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Isolation and Screening of Microorganisms from Municipal Solid Waste for Production of Amylase

***Jadhav A. V., Patil S. S. and Pathade G. R.**

Krishna Institute of Allied Sciences,
Krishna Vishwa Vidyapeeth, "Deemed to be University", Karad, M.S., India

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ABSTRACT

One of the major problems of the present world is the management of municipal solid waste which in produced in enormous amount. For sustainable management of the solid waste, the use of microbial enzymes can be a better alternative over chemical conventional methods. Amylase is of great importance in textile, food and pharmaceutical industry. It can be used for biotransformation of wastes. The municipal solid waste can be used to isolate amylase producing bacteria that can help in bio-transformation of irritant municipal waste. Here, we have isolated two potent amylase producing strains from municipal solid waste, OW1 and OW2. These isolates were studied for their morphological, cultural and biochemical characters. The isolates were screened for the production of enzyme amylase. Optimization of growth of the isolates with respect to pH and temperature. The potential of the isolates for waste degradation was checked by weight loss method. The isolates were then tentatively identified as *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Key words : *Municipal solid waste, Amylase producing bacteria, pH and temperature optimization, Pseudomonas aeruginosa, Bacillus subtilis.*

Introduction

There has been a considerable increase in municipal solid waste due to rapid population growth in many cities Mahjabeen *et al*. (2018). The municipal organic solid waste mainly composed of food waste, kitchen scraps, and yard waste is generated from markets and households. These are inadequately disposed in landfills causing the emission of greenhouse gases, their decomposition, affecting the ecosystem (Musa, *et al*., 2020; Gidarakos *et al*., 2006). The main composition of wastes composed of starch (Gao *et el*., 2015; Awasthi *et al.,* 2018). Starch, for their degradation into simple molecules, requires the extracellular enzymatic action of amylase (Saha and Santra, 2014; Al-Dhabi *et al*., 2019). Amylases of bacterial origin serves as an exquisite alternative to the hydrolysis of starch by chemical methods (Shaw *et al*., 1995). Bacterial amylase has gained profound importance in the field of textile industry, biotechnology, food processing and pharmaceutical industry (Pandey *et al*., 2000). Thus, the municipal solid waste management can be better achieved by using amylase producing bacteria. But the municipal waste might contain different toxic metal ions, irritant chemicals and radioactive materials. In this adverse condition, the bioconversion of waste is limited due to the hindered growth of microbes and restricted enzyme production. This predicament can be outmaneuvered by using the municipal solid waste as a source of amylase producing bacteria and the isolated strains tolerating the detrimental condition of municipal waste should produce enough amylase for their bio-conversion. The aim of the present study was to isolate

amylase producing bacteria from municipal solid wastes and to utilize them in large scale waste management. The isolates were further identified and characterized and the growth conditions were optimized.

Materials and Methods

Collection of sample: A sample was collected from municipal solid waste disposal site in sterile zip-lock plastic bags and was immediately brought to the laboratory and subjected to analysis. Serial dilution techniques were used for isolation of bacteria. Serial dilution of the samples was prepared by in the range of 10^{-1} to 10^{-4}.

Isolation of bacteria: 1ml from the stock was transferred to the 10^{-1} dilution blank using a fresh sterile pipette, 1ml from the 10^{-1} dilution was transferred to the 10^{-2} tube for each succeeding step then from the 10^{-2} to the 10^{-3} then from the 10^{-3} to the 10^{-4} tube. From each dilution tube 0.1 ml of dilution fluid was spread inoculated on Nutrient Agar culture media and incubated at 37 °C for 24 h. After the observation of colonies, the pure culture of bacteria was subcultured in NA slants; incubated at 37 °C to achieve vigorous growth and then preserved at 4 °C in refrigerator. Morphological, cultural and biochemical characterization of the strains were also carried out.

Screening of amylase production: All the isolated bacterial strains were screened qualitatively for the production of enzyme amylase. Each bacterial strain was streaked on the four corners of the Starch Agar. The petri plates were incubated overnight at 30^0C. Then the plates were flooded with indicator 0.1% iodine solution and the development of clear zone around the growth of organism was considered positive enzyme activity.

Production and optimization conditions: All the obtained isolates were suspended for optimal enzyme production with various physical parameters (pH and temp).

Waste degradation studies: The waste degradation potential of bacteria was studied by weight loss method. Waste samples were collected in sterile bags under aseptic condition and brought to the laboratory and sterilized in autoclave at 121 °C for 15 min. Sterilized waste samples were then weighted and inoculated with bacterial isolates and kept at 37 °C for 7, 15 and 21 days, respectively. After incubation, waste samples were washed with sterile water to remove inoculated microorganisms and then they were dried and weighted. The weight loss was calculated and from that very data, degradation potential of bacteria was determined.

Results and Discussion

The isolation and characterization of bacteria from Municipal solid waste dump site was undertaken in this study. Total 6 bacterial isolates were obtained of which two isolates were potent amylase producers.

The color of the isolate OW1 colony was pale yellow and isolate OW2 was blue. Both the colonies were circular, convex, opaque and moist. OW1 was Gram positive while OW2 was Gram negative in nature (Table 1).

The isolate OW1 was positive for all the tests except for indole production, methyl red test and urease production. The isolate OW2 was negative for most of the tests except for mannitol fermentation, nitrate reduction, urease, catalase and oxidase production (Table 2). Comparing these results with the Bergey's Manual of Bacteriology Volume I and II, the isolates were tentatively identified as,

1) OWI as *Bacillus subtilis*

2) OW2 as *Pseudomonas aeruginosa*

Isolates OW1 and OW2 produces maximum enzyme at pH 7.2. Isolate OW2 was seen to produce more enzyme units than isolate OW1 (Table 3).

Isolate OW1 produces maximum enzyme at temperature 37 °C. Maximum enzyme production by isolate OW2 was seen at temperature 33 °C.

Table 1. Colony and morphological characters of bacterial isolates OW1 and OW2

Isolate	Colony characters							Gram nature	Motility
	Size	Shape	Color	Margin	Elevation	Opacity	Consistency		
OW1	2mm	Circular	Pale yellow	Regular	Convex	Opaque	Moist	Gram positive rods	Motile
OW2	1mm	Circular	Blue	Regular	Convex	Opaque	Moist	Gram negative rods	Motile

Table 2. Biochemical tests of bacterial isolates OW1 and OW2.

Sr.No	Biochemical tests	OW1	OW2
1	Glucose (acid production)	+	-
2	Lactose (acid production)	+	-
3	Mannitol (acid production)	+	+
4	Sucrose (acid production)	+	-
5	Maltose (acid production)	+	-
6	Indole production	-	-
7	Methyl red test	-	-
8	Voges Proskauer test	+	-
9	Nitrate reduction test	+	+
10	Urease production test	-	+
11	Catalase production test	+	+
12	Oxidase production test	+	+

+ indicates positive test – indicates negative test

Table 3. Effect of pH on production of enzyme amylase by bacterial isolates OW1 and OW2.

pH	O. D. at 530 nm		Enzyme Unit/mL	
	OW1	OW2	OW1	OW2
5.2	0.03	0.06	2.7963	5.5926
6.5	0.04	0.07	3.7284	6.5247
7.2	0.05	0.09	4.6605	8.3889
8.9	0.04	0.05	3.7284	4.6605

Table 4. Effect of temperature on production of enzyme amylase by bacterial isolates OW1 and OW2.

Temperature	O. D. at 530 nm		Enzyme Unit/mL	
	OW1	OW2	OW1	OW2
25 ^{0}C	0.04	0.05	3.7284	4.6605
29 ^{0}C	0.06	0.08	5.5926	7.4568
33 ^{0}C	0.09	0.09	8.2894	8.2894
37 ^{0}C	0.12	0.06	11.1852	5.5926

The table 5 shows that the isolate OW1 have more degradative capability than isolate OW2.

Table 5. Weight of waste after inoculation of the bacterial isolates OW1 and OW2 after different periods.

Isolate	1st day	7th day	15th day	21st day
OW1	100 g	95.5 g	90.7 g	85.9 g
OW1	100 g	96.3 g	89.5 g	86.6 g

Summary and Conclusion

Municipal solid waste is a combination of different substrates; thus, it is an ideal enrichment media for cultivation of numerous microorganisms. Bacteria in this environment are metabolically active which leads to the production of various enzymes. The isolates obtained were subjected to morphological, biochemical and cultural characterization. The identification was done with OW1 - as *Bacillus subtilis* and OW2 as *Pseudomonas aeruginosa.* The Amylase yield was measured using assay method. The units of Amylase produced by *Bacillus subtilis* was found to be 0. 02 U/ml. The units of Amylase produced by *Pseudomonas aeruginosa* was found to be 0.055 U/ml.

The optimization studies with both the isolates on the production of enzyme amylase varying with pH and temperature showed that isolate OWI showed optimum growth for production of enzyme amylase at pH 7.2 and temperature 37°C than the isolate OW2.

The waste degradation potential from all the isolates showed a great significance. The isolate OW1 showed more waste degradation capacity. Thus, these isolates can be employed for the solid waste degradation at dump sites.

Conflict of Interest

No conflict of interest is there amongst the authors.

References

Awasthi, M.K., Wong, J. W. C., Kumar, S., Awasthi, S.K., Wang, Q., Wang, M., Ren, X., Zhao, J., Chen, H. and Zhang, Z. 2018. Biodegradation of food waste using microbial cultures producing thermostable á-amylase and cellulase under different pH and temperature. *Bioresource Technology.* 248(PtB):160–170.

Al-Dhabi, N.A., Esmail, G.A., Mohammed Ghilan, A.K. and Valan Arasu, M. 2019. Composting of Vegetable Waste Using Microbial Consortium and Biocontrol Efficacy of *Streptomyces* sp. Al-Dhabi 30 Isolated from the Saudi Arabian Environment for Sustainable Agriculture. *Sustainability*. 11(23): 6845. https://doi.org/10.3390/su11236845.

Gidarakos, E., Havas, G. and Ntzamilis, P. 2006. Municipal solid waste composition determination supporting the integrated solid waste management system in the island of Crete. *Waste Management (New York, N.Y.)*, 26(6): 668–679. https://doi.org/10.1016/j.wasman.2005.07.018.

Gao, W., Chen, Y., Zhan, L. and Bian, X. 2015. Engineering properties for high kitchen waste content municipal solid waste. *J. Rock Mech. Geotech. Eng.* 7(6): 646–658.

Pandey, A., Nigam, P., Soccol, C., Thomas, S. V., Singh, D. and Mohan, R. 2000. Advances in Microbial

Amylases. *Biotechnology and Applied Biochemistry.* 31(2): 135-52.

Mahjabeen, F., Khan, S., Choudhury, N., Hossain, M.M. and Khan, T.T. 2018. Isolation of Cellulolytic Bacteria from Soil, Identification by 16S rRNA Gene Sequencing and Characterization of Cellulase. *Bangladesh Journal of Microbiology*. 33(1-2): 17–22. https://doi.org/10.3329/bjm.v33i1.39598

Musa, A.M., Ishak, C.F., Karam, D.S. and Md Jaafar, N. 2020. Effects of fruit and vegetable wastes and biodegradable municipal wastes co-mixed composts on nitrogen dynamics in an oxisol. *Agronomy.* 10(10): 1609.

Saha, A. and Santra, S.C. 2014. Isolation and characterization of bacteria isolated from municipal solid waste for production of industrial enzymes and waste degradation. *J Microbiol Exp.* 1(1): 12-19.

Shaw, J.F., Lin, F.P., Chen, S.C. and Chen, H.C. 1995. Purification and Properties of an Extracellular á-Amylase from Thermus sp. *Botanical Bulletin of Academia Sinica.* 36(3): 195-200.