Plant Tissue Cult. & Biotech. **32**(1): 31-41, 2022 (June) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v32i1.60470

PTC&B

Biotechnologically Important Enzyme Producing Indigenous Bacteria Isolated from Fruit and Vegetable Wastes Samples Collected from different Local Markets

Probir Kumar Sharma, Zakya Sultana Jui, Tahsin Khan, Tahmina Islam and Mihir Lal Saha*

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Bacteria, Indigenous, Enzyme, Amylase, Protease

Abstract

Enzyme from bacterial sources is much stable and obtained cheaply. Amylases and proteases are among the most important enzymes. During this study, indigenous amylase and protease producing bacteria were isolated from common decayed fruits and vegetable wastes viz. Papaya, Brinjal, Cucumber, Potato and Snake Gourd. Bacterial load ranged in between 0.67×10^9 and 9.06×10^9 cfu/g on NA and 1.50×10^9 and 7.00×10^9 cfu/g on PYG agar medium. Maximum mean bacterial load on both NA ($6.19 \pm 2.60 \times 10^9$ cfu/g) and PYG agar ($4.54 \pm 1.03 \times 10^9$ cfu/g) were observed in decayed papaya. A total of 113 bacterial isolates were primarily isolated. Considering better amylase and protease activity 16 isolates were selected for detailed study. The starch hydrolysis ratio (SHR) of the isolates ranged in between 1.25 ± 0.37 and 2.47 ± 0.23 while casein hydrolysis ratio (CHR) ranged in between 2.35 ± 0.12 and 6.44 ± 1.16. The highest SHR was 2.47 ± 0.23 found in *Bacillus* sp. of snake gourd and the highest CHR was 6.44 ± 1.16 found in *Bacillus subtilis* also from snake gourd. Out of the 16 isolates 13 were Gram positive and 3 were Gram negative. Gram positive isolates were identified as *Bacillus acidocaldarius* (4), *B. firmus* (2), *B. lentus* (4), *B. subtilis* (2) and *B. alcalophilus* (1). Gram negative isolates were identified as *Edwardsiella hoshinae*, *Proteus myxofaciens* and *P. mirabilis*. Six isolates having higher SHR and CHR were authenticated through molecular identification and were identified as *Chryseobacterium* sp. S29.2, *Bacillus* sp. X8, *Bacillus* sp. strain GA1B, *Bacillus* sp.TdEND26, *Bacillus subtilis* strain BPA28 and *Bacillus subtilis* BAB-881. The maximum amylase (61.33 ± 2.14 U/ml) and protease (56.91 ± 0.23 U/ml) production were observed in *Bacillus* sp. TdEND26 rd. In case of co-production, the highest amount of amylase (54.13 ± 1.23 U/ml) protease (81.80 ± 4.54 U/ml) production was observed in *Bacillus* sp. TdEND26 at 24 hrs.

*Author for correspondence: <sahaml@du.ac.bd>.

Introduction

Enzymes are biological catalysts that facilitate the conversion of substrates by providing favorable conditions that lower the activation energy of the reaction. Microbial enzymes are known to be superior enzymes obtained from different microorganisms (Anbu et al. 2013). Microorganisms which found in fruit and vegetable wastes produced different enzymes *viz*. amylase, protease and pectinase. Some enzyme producing bacteria are *Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus megaterium, Bacillus subtilis, Pseudomonas sp., Aeromonas sp.* etc. Amylases have potential biotechnological applications in a wide range of industrial processes and they account for nearly 30% of the world's enzyme market (Rajagopalan and Krishnan 2008). The study of amylase production from microbial diversity has attracted the attention of researchers and industrialists in last decades.

This microbial amylase plays a significant role in various biotechnology companies for the manufacture of mass market consumer products (Horikoshi 1999). Amylase has a large scale of applications, such as different types of syrups, solubilization and saccharification of starch (Pandey et al. 2000). In detergent industries, amylases catalyze the hydrolysis of glycosidic linkages in stains and eliminate the starchy glue that combine with other stains and dirt. The α-amylase, an endo-amylase, is utilized primarily for laundry detergents, as exo-amylase activity is inefficient for stain removal. Currently, α-amylases are included in approximately 90% liquid detergent formulations (Hmidet 2009).

Huge percentages of the international market of industrially important enzymes are covered by proteases (Deng et al. 2010). Protease production is an inherent capacity of most microorganisms (Singh et al. 2015). Proteolytic enzymes support the natural healing process in local management of skin ulceration by efficient removal of necrotic material (Sjodahl et al. 2002). Proteases are useful and important components in biopharmaceutical products such as contact-lens, enzyme cleaners and enzymatic debrides (Deng et al. 2010). In the leather industries, proteases are useful in dehairing for the purpose of leather manufacture. Enzyme based dehairing processes using proteases help to reduce or even avoid those chemical and offer enormous environmental benefits (Khan 2013). The use of protease supplementation to detergent formulation significantly improves the cleansing of proteinaceous stains and delivers unique benefits that cannot otherwise be obtained with conventional detergents (Prabhavathy et al. 2013). The use of enzymes in detergent formulations is now common in developed countries with over half of all detergents contain enzymes. Proteases are also used in various industries such as silver recovery, food, protein hydrolysis, waste management and textile industries. Accordingly, proteases account for about 60% of the total enzyme sale in the world (Hamza 2017).

Amylases and proteases are used together in many industries such as food, pharmaceuticals, detergent industries etc. (Mitidieri et al. 2006). Among the wide range

of microbial species that secrete amylase and protease, their production from bacteria is cheaper and faster than other microbial sources. Considering facts and importance, the present study was aimed to isolate biotechnologically important enzymes like amylase and protease producing indigenous bacteria from local fruit and vegetable wastes.

Materials and Methods

Three different local markets of Dhaka City *viz.* Ananda Bazar, Hatirpool Bazar and Palashi Bazar were selected for sampling. Waste fruit and vegetable samples *viz.* Papaya, Brinjal, Cucumber, Potato and Snake Gourd were collected in sterile plastic bags. The pH of the samples was measured by a pH meter (ToA-Dkk, HM-31P, Japan) immediately after the samples were brought into the laboratory.

Nutrient agar (NA) (Eklund and Lankford 1967) and Peptone Yeast Extract Glucose agar (PYG) (Atlas 1997) media were used for the enumeration and isolation of bacteria associated with the collected samples with special reference to amylase and protease activities. The pH of the medium was adjusted to 7.0 ± 0.2 before the addition of agar and sterilization.

Serial dilution technique (Greenberg et al. 1992) was used for the isolation of bacteria. The inoculated plates were placed invertedly and incubated at 37 °C for 24 hrs for NA and 48 h for PYG in an incubator (Memmert GmbH + Co Kg 8540 Sehwabach). After 24 to 48 h of incubation, the plates having well discrete bacterial colonies were selected for counting and were counted by a colony counter (Digital colony counter, DC-8 OSK 10086, Kayagaki, Japan). Well discrete aerobic heterotrophic bacterial colonies having distinctive morphology were primarily selected immediately and were isolated aseptically on PYG agar slants. The selected isolates were purified through streak plate method.

Starch hydrolysis test (Claus 1995) on Starch Nutrient Agar (SNA) medium was performed to identify potential isolates showing amylolytic activity. Casein (Milk protein) hydrolysis test (Sneath et al. 1986) on Skim Milk Agar (SMA) medium was performed to identify proteolytic activity. Starch Hydrolysis Ratio (SHR) and Casein Hydrolysis Ratio (CHR) were calculated using the following formula to quantify amylolytic and proteolytic activity of the isolates.

$$\text{SHR or CHR} = \frac{\text{Zone Diameter}}{\text{Colony Diameter}}$$

Based on SHR and CHR, isolates were finally selected. Morphological and Biochemical tests (SAB 1957 and Sneath et al. 1986) were done and isolates were provisionally identified following Bergey's Manual of Systematic Bacteriology (Krieg and Holt 1984) and (Sneath et al. 1986).

Six bacterial potentials isolates were further identified using 16S rRNA based on molecular technique. 16S rRNA was amplified by using the universal primers CC(R): 5´-

CCAGACTCCTACGGGAGGCAGC-3´ and CD(F): 5´-CTTGTGCGGGCCCCCGTCAA TTC-3´. The genomic DNA was isolated from bacteria and PCR was performed. The amplified products were separated through gel electrophoresis on 1% agarose gel. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system (Microdoc DI-HD, MUV21-254/365, Cleaver Scientific). DNA sequencing was done in an automated gene sequencer (Macrogen, South Korea) and sequences were analyzed through NCBI-BLAST database (http://blast.ncbi.nlm.nih.gov/) and rRNA BLAST (http://bioinformatics.psb.ugent.be/cgi-bin/rRNA/blastform.cgi) programs.

The amylase production was enhanced using starch as inducer. 1 ml of Bacterial culture (inoculum) was inoculated into 49 ml of the production media (Padhiar and Kommu 2016) containing 1% Starch. Fermentation flasks were incubated at 37°C on rotary shaker (DAIHAN-LABTECH, South Korea) at 120 rpm for 24 h. After 24 h, the culture fluid was withdrawn and centrifuged at 9,000 rpm for 15 min in centrifuge (Thermo Scientific Sorvall ST 8R, Germany). The cell free supernatant was used for crude enzyme assay. Amylase activity was carried out using spectrophotometric method. Amylase activity was assayed using Dinitro Salicylic Acid (DNSA) method described by Senthilkumar et al. (2012).

Modified alkaline protease producing broth (APPB) (Horikoshi 1971) medium was used for protease production. 1 ml of bacterial culture as inoculum was inoculated into 49 ml of the production media of 1.0% glucose, 0.5% peptone, 0.5% yeast extract, 0.5% K_2HPO_4 and 0.01% $MgSO_4$. pH of the medium was adjusted to 8.5. Fermentation flasks were incubated at 37°C on rotary shaker (DAIHAN-LABTECH, South Korea) at 120 rpm for 24 hrs. After 24 hrs, the culture fluid was withdrawn and centrifuged at 8,000 rpm for 10 min in centrifuge (Thermo Scientific Sorvall ST 8R, Germany). The cell free supernatant was used for crude enzyme assay. Protease activity was determined using casein as substrate according to Sigma's Non-specific protease activity assay technique (Cupp-Enyard and Aldrich 2008).

For co-production of amylase and protease, modified broth media were used. 1 ml of bacterial culture was inoculated into 49 ml of the production media of 1.0% Starch, 0.5% Casein, 0.5% peptone, 0.5% Beef extract, 0.5% NaCl, 0.05% K_2HPO_4 and 0.05% $MgSO_4$. pH of the medium was adjusted to 7.0. Fermentation flasks were incubated at 37°C on rotary shaker (DAIHAN-LABTECH, South Korea) at 120 rpm for 24, 48, 72 hr After incubation, culture was centrifuged at 9,000 rpm for 15 min in centrifuge (Thermo Scientific Sorvall ST 8R, Germany). The cell free supernatant was used for crude enzyme assay. Amylase and protease activity assay in the co-production medium by the selected isolates was done following the techniques described earlier using the same cell free supernatant. Statistical analysis was performed with the statistical Package for the Social Sciences (SPSS) v.16.0 for windows (SPSS, SAS Institute Inc. Cary, USA).

Results and Discussion

During this study, the bacteria of interest were isolated from decayed fruits and vegetables samples of some local markets of Dhaka South City *viz.* Ananda Bazar, Hatirpool Bazar and Palashi Bazar. A good number of bacteria were found to be associated with decayed fruits and vegetables samples (Table 1). The bacterial load ranged in between 0.67×10^9 and 9.06×10^9 cfu/g on NA and 1.50×10^9 and 7.00×10^9 cfu/g on PYG agar medium, respectively. Maximum bacterial load was observed in the decayed papaya samples on both NA ($6.19 \pm 2.60 \times 10^9$cfu/g) and PYG agar ($4.54 \pm 1.03 \times 10^9$cfu/g).

Table 1. Bacterial load of studied wastes samples of some local markets.

Sample type	Scientific name	Bacterial load (cfu/g) on two media	
		NA	PYG
		Mean ± SD	Mean ± SD
Brinjal	*Solanum melongena*	$4.12 \pm 2.82 \times 10^9$	$4.45 \pm 2.27 \times 10^9$
Cucumber	*Cumumis sativus*	$3.79 \pm 1.96 \times 10^9$	$2.89 \pm 1.58 \times 10^9$
Papaya	*Carica papaya*	$6.19 \pm 2.60 \times 10^9$	$4.54 \pm 1.03 \times 10^9$
Potato	*Solanum tuberosum*	$2.68 \pm 0.95 \times 10^9$	$3.43 \pm 1.10 \times 10^9$
Snake Gourd	*Trichosanthes cucumerina*	$4.52 \pm 1.99 \times 10^9$	$3.67 \pm 0.64 \times 10^9$

A total of 113 bacterial isolates were isolated and screened finally 16 isolates were selected. Amylase and protease activities were evaluated by starch hydrolysis and proteolysis performances. Starch hydrolysis ratio (SHR) of the studied isolates ranged in between 1.25 ± 0.37 and 2.47 ± 0.23 and their casein hydrolysis ratio (CHR) ranged in between 2.35 ± 0.12 and 6.44 ± 1.16. The highest SHR (2.47 ± 0.23) was observed in *Bacillus* sp. BPA28 isolated from snake gourd of Hatirpool. The highest CHR was 6.44 ± 1.16 and observed in *Bacillus subtilis* BAB-881 isolated from snake gourd of Ananda Bazar. The SHR of the present findings found greater than that of reported by Padhiar and Kommu (2016) where they observed the highest SHR as 1.90. Oyeleke and Odiwole (2009) reported the highest SHR as 3.10 and bacteria associated with this SHR was *Bacillus subtilis* isolated from a cassava waste in Minna, Nigeria. The CHR was recorded higher than that of a previous work of Saha et al. (2011) where the highest CHR was 5.8 and the bacteria was associated with tannery waste.

All the isolated bacteria were found to be rod shaped. Both Gram positive and Gram negative bacteria were present in the collected samples. Morphological and biochemical characteristics of Gram positive bacteria were compared with the Bergey's Manual of Systematic Bacteriology (Krieg and Holt 1984, Sneath et al. 1986).Gram-positive isolates

were belonged to the single genus *Bacillus* while Gram negative bacteria were identified as the member of *Edwardsiella hoshinae*, *Proteus myxofaciens* and *P. mirabilis* (Table 2 and 3).

Table 2. Major biochemical characteristics of the selected bacterial isolates.

Isolate No.	V.P. test	M.R. test	Deep glucose agar	Utilization of		Tyrosine degradation	Lecithinase production	Nitrate reduction
				Citrate	Propio-nate			
HP/N/5/5	-	+	FA	-	-	+	-	-
HS/N/5/9	+	+	A	-	-	+	+	-
PB/N/3/5	-	+	A	-	-	+	+	-
APT/P/7/4	+	+	FA	-	-	-	-	+
AS/P/5/2	+	-	A	-	-	-	+	+
HP/P/7/2	+	-	A	-	-	-	+	+
PC/P/7/6	+	-	FA	+	+	-	-	+
PP/P/7/6	+	-	FA	-	+	-	-	+
HB/N/7/7	+	-	A	-	-	-	+	+
HS/P/7/8	+	-	A	-	-	-	+	+
HS/P/7/7	+	-	A	-	-	-	+	+
AS/P/6/7	+	+	A	-	-	-	+	+
AS/P/5'/6	-	-	A	-	-	-	+	+
AC/P/7/4	+	+	A	-	-	-	-	+
AC/P/7/5	**+**	**+**	A	-	-	-	**+**	**+**
AC/N/5/7	**+**	-	A	-	-	-	-	**+**

Table 3. Provisional identification of the bacterial isolates associated.

Isolate No.	Provisionally identified bacteria	Isolate No.	Provisionally identified bacteria
HP/N/5/5	*Proteus myxofaciens*	HB/N/7/7	*Bacillus lentus*
PB/N/3/5	*P. mirabilis*	HS/P/7/8	*B. lentus*
HS/N/5/9	*Edwardsiella hoshinae*	AS/P/5'/6	*B. lentus*
APT/P/7/4	*Bacillus acidocaldarius*	AC/N/5´/7	*B. lentus*
PP/P/7/6	*B. acidocaldarius*	HS/P/7/7	*Bacillus firmus*
HP/P/7/2	*B. acidocaldarius*	AS/P/5/2	*B. firmus*
PC/P/7/6	*B. acidocaldarius*	AC/P/7´/5	*Bacillus subtilis*
AC/P/7/4	*B. Alcalophilus*	AS/P/6/7	*B. subtilis*

Six isolates showing highest SHR and CHR were authenticated by molecular identification following 16S rRNA sequencing (Fig. 1). Here 5 Gram-positive provisionally identified isolates were found to be the same genus *Bacillus*. The isolate AS/P/6/7 was found to be same to molecular identification and identified as *Bacillus subtilis* (Table 4). The Gram negative isolate HP/N/5´/5 was provisionally identified as *Proteus myxofaciens* which was found to be different in case of molecular identification and identified as *Chryseobacterium* sp. S29.2. Overall, provisional identification could be considered as valid identification and could be recommended where molecular facilities are not available.

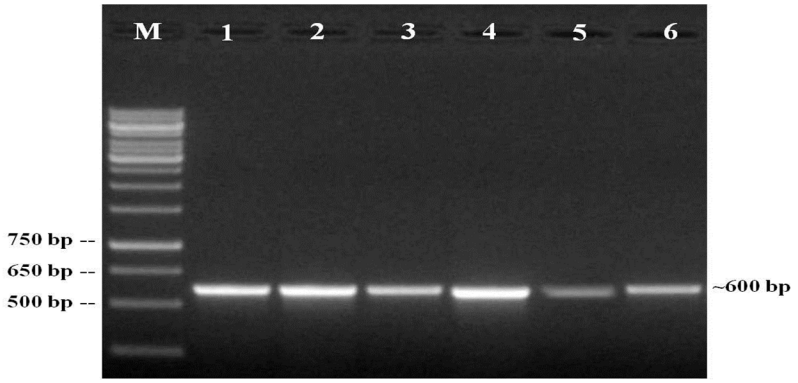

Fig. 1. PCR amplification of part of the 16S rRNA gene. Lane M is the 1.0 kb ladder and lanes 1-6 representing 6 (1=AS/P/5/2, 2=HP/P/7/2, 3=HP/N/5´/5, 4=HS/P/7/8, 5=HS/P/7/7 and 6= AS/P/6/7) different bacterial isolates.

Table 4. Comparison between provisional and molecular identification of the selected bacterial isolates.

Isolate No.	Provisional identification	Molecular identification				
		Scientific name	Strain	Max. coverage score	Evalue	Identity match (%)
AS/P/5/2	*Bacillus firmus*	*Bacillus* sp.	X8	1009	0.0	100%
HP/P/7/2	*Bacillus acidocaldarius*	*Bacillus* sp.	GA1B	1011	0.0	100%
HP/N/5/5	*Proteus myxofaciens*	*Chryseobacterium* sp.	S29.2	651	0.0	94%
HS/P/7/8	*Bacillus lentus*	*Bacillus* sp.	TdEND26	952	0.0	100%
HS/P/7/7	*Bacillus firmus*	*Bacillus subtilis*	BPA28	992	0.0	99%
AS/P/6/7	*Bacillus subtilis*	*Bacillus subtilis*	BAB-881	979	0.0	99%

In the present study, 6 bacterial isolates (*Bacillus* sp. X8, *Bacillus* sp. GA1B, *Chryseobacterium* sp. S29.2, *Bacillus* sp. TdEND26, *Bacillus subtilis* BPA28, *Bacillus subtilis* BAB-881) were found as good amylase and protease producers. In a study Janarthanan

et al. (2014) reported *Bacillus* sp. B17, *Micrococcus* sp. C3 and *Bacillus* sp. P1 as good amylase producer associated with vegetable waste. Samanta et al. (2013) found *Bacillus* spp. and *Cronobacter sakazakii* amylase producing bacteria from solid waste. In India, Verma et al. (2011) mentioned that most of the *Bacillus* species were good at amylase production. Choubey et al. (2015) worked on the production and partial purification of protease enzyme from bacterial isolate of different types of vegetable wastes and found different species of *Bacillus*. In a study Saha et al. (2011) reported alkaline protease producing bacteria from tannery effluents and the bacteria were *Bacillus subtilis*, *B. licheniformis*, *B. alcalophilus*, *B. badius*, *B. cereus*, *B. circulans*, *B. pumilus*, *B. alvei*, *B. brevis*, *B. coagulans*, *B. megaterium*, *B. polymyxa* and *Micrococcus varians.*

The amylase, the starch degrading enzymes are most important in the industries with huge application in food, fermentation, textile and paper. Prameela et al. (2016) focused on the isolation and characterization of amylase producing bacteria from the soil samples. In this study, two isolates *Chryseobacterium* sp. S29.2 and *Bacillus* sp. TdEND26 were selected for estimation of their amylase production following methodology of Padhiar and Kommu (2016) (Table 5). The highest amylase production was recorded as 61.33 ± 2.14 U/ml produced by the *Bacillus* sp. TdEND26. In a study Basma et al. (2015) reported maximum amylase production (72.5 U/ml) by the *Bacillus amyloliquefasciens* while Jogezai et al. (2011) reported maximum amylase activity as 79 U/ml by *B. subtilis.*

Table 5. Amylase and protease activity of two bacterial isolates.

Isolate No.	Bacteria	Amylase activity (U/ml) (Mean ± SD)	Protease activity (U/ml) (Mean ± SD)
HP/N/5/5	*Chryseobacterium* sp. X8	49.82 ± 2.43	47.81 ± 3.30
HS/P/7/8	*Bacillus* sp. TdEND26	61.33 ± 2.14	56.91 ± 0.23

Microbial proteases are one of the most important groups of enzymes, used in various industrial processes as food, pharmaceutical and detergent industries, as well as in the preparation of leather, textile and wool and others (Vadlamani and Parcha 2011). It has also promising application in medical sectors and management of industrial and household waste. Following methodology of Horikoshi (1971) the two selected isolates *Chryseobacterium* sp. S29.2 and *Bacillus* sp. TdEND26 were evaluated for protease production (Table 5). The maximum protease production was observed as 56.91 ± 0.23 U/ml by the *Bacillus* sp. TdEND26. Saha et al. (2011) reported that maximum protease production (94.8 and 119.3 U/ml) by *Bacillus pumilus* isolated from tannery effluent. Gill et al. (2016) reported maximum protease production as 36.79 U/ml and 34.89 U/ml by the *Bacillus subtilis* and *Bacillus megaterium*, respectively isolated from kitchen waste.

Table 6. Amylase and protease co-production by the two bacterial isolates.

Bacteria	Amylase activity (U/ml) Mean ± SD			Protease activity (U/ml) Mean ± SD		
	24 h	48 h	72 h	24 h	48 h	72 h
Chryseobacterium sp. X8	39.12 ± 2.74	45.44 ± 2.67	47.80 ± 1.03	54.48 ± 4.56	70.75 ± 2.55	72.08 ± 10.41
Bacillus sp. TdEND26	47.55 ± 4.01	50.97 ± 2.42	54.13 ± 1.23	70.87 ± 4.96	81.80 ± 4.54	77.22 ± 3.14

There were a few reports on co-production of protease and amylase by same bacterial strain (Hmidet et al. 2009). Protease and amylase are used together in many industries such as food industry, pharmaceuticals, detergent industries, etc. Detergent formulations are fortified with both proteases (Joo and Chang 2006) and amylases (Mitidieri et al. 2006). Amylases improve the washing capacity of the protease containing detergents and remove starchy food stains from fabrics which are difficult to remove under normal washing conditions. A major problem of using both enzymes together is proteolysis of amylase by protease. Therefore, the detergent formulations containing both activities together and amylase not being proleolyzed will have better applicability. Most of the commercial detergents are formulated by mixing amylase and protease either derived from different microbial sources, or produced by the same strain using different fermentation substrates (Hmidet et al. 2009). The selected isolates *Chryseobacterium* sp. S29.2 and *Bacillus* sp. TdEND26 were tested for their competence to produce amylase and protease enzyme together in one production medium. Interestingly, in case of co-production, increased protease activity of both the isolates was observed than that of amylase (Table 6). During co-production, the highest amylase activity (47.80 ± 1.03 U/ml) showed by the *Chryseobacterium* sp. S29.2 and 54.13 ± 1.23 U/ml in case of isolate *Bacillus* sp. TdEND26 whereas the highest protease activity (72.08 **±** 10.41 U/ml) showed by *Chryseobacterium* sp. S29.2 and 81.80 ± 4.54 U/ml by *Bacillus* sp. TdEND26. Hence, it would be worthy to consider the coproduction of enzymes as commercial levels of enzyme production.

Acknowledgements

The first author of this research work is grateful to the Ministry of Science and Technology, Govt. of the People's Republic of Bangladesh for providing partial financial support for this research work through National Science & Technology Fellowship program and also thanks to Plant Tissue Culture and Biotechnology Laboratory of the same Department for helping during molecular identification of the isolates.

References

Anbu P, Gopinath SCB, Cihan AC and **Chaulagain BP** (2013) Microbial enzymes and their applications in industries and medicine. BioMed. Res. Int. **2**.

Atlas RM (1997) Handbook of Microbiological Media (2nd ed.). CRC Press.Y. pp. 1706.

Basma T, Elhalem MABD, El-Sawy Rawia F, Gamal Khadiga A and **Taleb A** (2015) Production of amylases from *Bacillus amyloliquefaciens*under submerged fermentation using some agro-industrial by-products. Annal. Agricult. Sci. **60**(2): 193-202.

Choubey R, Bansal S and Gauri S (2015) Production and partial purification of protease enzyme from bacterial isolate of vegetable waste. Ind. J. Trop. Biodiv. **23**(1): 41-45.

Claus GW (1995) Understanding Microbes (4th ed.). W H Freeman and Co. New York. pp. 547.

Cupp-Enyard C and **Aldrich S** (2008) Sigma's Non-specific Protease Activity Assay - Casein as a Substrate. J. Vis. Exp. **19.**

Deng A, WU J, Zhang Y, Zhang G and **Wen T** (2010) Purification and characterization of a surfactant-stable high-alkaline protease from *Bacillus* spp. B001. Bioresour. Technol. **101**: 7100-7116.

Eklund C and **Lankford CE** (1967) Laboratory manual for general microbiology. Prentice-Hall International Inc. London. pp. 299.

Gill SS, Shrivastav A and **Jana AM** (2016) Isolation and Identification of Protease producing Bacteria through Biodegradation of Protein content of Kitchen Wastes in Gwalior, India. Int. J. Curr. Microbiol. App. Sci. **5**(10): 204-211.

Greenberg AE, Connors JJ, Jenkins D and **Franson MAH** (1998) Standard methods for examination of water and wastewater (20th ed.). APHA, Washington DC.

Hamza TA (2017) Bacterial Protease Enzyme: Safe and Good Alternative for Industrial and Commercial Use. Int. J. Chem. Biomol. Sci. **3**(1): 1-10.

Hmidet N, El-Hadj Ali N, Haddar A, Kanoun S, Alya S and **Nasri M** (2009) Alkaline proteases and thermostable α-amylase co-produced by *Bacillus licheniformis* NH1: Characterization and potential application as detergent additive. Biochem. Eng. J. **47**: 71-79.

Horikoshi K (1999) Alkaliphiles: Some applications of their their products for biotechnology. Microbiol. Mol. Biol. Rev. **63**: 735-750.

Horikoshi K (1971) Production of alkaline enzyme by alkalophilic microorganisms Part I. Alakaline protease produced by *Bacillus* no. 221. Agricult. Biol. Chem. **35**: 1407-1414.

Horikoshi K (1971) Production of alkaline enzyme by alkalophilic microorganisms Part II. Alakaline amylase produced by *Bacillus* no. A-40-2. Agricult. Biol. Chem. **35**: 1783-1791.

Janarthanan R, Prabhakaran P and **Ayyasamy PM** (2014) Bioremediation of vegetable wastes through biomanuring and enzyme production. Int. J. Curr. Microbiol. App. Sci. **3**(3): 89-100.

Jogezai A, Raza F, Abbas M, Bajwa D, Mohammad W, Kakar M and **Saeed AA** (2011) Optimization of cultural conditions for microbial α-amylase production. J. Microbiol. Antimicrob. **3**: 221-227.

Joo HS and **Chang CS** (2006) Production of an oxidant and SDS-stable alkaline protease from an alkalophilic *Bacillus clausii* I-52 by submerged fermentation: feasibility as a laundry detergent additive. Enzym. Microb. Technol. **38**: 176-183.

Khan F (2013) New microbial proteases in leather and detergent industries. Innovat. Res. Chem. **1**: 1-6.

Kreig NR and **Holt JG** (Eds.) (1984) Bergey's Manual of Systematic Bacteriology. (9th ed.).The Williams and Wilkins Company, Baltimore, USA.

Mitidieri S, Souza Martinelli AH, Schrank A and **Vainstein MH** (2006) Enzymatic detergent formulation containing amylase from *Aspergillus niger*: a comparative study with commercial detergent formulations. Biores. Technol. **97**: 1217-1224.

Oyeleke SB and **Oduwole AA** (2009) Production of amylase by bacteria isolated from a cassava waste dumpsite in Minna, Niger State, Nigeria. Afri. J. Microbiol. Res. **3**(4): 143-146.

Padhiar AR and **Kommu S** (2016) Isolation, Characterization and Optimization of Bacteria producing Amylase. Int. J. Adv. Res. Biol. Sci. **3**(7):1-7.

Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D and **Mohan R** (2000) Advances in microbial amylases. Biotechnol. Appl. Biochem.**31**: 135-152.

Prabhavathy G, Rajasekara M, Pandian and **Senthilkumar B** (2013) Identification of industrially important alkaline protease producing *Bacillus subtilis* by 16s rRNA sequence analysis and its applications. Int. J. Res.Pharma. Biomed. Sci. **4**: 332-338.

Prameela G, Dharshini KP, Chidanandappa M and **Kamala K** (2016) Isolation and characterization of amylase producing bacteria from Orange and Promegante peel. Ino. Americ. J. Pharma. Res. **6**(11).

Rajagopalan G and **Krishnan C** (2008) Alpha-amylase production from catabolite derepressed Bacillus subtilis KCC103 utilizing sugarcane bagasse hydrolysate. Bioresour Technol. **99**: 3044-3050.

SAB (Society of American Bacteriologists) (1957) Manual of microbiological methods. McGraw Hill Book Company Inc. New York. pp. 315.

Saha ML, Begum KJMH, Khan MR, and **Gomej DJ** (2011) Bacteria associated with tannery effluent and their alkaline protease activities. Plant tissue Cult. Biotechnol. **21**(1): 53-61.

Samanta A, Mitra D, Roy SN, Sinha C and **Pal P** (2013) Characterization and Optimization of Amylase Producing Bacteria Isolated from Solid Waste. J. Environ. Prot. **4**: 647-652.

Senthikumar PK, Uma C and **Saranraj P** (2012) Amylase Producing by *Bacillus* sp. Using Cassava as Substrate. Int. Pharm. Bio. Arch. **3**(2): 300-306.

Singh P, Rani A and **Chaudhary N** (2015) Isolation and characterization of protease producing *Bacillus* sp. from soil. Int. J. Pharm. Sci. Res. **6**(4): 633-639.

Sjodahl J, Emmer A, Vincent J and **Roeraade J** (2002) Characterization of proteinases from Antarctic krill (*Euphausia superba*), Protein Expr. Purif. **26**: 153-161.

Sneath PHA, Mair ME, Sharpe and **Holt JG** (Eds.) (1986) Bergey's manual of systematic bacteriology (9th ed.). The Williams and Wilkins Company, Baltimore, USA.

Vadlamani S and **Parcha SR** (2011) Studies on industrially important alkaline protease production from locally isolated superior microbial strain from soil microorganisms. Int. J. Biotechnol. Applicat. **3**: 102-105.

Verma V, Avasthi MS, Abhishek, Gupta R, Singh M and **Kushwaha A** (2011) Amylase Production & Purification from Bacteria Isolated from a Waste Potato Dumpsite in District Farrukhabad U.P State India. Europ. J. Expr. Biol. **1**(3): 107-113.

(Manuscript received on 15 February, 2022; revised on 29 March, 2022)