Screening of Soil Bacteria for Phytase Activity

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ABSTRACT

The aim of the present paper was to realize a screening for phytase producing bacteria isolated from soil where pig manure was stored. It was used PSM, PKA media and the molybdate-blue method in order to determine the presence of phytase- phosphate solubilizing- and protease- producing bacteria. About half of the bacterial isolates were phytase producers

Keywords: *bacteria, manure, phytase.*

INTRODUCTION

An important environmental problem is the insoluble phosphate from soil, being about 80% from the total content of soil phosphorus (Mukhametzhanova *et al.*, 2012). The most common form of phosphorus is phytic acid and phytates. Phytates are chelating agents, causing the formation of low degradable compounds. In nature, their hydrolysis is carried out by microbial phytases, which may potentially be used for innovative microbial technologies. Phytase (myo-inositol hexakisphosphate 3-phosphorilase) catalyses the sequential release of phosphate by the hydrolysis of phytic acid in a stepwise manner, releasing four inositol phosphates and inorganic phosphate. They can be synthesized both by microorganisms (bacteria, fungi and yeast) including the rumen microbes in ruminants, and soil microbes, and plants like wheat, barley, rice, corn or rye (Paik, 2003).

MATERIALS AND METHODS

Bacteria were isolated from soil sample collected from two households. Preliminary identification of the isolated strains was carried out on the basis of morphological analysis using Gram staining, microscopic observations and

culture characteristics. The screening method for phytase activity was performed by using PSM (Bae *et al.*, 1999). The PKA medium was used for testing inorganic phosphate solubilisation ability (Kaur and Reddy, 2013). The detection of clear zone surrounding the bacterial colonies is the sign of phosphate solubilising activity or phytase biosynthesis. The determination of phytase activity was made by molybdate-blue method. The experiment was realized with three repetitions in order to obtain accurate results. The detection of clear zone surrounding the bacterial colonies is the sign of phosphate solubilising activity or phytase biosynthesis. The efficiency of hydrolysis was determined by the formula $Z-C/C$, where Z = halo diameter and C = colony diameter.

RESULTS AND DISCUSSIONS

Most of the isolated bacterial strains are Gram-. From 55 isolates, 27 are able to synthesize phytases, from which 17 presented high enzymatic activities (according to halo dimension and enzymatic assay). Additionally, 38 bacterial isolates have proteolytic activity (detected on casein containing medium) and only one proved to solubilise the inorganic phosphate (Tab.1).

Tab. 1. The results obtained after screening

Bacteria	Phytases synthetization	Phosphate solubilization	Proteolytic activity	Bacteria	Phytases synthetization	Phosphate solubilization	Proteolytic activity
1	-	-	-	31	-	-	-
2	-	-	+	32	+++	-	++
3	-	-	-	33	-	-	+
4	-	-	+	34	+	+	++++
5	-	-	++++	35	++++	-	+
6	-	-	+	36	+	-	+
7	-	-	+	37	-	-	+++
8	-	-	+++	38	-	-	++++
9	+	-	++++	39	-	-	+++
10	+++	-	+	40	-	-	++++
11	++++	-	++++	41	-	-	+
12	++++	-	++++	42	++	-	-
13	++++	-	+	43	-	-	-
14	+++	-	+	44	-	-	+++++
15	+	-	+	45	+	-	+++
16	+	-	-	46	-	-	+++
17	+	-	++++	47	-	-	++
18	+++	-	++	48	-	-	+++
19	+	-	++	49	-	-	+
20	+	-	+++	50	++++	-	++++
21	++	-	++++	51	-	-	-
22	-	-	+++	52	-	-	-
23	+++	-	+++	53	-	-	-
24	++	-	++	54	-	-	-
25	++++	-	+	55	-	-	-
26	++++	-	+				
27	+++	-	++				
28	+++	-	+				
29	-	-	+++				
30	+++	-	+++				

CONCLUSIONS

According to the screening test, about half of the bacterial isolates were phytase producers. The best producers may be used in further studies for the remediation of soil polluted with insoluble phosphorus compounds.

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REFERENCES

1. Bae HD, Yanke LJ, Cheng KJ, Selinger LB (1999). A novel staining method for detecting phytase activity. *J Microbiol Methods* 39(1):17- 22.
2. Kaur G, Reddy MS (2013). Phosphate solubilizing rhizobacteria from an organic farm and their influence on the growth and yield of maize (*Zea mays* L.), *J. Gen. Appl. Microbiol.* 59: 295-303.
3. Mukhametzyanova AD, Akhmetova AI, Sharipova MR (2012). Microorganisms as Phytase Producers. *Microb.* 81(3):267-275
4. Paik I (2003). Application of Phytase, Microbial or Plant Origin, to Reduce Phosphorus Excretion in Poultry Production. *Asian-Aust. J. Anim. Sci.* 16(1):124-135.