



Biodegradation by Proteolytic Bacteria: An Attractive Alternative for Biological Waste Treatment

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Nat. Env. Poll. Tech.
ISSN: 0972-6268
www.neptjournal.com

Key Words:

Proteases enzyme
Proteolytic bacteria
Proteinaceous waste
Waste treatment

ABSTRACT

Protease enzyme found in proteolytic bacteria brings about proteolysis by hydrolysis of peptide bond in the polypeptide chain. Evidence for extracellular proteolytic activity was demonstrated for *Pseudomonas aeruginosa* and *Bacillus cereus* isolated from soil. The two bacterial isolates were screened for proteases production based on their growth in liver particle medium and further evaluated for proteases activity against proteinaceous substrates like gelatin, milk protein, soya protein, bovine meat protein and egg protein. The proteolytic activity of cell-free extracts of strains varied. Furthermore, difference between hydrolysing activities of the bacterial isolates towards all substrates indicates the presence of powerful extracellular proteolytic activity. The experiment was designed to evaluate the ability of proteolytic bacteria to degrade organic waste components. The study hints towards a practical and economic solution for hydrolysing most solid proteinaceous waste generated from industries like meat industries, oil refineries, dairy processing industries, egg and poultry industries and food industries.

INTRODUCTION

Annually, great amounts of proteinaceous wastes, which could be measured in many billions of tons, are produced worldwide as residues from industrial processing. However, as the price of customary disposal or treatment options have risen, and ever more stringent legislation been imposed, alternative technologies like microbial methods have become increasingly attractive in the light of their greater relative cost-effectiveness (Gareth et al. 2003).

Proteases bring about proteolysis by hydrolysis of peptide bond in the polypeptide chain (Barrett et al. 2003). Proteases, occurring naturally in all organisms, are essential for cell growth and differentiation and execute a large variety of functions having important biotechnological applications (Beynom & Bond 1989). Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity and their susceptibility to genetic manipulation (Rangaswami & Bagyaraj 2004).

A preliminary study on assessment of proteinaceous waste by-products generated from meat industries, oil refineries, dairy processing industries, egg and poultry industries and food industries was performed. The microbial biotreatment method was designed to enlighten the ability of proteolytic bacteria to hydrolyse these solid proteinaceous wastes produced from the industries. In the present study isolation of bacterial isolates from soil and their action against various proteinaceous compounds to determine their proteolytic activity was made.

When microbes were cultured on the proteinaceous substrates like gelatin, milk protein, soya protein, bovine meat protein and egg protein, protein was solely available for bacterial growth. The nitrogen, carbon and energy are locked in protein. In order to set these growth factors free for use, the

first process that must take place is enzymatic hydrolysis of proteins. Proteins are degraded by these proteolytic bacteria and they utilize the degradation products like amino acids as nutrients for their growth. The extent of proteolytic activity by microorganisms can be determined by the amount of enzyme that releases in substrate under incubation period.

MATERIALS AND METHODS

Isolation and identification of proteolytic bacteria: Two important bacterial species, *Pseudomonas aeruginosa* and *Bacillus cereus* were isolated from soil using substrates corresponding to that present in industrial wastes. The individual bacterial isolates were identified on the basis of morphology, biochemical and cultural characteristics, and cultivated on their specific media.

Screening of proteolytic bacteria: Individual colonies were screened for protease enzyme production on liver particle medium. The inoculated liver particle medium was incubated at 37°C for 24 h and observed for turbidity, which indicates proteolytic activity of microorganism (Ronald et al. 2001).

Determination of proteolytic activity: For determining the efficacy of isolates to digest the protein, proteinaceous substrates corresponding to the proteinaceous wastes generated from industries, were prepared as 5% solutions in distilled water and sterilized by appropriate technique. The pH was adjusted in range of 7.2-7.7. Inoculums of 0.5 mL of each isolate was inoculated in 5 mL of substrate solution thus prepared. Incubation was done at 37°C for 24 hrs.

McFarland standards were used as a reference to adjust the turbidity of bacterial suspensions so that the same numbers of colony forming units are present in 5 mL of substrate solution. The standard can be compared visually to bacterial suspension (Koch 1970, 1994, Lisa et al. 1999). The types of proteinaceous wastes generated from the industries (Vasso Oreopoulou & Winfried Russ 2007) are given in Table 1.

Total protein assay: The two bacterial isolates inoculated in the respective substrates were later observed for their proteolytic activity. Each time one uninoculated sample was used as control. The test samples were centrifuged and the supernatant, thus, collected was estimated for total protein content by Folin and Lowry method (Lowry et al. 1951).

RESULTS AND DISCUSSION

The study demonstrates the extracellular proteolytic activity for *Pseudomonas aeruginosa* and *Bacillus cereus*. Extracellular proteases activity for *Bacillus* was reported by Glenn (1976), Priest (1977), Ward (1985) and Pastor et al. (2001). Protease activities of *Pseudomonas* were found out by Morihara (1963, 1964), Glenn (1976) and Bayouth (2000).

There are reports on proteases enzyme available for study of optimization of pH range (Kalaiarasi & Sunitha 2009, Kumar et al. 2002, Borriss 1987), optimum temperature (Fujiwara & Yamamoto 1987, Kalaiarasi & Sunitha 2009) and maximum growth supporting incubation period (Kalaiarasi & Sunitha 2009, Kumar et al. 2002). Taking into consideration the above references, pH was adjusted in range of 7.2-7.7 and incubation was done at 37°C for 24 hrs.

Taking into consideration the work done on proteolytic activity of microorganisms, the present work has been confined to evaluate the ability of proteolytic bacteria *Pseudomonas aeruginosa* and *Bacillus cereus* to degrade organic waste components.

Proteolytic activity of bacterial isolates, studied in accordance to Folin-Lowry method, depicts the following results.

Table 1: Industries, types of proteinaceous wastes with protein content and corresponding substrates used in the experiment.

Industry	Type of Proteinaceous wastes with protein content (%)	Corresponding substrates used in experiment
Food & Pharma Industry	Gelatin (98 -99%)	Gelatin
Dairy Processing Industry	Whey containing milk protein (12.1-17.9 %)	Casein or Milk Protein
Soya Oil Refineries	Cracked soyabeans, malt (38-42%)	Soya Protein
Meat Industry	Slaughterhouse waste (34.6%)	Bovine Meat Protein
Egg & Poultry Industry	Egg, Egg shells (12.9%)	Egg Protein

Table 2: The estimation of gelatin degradation.

	B	1	2	3	4	5	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>
O.D. for gelatin	0.00	0.03	0.06	0.09	0.12	0.15	0.09	0.06
Gelatin degraded (mg/dl)	0.00	4	8	12	16	20	12	8

Table 3: The estimation of casein or milk protein degradation.

	B	1	2	3	4	5	<i>Pseudomonas aeruginosa</i>	<i>Bacillus ereus</i>
O.D. for casein	0.00	0.02	0.04	0.06	0.08	0.10	0.06	0.07
Casein degraded (mg/dl)	0.00	4	8	12	16	20	12	14

Table 4: The estimation of soy protein degradation.

	B	1	2	3	4	5	<i>Pseudomonas aeruginosa</i>	<i>Bacillus ereus</i>
O.D. for soya protein	0.00	0.02	0.04	0.06	0.08	0.10	0.06	0.08
Soya protein degraded (mg/dl)	0.00	4	8	12	16	20	12	18

Table 5: The estimation of bovine meat protein degradation.

	B	1	2	3	4	5	<i>Pseudomonas aeruginosa</i>	<i>Bacillus ereus</i>
O.D. for bovine meat protein	0.00	0.02	0.04	0.06	0.08	0.10	0.07	0.07
Bovine meat protein degraded (mg/dl)	0.00	4	8	12	16	20	14	14

Table 6: The estimation of egg protein degradation.

	B	1	2	3	4	5	<i>Pseudomonas aeruginosa</i>	<i>Bacillus ereus</i>
O.D. for egg protein	0.00	0.02	0.04	0.06	0.08	0.10	0.09	0.11
Egg protein degraded (mg/dl)	0.00	4	8	12	16	20	9	11

Estimation of gelatin degradation: The study on gelatin degradation suggests that *Pseudomonas aeruginosa* utilizes the substrate most followed by *Bacillus cereus* as shown in Table 2. The results coincide with the findings of Singh & Venkataramana (1998), who reported that gelatin was more suitable nitrogen source for growth as well as for production of enzymes by the *Pseudomonas* sp.

Estimation of casein degradation: The study on casein degradation suggests that *Bacillus cereus* utilizes this substrate most followed by *Pseudomonas aeruginosa* as given in Table 3. This finding is in agreement with findings of Wang & Hsu (2005) who found that casein was better nitrogen source of protease production. McConn et al. (1964) reported similar results.

Estimation of soya protein degradation: The study on soya protein degradation suggests that *Bacillus cereus* utilizes the substrate most followed by *Pseudomonas aeruginosa* (Table 4). The results concur with Sinha & Satyanarayana (1999) who reported similar results for *Bacillus* stating that soya meal was best nitrogen source for protease production. Barindra Sana et al. (2006) accounted the commercial applications of soya protein as substrate for protease production.

Estimation of bovine meat protein degradation: The study on bovine meat protein degradation suggests the equivalent utilization by *Pseudomonas aeruginosa* and *Bacillus cereus* as given in Table 5. The results find correlation with the findings of Dalev (1994), Chen Qihe et al. (2006), and Kalaiarasi & Sunitha (2009).

Estimation of egg protein degradation: The study on egg protein degradation suggests that *Bacillus cereus* utilizes the substrate most followed by *Pseudomonas aeruginosa* as given in Table 6. The results agree with the findings of Lokendra Singh and Venkataramana (1998) who reported that egg protein yields high growth of *Bacillus*.

There are different reports available on proteolytic activity against a wide variety of substrates reported by Morihara (1963), Singh & Venkataramana (1998), Barindra Sana et al. (2006) and Kalaiarasi & Sunitha (2009). The efficacy of *Pseudomonas aeruginosa* and *Bacillus cereus* isolated from soil to digest different proteinaceous substrates is shown in Fig. 1 and Fig. 2 respectively.

It can be concluded from the study that the proteolytic bacteria are of considerable interest in view of their activity against solid proteinaceous waste substrates like gelatin, milk protein, soya protein, bovine meat protein and egg protein. *Pseudomonas aeruginosa* and *Bacillus cereus* have strong proteolytic activity on the basis of their efficacy for digestion of different protein substrates. *Pseudomonas aeruginosa* and *Bacillus cereus* have the potential for both degrading solid proteinaceous waste generated from industries as well as the potential for protease production. Hence, study on

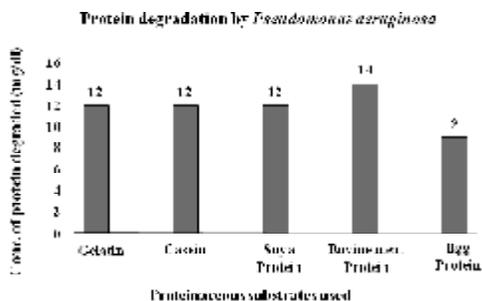


Fig. 1: Efficacy of *Pseudomonas aeruginosa*.

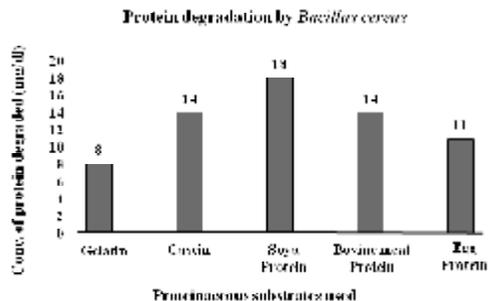


Fig. 2: Efficacy of *Bacillus cereus*.

microbial techniques of proteolytic microbes becomes imp en-route towards sustainable development.

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