1964) *Proc Soc Exp Biol Med*, 115, pp. 391-393. , 1:CAS:528:DyaF2cXnslentQ%3D%3D 14121619
- Maenz, D.D., Engele-Schaan, C.M., Newkirk, R.W., Classen, H.L., The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal (1999) *Animal Feed Science and Technology*, 81 (3-4), pp. 177-192. , DOI 10.1016/S0377-8401(99)00085-1, PII S0377840199000851
- Mallin, M.A., Impact of industrial animal production on river and estuaries (2000) *Am Sci* (January-February), 88, pp. 26-73
- Miksch, G., Kleist, S., Frieh, K., Flaschel, E., Overexpression of phytase from *Escherichia coli* and its extracellular production in bioreactors (2002) *Appl Microbiol Biotechnol*, 59, pp. 685-694. , 10.1007/s00253-002-1071-z 1:CAS:528:DC%2BD38XoslyktLk%3D 12226725
- Muller-Hill, B., Rickenberg, H.V., Wallenfels, K., Specificity of the induction of the enzymes of the lac operon in *Escherichia coli* (1964) *J Mol Biol*, 10, pp. 303-318. , 10.1016/S0022-2836(64)80049-8
- Ni, Y., Chen, R., Extracellular recombinant protein production from *Escherichia coli* (2009) *Biotechnol Lett*, 31, pp. 1661-1670.

- , 10.1007/s10529-009-0077-3 1:CAS:528:DC%2BD1MXht1eksb7O 19597765
- Nolan, K.B., Duffin, P.A., Mc Weeny, D.J., Effect of phytate on mineral bioavailability. in vitro studies on Mg²⁺, Ca²⁺, Fe³⁺, Cu²⁺ and Zn²⁺ (also Cd²⁺) solubilities in the presence of phytate (1987) *J Sci Food Agric*, 40, pp. 79-85. , 10.1002/jsfa.2740400110 1:CAS:528:DyaL2sXltleisbc%3D
 - Oh, B.-C., Choi, W.-C., Park, S., Kim, Y.-O., Oh, T.-K., Biochemical properties and substrate specificities of alkaline and histidine acid phytases (2004) *Applied Microbiology and Biotechnology*, 63 (4), pp. 362-372. , DOI 10.1007/s00253-003-1345-0
 - Pages, J.M., Anba, J., Lazdunski, C., Conditions leading to secretion of a normally periplasmic protein in *Escherichia coli* (1987) *J Bacteriol*, 169, pp. 1386-1390. , 1:CAS:528:DyaL2sXhslalurk%3D 3549684
 - Vats, P., Banerjee, U.C., Production studies and catalytic properties of phytases (myo-inositolhexakisphosphate phosphohydrolases): An overview (2004) *Enzyme and Microbial Technology*, 35 (1), pp. 3-14. , DOI 10.1016/j.enzmictec.2004.03.010, PII S0141022904000870
 - Ramchuran, S.O., Nordberg Karlsson, E., Velut, S., De Mare, L., Hagander, P., Holst, O., Production of heterologous thermostable glycoside hydrolases and presence of host-cell proteases in substrate limited fed-batch cultures of *Escherichia coli* BL21(DE3) (2002) *Appl Microbiol Biotechnol*, 60, pp. 408-416. , 10.1007/s00253-002-1132-3 1:CAS:528:DC%2BD38Xpt1Cjurs%3D 12466880
 - Ramchuran, S.O., Nordberg Karlsson, E., Holst, O., Effect of post induction, nutrient feed composition and use of lactose as inducer during production of thermostable xylanase in *Escherichia coli* (2003) *J Biosci Bioeng*, 99, pp. 477-484. , 10.1263/jbb.99.477
 - Decs, R., Rao, K.V., Reddy, V.D., Cloning and expression of *Bacillus* phytase gene (phy) in *Escherichia coli* and recovery of active enzyme from the inclusion bodies (2008) *J Appl Microbiol*, 105, pp. 1128-1137. , 10.1111/j.1365-2672.2008.03833.x 1:CAS:528:DC%2BD1cXht12lurnE 18479345
 - Sambrook, J., Fritsch, E.F., Maniatis, T., (1989) *Molecular Cloning: A Laboratory Manual*, , Cold Spring Harbor Laboratory Press Cold Spring Harbor
 - Scott, J.J., Loewus, F.A., A calcium-activated phytase from pollen of *Lilium longiflorum* (1986) *Plant Physiol*, 82, pp. 333-335. , 10.1104/pp.82.1.333 1:CAS:528:DyaL28XlvKnrY%3D 16665018
 - Shah, P., Bhavsar, K., Soni, S.K., Khire, J.M., Strain improvement and up scaling of phytase production by *Aspergillus niger* NCIM 563 under submerged fermentation conditions (2009) *J Ind Microbiol Biotechnol*, 36, pp. 373-380. , 10.1007/s10295-008-0506-7 1:CAS:528:DC%2BD1MXitlWrsL0%3D 19082644
 - Shimizu, M., Purification and characterization of phytase from *Bacillus subtilis* (nato) N-77 (1992) *Biosci Biotech Biochem*, 56, pp. 1266-1269. , 10.1271/bbb.56.1266 1:CAS:528:DyaK38Xls1Kns74%3D
 - Simon, O., Igbasan, F., In vitro properties of phytases from various microbial origins (2002) *International Journal of Food Science and Technology*, 37 (7), pp. 813-822. , DOI 10.1046/j.1365-2621.2002.00621.x
 - Stahl, C.H., Wilson, D.B., Lei, X.G., Comparison of extracellular *Escherichia coli* AppA phytases expressed in *Streptomyces lividans* and *Pichia pastoris* (2003) *Biotechnology Letters*, 25 (10), pp. 827-831. , DOI 10.1023/A:1023568826461
 - Sunitha, K., Lee, J.K., Oh, T.K., Optimization of medium components for phytase production by *E. coli* using response surface methodology (1999) *Bioproc Eng*, 21, pp. 477-481. , 1:CAS:528:DyaK1MXnsVajs7c%3D
 - Suominen, I., Karp, M., Lahde, M., Kopio, A., Glumoff, T., Meyer, P., Mantsala, P., Extracellular production of cloned α -amylase by *Escherichia coli* (1987) *Gene*, 61 (2), pp. 165-176. , DOI 10.1016/0378-1119(87)90111-9
 - Viitanen, M.I., Vasala, A., Neubauer, P., Alatosava, T., Cheese whey-induced high-cell-density production of recombinant proteins in *Escherichia coli* (2003) *Microbial Cell Factories*, 2, p. 2. , <http://www.microbialcellfactories.com/content/2/1/2>, DOI

10.1186/1475-2859-2-2

- Vohra, A., Satyanarayana, I., A cost-effective cane molasses medium for enhanced cell-bound phytase production by *Pichia anomata* (2004) *Journal of Applied Microbiology*, 97 (3), pp. 471-476. , DOI 10.1111/j.1365-2672.2004.02327.x
- Vuolanto, A., Von Weymarn, N., Kerovuo, J., Ojamo, H., Leisola, M., Phytase production by high cell density culture of recombinant *Bacillus subtilis* (2001) *Biotechnology Letters*, 23 (10), pp. 761-766. , DOI 10.1023/A:1010369325558
- Wyss, M., Brugger, R., Kronenberger, A., Remy, R., Fimbel, R., Oesterhelt, G., Lehmann, M., Van Loon, A.P., Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): Catalytic properties (1999) *Appl Environ Microbiol*, 65, pp. 367-373. , 1:CAS:528:DyaK1MXpvFCntw%3D%3D 9925555
- Yamabhai, M., Emrat, S., Sukasem, S., Pesatcha, P., Jaruseranee, N., Buranabanyat, B., Secretion of recombinant *Bacillus* hydrolytic enzymes using *Escherichia coli* expression systems (2008) *Journal of Biotechnology*, 133 (1), pp. 50-57. , DOI 10.1016/j.jbiotec.2007.09.005, PII S0168165607015714