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International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Characterization of Salt Tolerant Microbial Strains Isolated from Agricultural Field Soils of Andhra Pradesh

Darsi Phebe Sarah *

Department of Botany & Microbiology, Andhra Christian College, Guntur, Andhra Pradesh, India.

ABSTRACT

This paper describes production and characterization of Amylase, Chitinase, L- Asparagine and Lipases from Bacillus species isolated from chilli rhizosphere in the vicinity of Guntur, Andhra Pradesh. On the basis of zone formed, ten bacterial strains was selected and identified as *Bacillus* species and names as *Bacillus sp.* 231 to *Bacillus sp.* 240 This study investigates the optimization and production of Amylase, Chitinase L- Asparagine and Lipase. Days of incubation, temperature, pH, supplementary carbon and nitrogen sources on the production of amylase, chitinase, L- Asparagine and Lipase was studied and accordingly optimum conditions were determined. The isolated soil Bacillus *species* produces high levels of Amylase, chitinase and L- Asparagine, under optimized culture conditions, *viz.*, on the third day of incubation at an optimum pH of 7.5 and temperature of 37º C. Enzyme production was recorded with different concentrations of Nacl 0.25, 0.5, 0.75,1.0, 1.25, 1.50 and 2.0%. Maximum enzyme production was observed at 1.0% NaCl Concentrations. The isolate *Bacillus* sp. 265 showed highest enzyme Amylase 21 IU/mg, Chitinase 23 IU/mg, L- Asparginase production 22 IU/mg and Lipase production 25 IU/mg respectively. These enzymes are crucial to making cheese, brewing beer, baking bread, extracting fruit juice, tanning leather, and much more.

Keywords: Amylase, Chitinase**,** L- Asparginase, Lipase, Bacillus

Introduction

Enzymes are one of the essential products acquired for the needs of human through microorganisms. Enzymes like Amylases, Lipases, L- Asparginase, Glutaminase, Proteases and Cellulases has great industrial value in the present-day market. Among these Amylases plays a crucial role in enzyme market. Most of the prominent enzymes like protease, cellulases and amylase were used in many industries. L-asparaginase belongs to an amidase group that produces aspartic acid and ammonia by asparagine hydrolysis (1,2). The search for other asparaginase sources, like eucaryotic microorganisms, can lead to an enzyme with less adverse effects. The importance of microorganisms as L-asparaginase sources has been focused since the time it was obtained from *Escherichia coli* and its antineoplastic activity demonstrated in guinea pig serum (2,3,4,5,6,7).

This enzyme is widely distributed, being found in L-asparaginase is widely distributed, being found in animal, microbial and plant sources. Large number of microorganisms that include *Erwinia carotovora*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa* and *E. coli*. It has been observed that eukaryotic microorganisms like yeast and fungi have a potential for asparaginase production. For example, the mitosporic fungi genera such as *Aspergillus*, *Penicillium*, and *Fusarium*, are commonly reported in scientific literature to produce asparaginase (8,9,10).

Plant Growth Promoting Rhizobacteria (PGPR) have been found to be effective in reducing abiotic stresses in plants, thereby promoting sustainable agriculture. In recent years, researchers have been exploring the potential of PGPR in enhancing plant growth under salt stress (17,18,19). The interaction between microbes and plants provides multiple mechanisms that induce salt stress resistance in plants. Previous studies have shown that PGPRs like Pesudomonas spp. and Bacillus spp. isolated from saline soil can promote plant growth under saline conditions. This has led to increased interest in using PGPR as a bio-resource to reduce the effects of salinity and improve plant growth in saline areas (20,21,22,23,24).

Recent studies have reported significant results in using PGPR to reduce salt stress in wheat plants, leading to enhanced growth and productivity in saline areas. PGPR or certain compounds can help plants withstand salt stress by modulating hormonal, photosynthetic, and ROS scavenging pathways. Among PGPR, Bacillus strains are particularly promising due to their gram-positivity, ability to make spores, colonize roots, and promote plant growth (25,26). Bacillus strains have been shown to alleviate abiotic stresses in plants, but their genetic features enabling them to show resistance under extreme conditions have been understudied (27,28). Therefore, it is crucial to develop high salinity-tolerant Bacillus strains with salt-resistant genetic features that can produce bioactive osmotic compatible solutes to cope with harsh salt stress conditions.

Materials and methods

Isolation

In the process of isolation, one gram of soil sample was taken and suspended in 10ml of sterile distilled water and then shaken thoroughly for 10 minutes. Nutrient Agar Medium was used in Serial dilution plate technique for the isolation of microorganisms from the collected samples. Sterilized water was utilized for the preparation of serial dilutions up to 10^{-5} for each sample. These dilutions were placed on plates containing Nutrient Agar Medium (NAM), triplicates were maintained for each plate, and then were incubated at 35°C for 24 to 48 h. pure colonies were selected and maintained on Nutrient Agar Medium (NAM) slants at 4°C and further estimated for enzyme production in liquid medium (12).

Enzyme Assay:

Assay of enzyme was carried out as per Imada *et al*. (13). 0.5 ml of 0.04 M asparagine was taken in a test tube, to which 0.5 ml of 0.5 M buffer (acetate buffer pH 5.4), 0.5 ml of enzyme and 0.5 ml of distilled water was added to make up the volume up to 2.0 ml and incubate the reaction mixture for 30 min. After the incubation period the reaction was stopped by adding

0.5 ml of 1.5 M TCA (Trichloroacetic acid). 0.1 ml was taken from the above reaction mixture and added to 3.7 ml distilled water and to that 0.2 ml Nessler's reagent was added and incubated for 15 to 20 min. The OD was measured at 450 nm. The blank was run by adding enzyme preparation after the addition of TCA. The enzyme activity was expressed in International unit (11,14,15).

Statistical Analysis

All measurements were carried out in triplicate. Statistical analyses were performed using one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple range tests. Differences at $P < 0.05$ were considered statistically significant.

Results and Discussion

A total of 10 isolates were obtained from chilli rhizosphere in the vicinity of Guntur, Andhra Pradesh. The preliminary characterization like cultural and biochemical characteristics was done by Bergey's manual of systemic bacteriology. All the isolates belong to *Bacillus* species according to their preliminary and biochemical studies. All the isolates were designated as Bacillus sp. PS231 to Bacillus sp. PS240 and tested for amylase, chitinase activity, L- Asparginase and lipase were studied. Amylase production was recorded with different concentrations of Nacl 0.25, 0.5, 0.75,1.0, 1.25, 1.50 and 2.0%. Maximum enzyme production was observed at 1.0% NaCl Concentrations. (Table-1). In this study enzyme production of each strain is based on the specific growth rate of the strain.

Table-1: Amylase production (IU/mg) from different concentrations of NaCl

Strain no.	Nacl (0.25%)	Nacl (0.5%)	Nacl (0.75%)	Nacl (1.0%)	Nacl (1.25%)	Nacl (1.5%)	Nacl (2.0%)
Bacillus Sp. PS231	0.12	0.15	0.17	0.20	0.13	0.07	-
Bacillus Sp. PS232	0.15	0.17	0.15	0.17	0.11	0.05	-
Bacillus Sp. PS233	0.11	0.14	0.15	0.17	0.11	0.07	-
Bacillus Sp. PS234	0.13	0.14	0.14	0.21	0.10	0.10	0.10
Bacillus Sp. PS235	0.05	0.07	0.07	0.22	0.08	0.07	-
Bacillus Sp. PS236	0.04	0.07	0.09	0.12	0.08	0.08	0.05
Bacillus Sp. PS237	0.07	0.09	0.07	0.14	0.10	0.10	0.05
Bacillus Sp. PS238	0.11	0.14	0.14	0.21	0.14	0.12	0.05
Bacillus Sp. PS239	0.05	0.07	0.09	0.13	0.10	0.08	-
Bacillus Sp. PS240	0.04	0.06	0.08	0.15	0.10	0.08	-

*The overall model is significant with p<0.05

Chitinase production was studied from different concentrations of NaCl 0.25 to 2.0% (Tabl-2). Chitinase activity was observed by all the ten species of bacillus designated as Bacillus sp.231 to Bacillus PS 240. Maximum chitinase activity observed in 1.0% NaCl concentration. The pervious reports supports to this study (19).

Table-2: Chitinase production (IU/mg) from different concentrations of NaCl

Strain no.	Nacl (0.25%)	Nacl (0.5%)	Nacl (0.75%)	Nacl (1.0%)	Nacl (1.25%)	Nacl (1.5%)	Nacl (2.0%)
Bacillus Sp. PS231	0.04	0.09	0.14	0.17	0.10	0.04	-
Bacillus Sp. PS232	0.07	0.09	0.12	0.18	0.10	0.06	-
Bacillus Sp. PS233	0.04	0.08	0.10	0.15	0.08	0.04	-

Bacillus Sp. PS234	0.11	0.14	0.17	0.23	0.12	0.08	0.05
Bacillus Sp. PS235	0.05	0.09	0.12	0.15	0.11	0.05	0.02
Bacillus Sp. PS236	0.06	0.11	0.14	0.20	0.13	0.06	0.04
Bacillus Sp. PS237	0.08	0.12	0.14	0.14	0.12	0.08	0.06
Bacillus Sp. PS238	0.10	0.14	0.16	0.22	0.12	0.10	0.07
Bacillus Sp. PS239	0.07	0.12	0.14	0.15	0.10	0.07	0.06
Bacillus Sp. PS240	0.10	0.12	0.15	0.15	0.10	0.10	-

***The overall model is significant with p<0.05**

The L- Aspargine production was studied from different concentrations of NaCl 0.25 to 2.0% (Tabl-3). L- Aspargine activity was observed by all the ten species of bacillus designated as Bacillus sp.231 to Bacillus PS 240. Maximum chitinase activity observed in 1.0% NaCl concentration. Our results coincide with the other reports (17,18,19,26).

Table-3: L- Aspargine production (IU/mg) from different concentrations of NaCl

Strain no.	Nacl (0.25%)	Nacl (0.5%)	Nacl (0.75%)	Nacl (1.0%)	Nacl (1.25%)	Nacl (1.5%)	Nacl (2.0%)
Bacillus Sp. PS231	0.08	0.11	0.15	0.20	0.15	0.05	0.02
Bacillus Sp. PS232	0.12	0.12	0.16	0.18	0.14	0.07	-
Bacillus Sp. PS233	0.11	0.12	0.15	0.21	0.13	0.10	0.03
Bacillus Sp. PS234	0.11	0.14	0.17	0.22	0.12	0.10	-
Bacillus Sp. PS235	0.07	0.12	0.12	0.15	0.11	0.07	-
Bacillus Sp. PS236	0.08	0.11	0.15	0.17	0.12	0.08	0.03
Bacillus Sp. PS237	0.09	0.12	0.14	0.14	0.10	0.09	0.05
Bacillus Sp. PS238	0.11	0.14	0.12	0.21	0.11	0.08	-
Bacillus Sp. PS239	0.04	0.12	0.14	0.15	0.11	0.06	0.04
Bacillus Sp. PS240	0.08	0.10	0.12	0.15	0.12	0.07	0.04

*The overall model is significant with p<0.05

The Lipase production was studied from different concentrations of NaCl 0.25 to 2.0% (Tabl-4). Lipase activity was observed by all the ten species of bacillus designated as Bacillus sp.231 to Bacillus PS 240. Maximum lipase activity observed in 1.0% NaCl concentration. Our results coincide with the other reports (1,14,23).

Table-4: Lipase production (IU/mg) from different concentrations of NaCl

Strain no.	Nacl (0.25%)	Nacl (0.5%)	Nacl (0.75%)	Nacl (1.0%)	Nacl (1.25%)	Nacl (1.5%)	Nacl (2.0%)
Bacillus Sp. PS231	0.05	0.08	0.11	0.18	0.10	0.12	-
Bacillus Sp. PS232	0.07	0.09	0.12	0.16	0.10	0.15	-
Bacillus Sp. PS233	0.09	0.10	0.15	0.15	0.08	0.11	-
Bacillus Sp. PS234	0.05	0.08	0.12	0.25	0.12	0.13	0.04
Bacillus Sp. PS235	0.07	0.09	0.15	0.17	0.11	0.05	0.02
Bacillus Sp. PS236	0.06	0.10	0.15	0.16	0.13	0.04	0.04
Bacillus Sp. PS237	0.08	0.10	0.14	0.21	0.12	0.07	0.05
Bacillus Sp. PS238	0.09	0.10	0.12	0.22	0.12	0.11	0.04
Bacillus Sp. PS239	0.06	0.08	0.12	0.13	0.10	0.05	0.06
Bacillus Sp. PS240	0.07	0.10	0.14	0.14	0.10	0.04	-

Conclusion

From the results the salt-tolerant plant growth-promoting rhizobacteria (PGPR) isolated from saline soils in the vicinity of Guntur, collected from agriculture filed soils. And these soils contain maximum salinity and it can be used to overcome the detrimental effects of salt stress on plants, with beneficial effects of physiological functions of plants such as growth and yield, and overcome disease resistance. Therefore, application of microbial inoculants to alleviate stresses and enhance yield in plants could be a low cost and environmental friendly option for the management of saline soil for better crop productivity in and around Guntur.

Acknowledgements

Author would like to greatly thankful the department of Botany and Andhra Christian College, Guntur for providing necessary facilities to complete this research work.

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