Journal of Bio-Molecular Sciences (JBMS) (2015) 3(1): 18-24.

Journal of Bio-Molecular Sciences
(JBMS)
ISSN: 2311-4630
www.directsciences.com/jbms

Isolation, Screening and Identification of Protease Producing Bacteria from Soil of Karak, Khyber Paktunkhwa, Pakistan

Nayab[1], Muhammad Qasim [1*], Allah Nawaz[1], Hazir Rahman[1], Anum Tabassum[1], Tilawat Khan[1] and Noor Mohammad[2]

[1]Department of Microbiology, Kohat University of Science and Technology, Kohat, Pakistan
[2]Department of Biotechnology and Genetics Engineering, Kohat University of Science and Technology, Kohat, Pakistan

Received 17 Fab. 2015; Accepted 05 March 2015; Published 31 March 2015

Abstract: Microorganisms are important source of proteases which are employed in detergent, food, pharmaceutical, leather and photographic industries. Among microorganisms, bacteria have been widely explored globally for their protease production that leads to discovery of several novel proteases. The present research work was carried out to explore the indigenous protease producing soil bacterial flora of Karak, Khyber Pakhtunkhwa, Pakistan. A total of 60 bacteria were isolated from 16 soil samples collected from different sites in district Karak. The isolates were screened for protease production by using skimmed milk agar. Among all the isolates, 18 bacterial isolates were found protease producers with various degree of protease activity. These isolates were further identified through their morphological, microscopic and biochemical examination. It was concluded that soil of Karak is rich in protease producing bacteria that exhibit diversified proteolytic activities and biochemical features. Further optimization of protease activity and their structure elucidation may be performed which will be helpful to identify novel protease.

Key words: Soil sample, Protease producing bacteria, Karak, Skim milk agar

Introduction

Proteases are hydrolytic enzymes which catalyze the cleavage of peptide bonds in proteinaceous substrates to form a polypeptide chain in a particular protein (Gupta et al., 2005). Proteases have wide industrial applications as it account for two thirds of the total enzymes consume in various industries. Proteases are routinely used in detergent, food, pharmaceuticals, leather and photographic industries (Chu, 2007; Freddi et al., 2003; Harley, 1960; Kumar et al., 2002; Kumar and Takagi, 1999). In leather industries proteases are used in the process of batting and soaking (Anwar and Saleemuddin, 1998). Furthermore, proteolytic enzymes help to reduce the inflammation by neutralizing the biochemical inflammation (Miller, 1956).

*Correspondence to: Muhammad Qasim, Department of Microbiology, Kohat University of Science and Technology, 26000 Kohat, Pakistan, E-mail: qasim89@gmail.com

Protease can be derived from different sources such as plants and animals but proteases from microbial source are preferred over the other sources because microbial proteases possess all the characteristics required for biotechnological applications such as rapid broad biochemical diversity, grow in limited space and excellent feasibility to form various enzymes for different chemical processes (Checkirb et al., 2009; Rao et al., 1998). The present study documented for the first time the isolation and identification of bacteria that exhibited diverse proteolytic activities from the soil of Karak, Pakistan.

Materials and Methods

Study area

The present study was conducted on the soil samples of Karak which is situated in Khyber Pakhtunkhwa region of Pakistan. The climate is hot during the summers, with temperature range of 40-45^{o}C.

Samples collection

Total 16 different soil samples were collected about 2-3 centimetres below from surface of earth using sterile bottles from various sites of Karak such as hills, soil compost, slaughter houses, gardens, gas plant, agriculture fields, and petrol pumps.

Cultivation of Bacteria

One gram (dry weight) soil of every sample was serially diluted in sterilized distilled water, and 100µl diluted soil sample were spread on nutrient agar plates and incubated at 37^{o}C for 24 hours to obtain different bacterial colonies.

Purification of the isolates

The pure culture of isolates were obtained through subsequent sub-culturing on nutrient agar by following streak plate method at 37°C for 24 hours, and pure culture was confirmed through microscopic examination (Habib et al., 2012).

Screening of the isolates for the protease production

The bacterial isolates were screened for the yield of protease by skim milk agar plate method as described earlier (Fikret Uyar et al., 2011). Briefly, the bacterial colonies were streaked on skimmed milk agar plate and incubated at 37^{o}C for 24 hours. The Formation of clear zone around the colonies indicated the proteolytic activities of bacteria resulting from milk protein hydrolysis.

Identification of protease producing bacteria

Bacterial colonies showing protease activity were further characterized using cultural characteristics (colony color, shape, and transparency), microscopic findings (Gram staining and spore staining) (Habib et al., 2012) and biochemical features. (catalase, oxidase, voges proskauer, starch hydrolysis test) (Folasade, 2005).

Results and discussion

A total of 16 soil samples from different sites of Karak region were screened for protease producing bacteria. There was no significant difference among the pH and temperature of soil samples that ranged from 7.06-7.89 and 27-30^{o}C respectively. Soil consistency was varied such as soft, hard, sandy and muddy. Colour of soil samples were also different such red, brownish and gray (Table. 1). Isolation of protease producing bacteria was first carried out using nutrient agar media and further screened for protease production on screened on skim milk agar plates. Formation of clear zones around the colonies was considered as indication of protease production. In 16 soil samples, we obtained 60 various types of bacterial colonies having different culture characteristics.

Table 1. Soil samples distribution and characteristics collected from various sites of Karak.

S. No	Sites	pH	Consistency	Temperature	Colour
1	Hill (Ahmadi banda)	7.76	Soft	29 ^{o}C	Red
2	Hill (Takht-e-Nasrati)	7.77	Hard	29 ^{o}C	Brownish
3	Petrol Pump (Ahmadi banda)	7.08	Sandy	27 ^{o}C	Brownish
4	Petrol Pump (Takht-e-Nasrati)	7.06	Soft	27 ^{o}C	Brownish
5	Soil compost (Ahmadi banda)	7.86	Soft	30 ^{o}C	Gray
6	Soil compost (Takht-e-Nasrati)	7.84	Sandy	27 ^{o}C	Brownish
7	Garden (Ahmadi banda)	7.50	Sandy	28 ^{o}C	Brownish
8	Garden (Takht-e-Nasrati)	7.50	Sandy	28 ^{o}C	Brownish
9	Slaughter house (Ahmadi banda)	7.89	Sandy	30 ^{o}C	Gray
10	Slaughter house (Takh Nasrati)	7.88	Sandy	30 ^{o}C	Gray
11	Gas plant (Ahmadi banda)	7.65	Muddy	26 ^{o}C	Gray
12	Gas plant (Takht-e-Nasrati)	7.65	Hard	27 ^{o}C	Gray
13	Detergent effluent (Ahmadi banda)	7.48	Soft	28 ^{o}C	Gray
14	Detergent effluent (Takht-e-Nasrati)	7.74	Muddy	28 ^{o}C	Brownish
15	Agriculture soil (Ahmadi banda)	7.46	Hard	29 ^{o}C	Brownish
16	Agriculture soil (Takht-e-Nasrati)	7.47	Hard	30 ^{o}C	Brownish

Out of 60 isolates only 18 isolates showed zone around the colonies (Figure 1 and Figure 2). A diversified proteolytic activity was observed ranges from a large clear zone (strong proteolytic activity) to small zone (weak proteolytic activity). Those bacterial isolates which showed clear zone of protease production were further explored for their culture, microscopic and biochemical characteristics.

Figure 1. Screening of protease producing bacterial isolates on skimmed milk agar. Bacteria were grown on skimmed milk agar and incubated for 24hr at 37^0C. Clear zone around the colony indicates the protease activity.

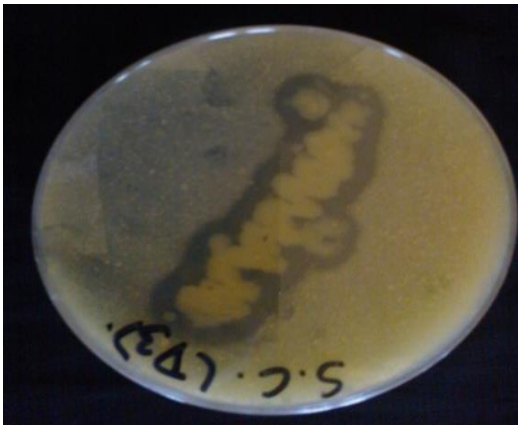
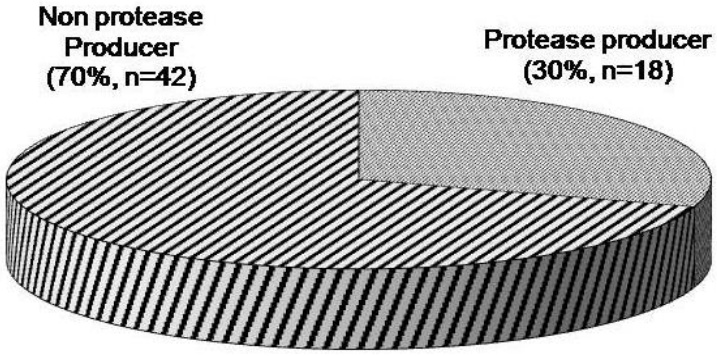


Figure.2. Percentage of protease producing bacteria isolated from the soil (n=16) of Karak.



The findings of morphological and biochemical tests are shown in Table 2. Out of 18 isolates 14 were Gram positive rods. These isolates were positive for VP and starch hydrolysis test while negative for catalase and oxidase test. All these Grams positive isolates were spore producers (Table 2).

The 4 isolates (Pd3 1, SCd3 1, Gd1 1 and Gd3 1) were Gram negative rods and were non-spore forming. All these isolates were catalase and oxidase producers. Furthermore, these Grams negative isolates were negative for starch hydrolysis and VP test (Table. 2).

Table 2. Morphological and Biochemical characterization of Protease producing bacteria isolated from different soil samples of Karak.

Isolate	Colony morphology	Microscopy	Biochemical tests				
		Gram staining	Spore staining	Oxidase	Catalase	Starch hydrolysis	VP test*
Pd31	Green colored, round & Opaque	Gram –ve rods	-ve	+ve	+ve	-ve	-ve
SCd31	Light green,rough, & opaque	Gram –ve rods	-ve	+ve	+ve	-ve	-ve
Gd1 1	Green,smooth, & opaque	Gram –ve rods	-ve	+ve	+ve	-ve	-ve
Gd3 1	Green color, rough & translucent	Gram –ve rods	-ve	+ve	+ve	-ve	-ve
Pd3 2	Cream colored, irregularly shaped & transparent	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
Pd1 1	Yellow colored,flat & translucent	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
Pd3 3	Cream colored , circular & opaque	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
SHd1	Cream colored , circular & translucent	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
SHd3	Yellow colored,irregular & opaque	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
SCd1	Cream colored, flat & spreading	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
SCd3	Yellow colored & circular	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
SCd1 2	Irregularly shaped,yellow colored &translucent	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
Gd1 2	Yellow colored, flat & spreading	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
Hd1 2	Yellow & Irregular & spreading	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
Dtd2 1	White colored & Irregular	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
Dtd2 2	White colored & Irregular	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
Hd1	Cream colored & flat	Gram +ve rods	+ve	-ve	-ve	+ve	+ve
Gd3 2	Yellow colored & irregular	Gram +ve rods	+ve	-ve	-ve	+ve	+ve

*Voges–Proskauer;

.

Several investigations have been done for screening new bacterial isolates for protease production. A previous study performed by Gupta et al., (2005) isolated several protease producing bacteria and found S*treptomycin s*pp. as best protease activity exhibiting bacteria. Another study conducted on isolation of protease producing bacteria from tannery effluent revealed Alcaligenes sp., Aeronomas sp., Bacillus sp., Staphylococcus sp. and Pseudomonas sp as protease producer (Muthuprakash and Abraham, 2011). While another study found *Bacillus licheniformis* (PB1) as potent protease producing bacteria (Ghumro el al., 2010). In addition, another study performed on soil samples revealed significant number of protease producing bacteria including *Bacillus licheniformis* (Rani et al., 2012).

It was concluded that the soil of Karak is rich with proteolytic bacteria that exhibit diversified proteolytic activities and biochemical features. The soil can be used for the isolation of protease producing bacteria for the commercial extraction of proteases. Further investigations on molecular characterization and protease production optimization at various growth conditions are needed to improve enzyme production quality for its industrial use.

References

Anwar, A. and Saleemuddin, M. 1998. Alkaline proteases: A review. Bioresour. Technol. 6: 175-183.

Checkirb, D., Tahar, A. and Cochet, N. 2009. Acid protease production by isolated species of penecillium. Eur. J. Sci. Res. 25: 469-477.

Chu, W. H. 2007. Optimization of extracellular alkaline protease production from species of *Bacillus*. J. Ind. Microbiol. Biotech. 34: 241-245.

Fikret, U., Ilknur, P., Göksel, K., and Ebru Ince ,Y. 2011. Optimal conditions for production of extracellular protease from newly isolated *Bacillus cereus* strain CA15 EurAsia. J. Bio. Sci. 5: 1-9.

Folasade, M., Olajuyigbe and Joshua, O. A. 2005. Production dynamics of extracellular protease from Bacillus species. Afr. J. Biotech. 4: 776-779.

Freddi, G., Mossotti, R. and Innocenti, R. 2003. Degumming of silk fabric with several proteases. J. Biotech. 10: 101–112.

Ghumro, P. B., Shafique M., Ali, M. I., Javed, I., Ahmad, B., Jamal, A., Ali, N. and Hameed, A. 2011. Isolation and screening of protease producing thermophilic *Bacillus* strains from different soil types of Pakistan. Afri. J. Microbiol. Res. 5: 5534-5539.

Gupta, A., Roy, I., Patel, R. K., Singh, S. P., Khare, S. K. and Gupta, M. N. 2005. One-step purification and characterization of an alkaline protease from haloalkaliphilic *Bacillus sp*. J. Chromatogr. A. 1075: 103-108.

Harley, B. S. 1960. Proteolytic enzymes. Annu. Rev. Biochem. 29: 45-72.

Habib, S. M. A., Fakhruddin, A. N. M., Begum, S. and Ahmed, M. M. 2012. Isolation and Screening of Thermostable Extracellular Alkaline Protease Producing Bacteria from Tannery Effluents. J. Sci. Res. 4: 515-522.

Kumar, A., Sachdev, A., Balasubramanyam, S.D., Saxena, A.K. and Lata, A. 2002. Optimization of conditions for production of neutral and alkaline protease from species of Bacillus and Pseudomonas. Ind. Micro. 42: 233-236.

Kumar, C. G. and Takagi, H. 1999. Microbial alkaline proteases from a bioindustrial view point. Biotechnol. Adv. 17: 561-594.

Miller, K. 1956. Proteolytic enzymes in inflammation rationale for use. Postgrad Med. 19: 16-22.

Muthuprakash, K .M. S. and Abraham, J. 2011. A comparative analysis of protease

producing microbes isolated from tannery effluent. Int. J. Sci. Nature. 2: 110-113.

Rani, M. R, kumara B. L, Hanuma, M. S. and Siva, P. D. 2012. Isolation and screening of alkaline protease producing bacteria and induction of over producing Bacillus licheniformis mutants through UV irradiation. IOSR Pharma. 1(1):001-014.

Rao, M. B., Tanksale, A. M., Ghatge, M. S. and Deshpande, V. V. 1998. Molecular and Biotechnological aspects of Microbial Proteases. Microbiol. Mol. Biol. Rev. 62: 597-635.