Screening for phytase-producing bacteria from hydrothermal environments

Stefanie Gabler¹, Nitza Inostroza², Jacquelinne Acuña², Daniel Menezes-Blackburn³, Ralf Greiner¹ and <u>Milko Jorquera²</u>

¹Max Rubner-Institut, Federal Research Institute of Nutrition and food, Karlsruhe, Germany ²Scientific and Technological Bio-resource Nucleus, Universidad de La Frontera, Temuco, Chile, email: nitza.inostroza@gmail.com

³Lancaster Environment Centre, Lancaster University, Lancaster, UK.

Phytases are ecologically important enzymes involved in the organic phosphorus cycling in nature. These enzymes are widely used as feed additives to improve mineral bioavailability in animal diets [1]. For their biotechnological application, thermal tolerance is, among others, a desired property of phytases. However, so far thermal tolerance has scarcely been included into phytase-screening programs. The objective of this study was isolate and characterize thermo-tolerant bacterial phytases from Chilean hydrothermal environments (Liquiñe hotspring [39°44'S; 71°51'W] and El Tatio geyser [22°19'S, 68°0'W]). Screening of 69 thermotolerant (60°C) culturable bacteria was carried out and the strains Bacillus sp. 9B and Geobacillus sp. 15 were selected and identified (16S rRNA gene) as producers for intra- and extracellular phytases. The results also indicate that both strains are able to synthesize more than one individual phytase. The characterization of intracellular phytase revealed that Bacillus sp. 9B produces phytase that shows an optimum pH at 5.0. This result suggests a novel property for phytases from genus Bacillus which are mostly characterized by Bpropeller phytases with optimal pH in the range of 6.5 to 8.0. An optimum at pH ~5.0 is known for other phytase classes such as histidine acid phosphatase and purple acid phosphatase [2]. The strain Geobacillus sp. 15 also produces phytase that shows optimum pH at 5.0, but with an unusual residual activity at low pH (12% and 30% at pH 1.0 and 4.0, respectively). Most microbial phytases are inactive at low pH, except Aspergillus niger and Escherichia coli phytase [3,4]. The characterization of an acidic thermo-tolerant phytase produced by Geobacillus stearothermophilus from hot-spring has recently been reported [5]. The temperature optimum for phytate dephosphorylation was determined to be 60°C for the phytase from Bacillus sp. 9B and 50°C for the Geobacillus sp. 15 phytase. Interestingly, the phytase from Geobacillus sp. 15 shows a residual activity of 46% after incubation at 90°C for 20 min. Our study demonstrates that Chilean hydrothermal environments represent an unexplored source of novel thermo-tolerant bacterial phytases with more favorable properties for biotechnological applications, for example in food processing or as feed additives.

References:

- [1] Menezes-Balckburn, D., Greiner, R., "*Enzymes Used in Animal Feed: Leading Technologies and Forthcoming Developments*", In: Cirillo, G., Gianfranco-Spizzirri, G., and Lemma, F. (eds.) Functional Polymers in Food Science (Scrivener Publishing LLC, 47-73, 2015).
- [2] Lei, X., Porres, J., Mullaney, E., Brinch-Pedersen, H., "Phytase: Source, Structure and Appication", In: Polaina, J, MacCabe, A. (eds.), Industrial Enzyme/Seconday Phytase: Source, Structure and Appication (Springer, 505-529, 2007).
- [3] Greiner, R., Konietzy, U., Jany, K.D., "Purification and characterization of two phytases from Escherichia coli", Arch. Biochem. Biophys., 303 (1),107-13, 1993.
- [4] Wyss, M., Brugger, R., Kronenberger, A., Remy, R., Fimbel, R., Oesterhelt, G., Lehmann, M., Van Loon, A., "*Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phorsphohydrolases): cataclytic properties*", Appl. Environ. Microb., 65, 2, 367-373, 1999.
- [5] Parhamfar, M., Badoei-Dalfard, A., Khaleghi, M., Hassanshahian, M., "Purification and characterization of an acidic, thermophilic phytase from a newly isolated Geobacillus stearothermophilus strain DM12", Prog. Biol. Sci., 5, 1, 61-73, 2015.