Published by :
http://www.ijert.org

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 10, October-2016

Quantitative Estimation of Protease Produced by Bacterial Isolates from Food Processing Industries

I. S. Sony* and V. P. Potty
Department of Microbiology,
Cashew Export promotion Council of India (CEPCI),
Mundakkal, Kollam, Kerala, India

***Abstract* - To quantitatively estimate the protease produced by bacterial isolates from soil and water samples collected from food processing industries. In the present study, quantitative estimation of protease produced by selected bacterial isolates from soil and water samples collected from food processing industries was carried out and best protease producing organism can be used for the degradation of proteinaceous waste material from food manufacturing units leading to recycling of food industry waste. Protease activity was qualitatively determined using Gelatine clear zone method. Quantitative estimation of protease was performed for five bacterial isolates such as *Cedecea davisae* (TKMFT8), *Staphylococcus intermedius* (TKMFT10), *Proteus mirabilis* (TKMFT19), *Enterobacter asburiae* (TKMFT25) and *Alloiococcus otitis* (TKMFT61). Among all studied bacterial isolates, the maximum protease activity (138.23±1.50) was recorded in *Cedecea davisae* (TKMFT8).**

Key-Words:* *Protease, Protease activity, Quantitative estimation

1.INTRODUCTION

Proteases can be defined as enzymes which catalyze the breakdown of hydrolytic bonds in proteins thereby releasing amino acids and/or peptides (Louwrens W.Theron & Benoit Divol, 2014).Proteases represent a distinct subgroup of hydrolytic enzymes which catalyze the peptide bond breakdown in proteinous substrates (Younes Ghasemi et al, 2011; Bhatnagar *et al*., 2010; Yadav *et al*., 2011; Sankeerthana *et al*., 2013). Among enzymes, proteases are one of the major classes occupying 60% of total enzyme market (Chu W.H 2007; Kunamneni et al, 2003; Merheb-Dini et al, 2009, Verma O D et al.2011). Proteases are involved in every aspect of function of organisms and are among the oldest and most diverse enzyme families known.

Owing to their broad substrate specificity, proteases have tremendous applications in baking, brewing, waste treatment, leather processing, detergent formulations, meat tenderization, peptide synthesis, cheese manufacture, protein hydrolysate, soysauce production, pharmaceutical industry, silk industry, organic synthesis, silver recovery from waste photographic film and analytical tools in basic research and have prominent commercial value (Emtiazi et al.2005;Beheshti et al.2009;Sumantha et al.2006;Ward et al.2009).Moreover, baking industries use wheat flour (or Bakery dough) as a prime component in baking processes. This compound consists of an insoluble protein called gluten which is hydrolyzed by protease (Sumantha *et al*., 2005). In addition, the addition of proteases decreases the mixing time and results in 4 increased loaf volumes (Leng and Xu, 2011).

Generally, proteolytic enzymes (proteases) are categorized into peptidases and proteinases. Protein degradation into smaller peptide fractions is catalyzed by proteinases; while hydrolysis of specific peptide bonds or completely breakdown peptides to amino acids are catalyzed by peptidases or peptide bond hydrolase (Michael G. Ga¨nzle et al, 2008; Anita, 2010; Vasantha and Subramanian, 2012).Proteases are classified under the subgroup 4 of Group 3 (hydrolases) and this classification is according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. Proteases are grouped as endo-or exo-enzymes based on their site of action on protein substrates (Rao et al, 1998). Depending on their catalytic mechanism, they are further classified (Enzyme Commission (E.C.) 3.4. 21- 25) as aspartic proteases, serine proteases, cysteine proteases or metalloproteases (Barett, 1994; Barett, 1995).) Based on the p^H optima, there are 3 different types of proteases such as acidic, neutral and alkaline and among these, alkaline protease is the most commonly used industrial enzyme due to their stability and activity at alkaline PH (Onkar Nath Tiwari et al, 2015).

Proteases are present in all forms of life such as plants, animals and microorganisms. The major sources of proteases were from plants (eg.pineapple, fig and papaya), animals (eg.calf stomach) and microbes (eg.*Pseudomonas spp, Bacillus spp).*). Plant proteases composed of zingiabain, papain, ficin and bromelain (Adulyatham and Owusu- Apenten, 2005) and animal proteases include chymotrypsin, pepsin, Rennin, Trypsin etc. Microbial proteases are distributed in diverse microbial population viz.bacteria, fungi, viruses and actinomycetes. Microorganisms serve as a preferred source of protease and account for around two-thirds of commercial protease production worldwide. Current world demand for microbial protease are increasing due to their cost effectiveness, rapid growth and the ease with generating high yielding genetically modified strains(Sankeerthana et al.2013).The major bacterial genera which contribute to proteases include *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Halomonas*, *Pseudomonas* and *Serratia* (Shafee N et al,

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 10, October-2016

2005; Rao et al.1998).In addition to this ,other bacteria producing protease include *Alteromonas* sp.*(* Yeo, I.O., et al,1995*), Brevibacterium linens(*Rattray, F.P., 1995*), Hyphomonasjannaschiana* VP 3(Shi, J., et al,1997) *Lactobacillus helveticus(*Valasaki, K., et al.2008*) Microbacterium* sp. (Gessesse, A. and Gashe, B.A. (1997))*Pimelobacter* sp.*z-483*(Oyama, H., et al.1997*) Salinivibrio* sp. Strain AF-2004 (Heidari, H.R.K., et al.2007) *Streptomyces* isolate EGS-5(Ahmad, S.M. (2011)) *Streptomyces microflavus* (Rifaat, H.M., et al.2006) *Streptomyces rimosus* (Yang, S.S. and Wang, J.Y. 1999) *Thermoactinomyce* ssp. (Lee, J.K., et al.1996) *Thermoactinomycesthalpophilus* THM1 (Anderson, J.K., et al.1997).In the present study ,quantitative estimation of protease produced by selected bacterial isolates from soil and water samples collected from food processing industries was carried out and best protease producing organism can be used for the degradation of proteinaceous waste material from food manufacturing units leading to recycling of food industry waste.

2. MATERIALS AND METHODS

Isolation and identification of Protease producing bacteria

Isolation of protease producing bacteria from soil and water samples collected from food processing industries was performed by serial dilution technique (Sjodahl *et al.* (2002)). A total of 87 dissimilar colonies from nutrient agar plates were selected and each isolate was given a reference number (TKMFT 01 to TKMFT87) and each isolate was subjected to primary screening for the production of protease by plate assay using gelatine agar (Abdel Galil, 1992). Selection of protease producing organisms was carried out by measuring the zone diameter on Gelatine agar medium. According to the results obtained, the maximum zone diameter was obtained for TKMFT25 (15mm) on Gelatine Agar medium which was followed by TKMFT39 (14mm), TKMFT22 (22mm), TKMFT10 (10mm), TKMFT53 (9mm), TKMFT19 (8mm), TKMFT 8 & TKMFT61 (7mm).Selected isolates were identified based on the morphological and biochemical identification using Biomerieux Vitek-2 compact automated system.

Qualitative test for Protease

Proteolytic activities of selected bacterial isolates were detected on the basis of formation of clear zones around the bacterial isolates. Bacterial colonies appeared on agar plates were screened for evaluating their proteolytic potential by inoculating them in gelatin agar medium .Out of 87 isolates, 8 isolates were used in present study for thorough investigation as it exhibited most prominent zones of proteolysis around the colony. Protein hydrolysis was expressed as diameter of clear zone in millimetre (mm). Based on the results obtained in the biochemical identification of 8 isolates using Biomerieux VITEK 2 System, 5 isolates were selected for quantitative test for protease activity.

Quantitative estimation of Protease

The five bacterial isolates were inoculated separately to five conical flasks containing 100 ml MRS (De Man, Rogosa and Sharpe) broth and incubated over a 10 days period at 37^0 C in a rotary shaker. MRS broth without bacterial inoculation kept as control. The contents of the flasks were collected in a centrifuge tube and it was centrifuged at 10000 rpm for 10 minutes at 4^0 C in order to get the cell free supernatant containing crude enzyme. The protease assay was carried out from the first day onwards to till 10 th day to find out the day with maximum enzyme production.

Fig.II . MRS broth inoculated with bacterial isolates for Quantitative estimation of Protease

Measurement of activity of Enzyme

Protease activity in the culture supernatant was determined according to the method of Tsuchida et al. (1986) by using casein as a substrate. A mixture of 500 µl of 1% (w/v) of casein in 50 mM phosphate buffer, pH 7 and 200 µl crude enzyme extract were incubated in a water bath at 40°C for 20 minutes. After 20 minutes, the enzyme reaction was terminated by the addition of 1 ml of 10% (w/v) trichloroacetic acid (TCA) and was kept at room temperature for 15 minutes. Then, the reaction mixture was centrifuged to separate the unreacted casein at 10,000 rpm for 5 minutes. The supernatant mixed with 2.5 ml of 0.44M Na2CO3. 1 ml of 3-fold diluted Follin Ciocalteus phenol reagent was adding. The resulting solution was incubated at room temperature in the dark for 30 minutes and absorbance of the blue colour developed was measured at 660 nm against a reagent blank using a tyrosine standard (Lowry et al. 1951). One unit of protease is defined as the amount of enzyme that releases 1 µg of tyrosine per ml per minute under the standard conditions of supernatant solution.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 10, October-2016

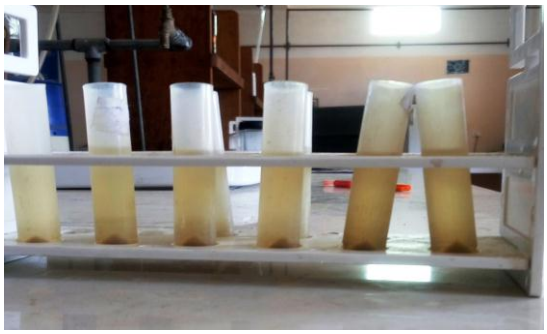

Fig.III. Supernatant solution containing

Fig.IV. Standard Protein assay by Folin Lowry's method crude protease enzyme for quantitative estimation of Protease

Quantitative assay of protein: The total protein content of the samples was determined by by Lowry method using Bovine serum Albumin as standard (Lowry et al. 1951).

3. RESULT AND DISCUSSION

Bacteria were isolated from soil and water samples collected from food processing industries. The proteolysis ability of 87 bacterial isolates from soil and waste water samples were evaluated using Gelatine agar medium.

Following inoculation and incubation of the Gelatine agar plates, organisms secreting protease enzyme exhibited a zone of proteolysis which was shown by a clear area around bacterial colonies. Among 87 isolated bacteria, 27 isolates were protease producer based on zone of hydrolysis and out of them 8 isolates (TKMFT 8, TKMFT10, TKMFT19, TKMFT22, TKMFT25, TKMFT39, TKMFT53, and TKMFT61) were chosen for further studies based on the diameter of zone of hydrolysis.

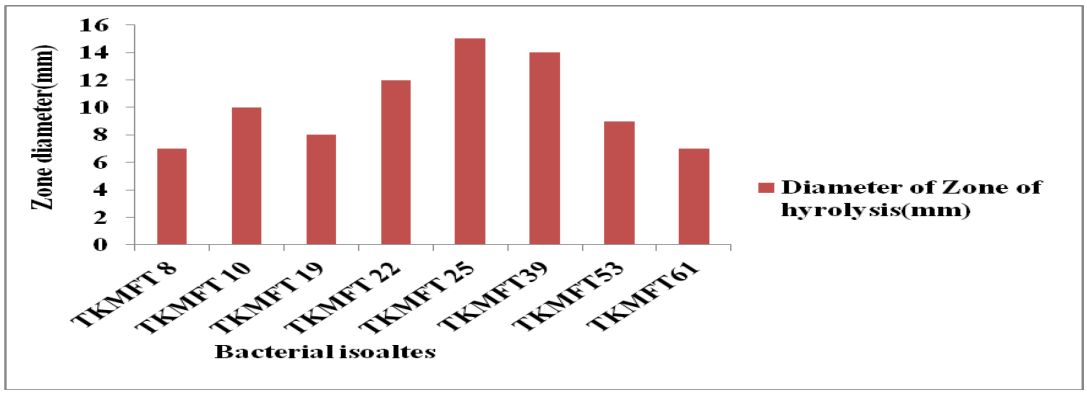

Fig.I. Zone of Diameter of protease producing Bacterial isolates

The selected 8 isolates were identified using cultural characterization, microscopic observation and biochemical identification using Biomerieux VITEK 2 system. Among the 8 isolates, TKMFT22, 10, 53 are representing *Staphylococcus intermedius*, TKMFT25, 39 are representing *Enterobacter asburiae*, and TKMFT8, TKMFT 19, and TKMFT61 are representing *Cedecea davisae*, *Proteus mirabilis* and *Alloiococcus otitis* respectively according to the test results.

The bacterial isolates were screened for their abilities to produce extracellular protease during their growth on protease production medium (Folasade and Joshua 2005).In order to select the bacterial isolate with highest potential of protease production, the selected 5 isolates were tested for extracellular protease production in liquid medium (MRS broth). Protease activity in the crude enzyme extract was determined (Carrie Cupp Enyard, 2008). The selected 5 isolates (TKMFT8, TKMFT10, TKMFT19, TKMFT25, and TKMFT61) were inoculated into MRS broth and incubated over a 10 days period at 37^0 C in a rotary shaker and quantitatively determined the production of extracellular protease. The protease assay was carried out from the first day onwards to till 10 th day to find out the day with maximum enzyme production .The results and observations are summarized in Table.I.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 10, October-2016

Table. I. Protease activity (U/ml) of bacterial isolates and protein content mg/ml

Incubation day	Protease activity(u/ml) & protein content mg/ml					
		TKMFT8	TKMFT10	TKMFT19	TKMFT25	TKMFT61
1	PA	6.93±0.75	45.5 ±1.3	15.16±1.98	6.93±0.75	16.46±1.98
	PC	2.73	1.97	2.25	1.89	0.89
2	PA	17.33 ±0.75	57.63 ±0.75	35.1±1.3	14.73±1.98	39±2.25
	PC	2.52	1.95	2.13	2.23	0.85
3	PA	45.06±1.98	70.2±1.3	41.16±0.75	17.76±0.75	46.36±1.50
	PC	2.37	1.75	1.98	1.78	0.72
4	PA	61.1±1.3	80.16±0.75	54.6±1.3	26.86±1.98	83.2±2.25
	PC	1.98	1.32	1.77	1.65	0.65
5	PA	91.43±1.98	88.83±0.75	78.43±2.70	42.03±2.70	103.56±1.98
	PC	1.81	1.1	1.78	1.63	0.64
6	PA	138.23±1.50	125.66±1.98	95.76±0.75	61.53±1.98	97.5±2.25
	PC	1.8	0.95	1.53	1.32	0.63
7	PA	113.06±1.98	117.43±1.98	126.96±1.98	56.33±075	91±2.6
	PC	1.79	0.94	0.87	1.31	0.52
8	PA	91 ±1.3	108.76±1.98	114.4±1.3	38.13±2.25	74.96±0.75
	PC	1.78	0.94	0.89	1.05	0.5
9	PA	84.5±1.3	89.26±1.98	109.63±2.70	31.63±0.75	61.96±0.75
	PC	1.59	0.97	0.72	0.97	0.45
10	PA	77.56 ±0.75	83.2 ±1.3	104.43±0.75	22.96±1.98	55.9±1.3
	PC	1.51	0.98	0.75	0.98	0.43

Protease activity expressed in U/ml.

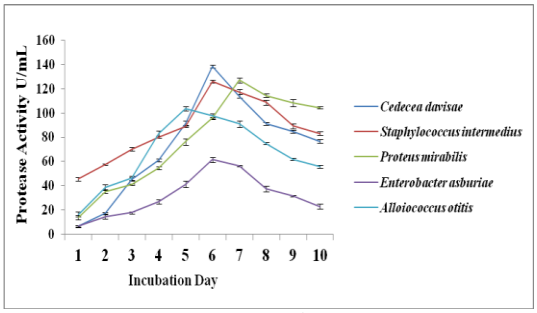

Fig.V.Comparison of Protease activity (U/mL)by different bacterial isolates

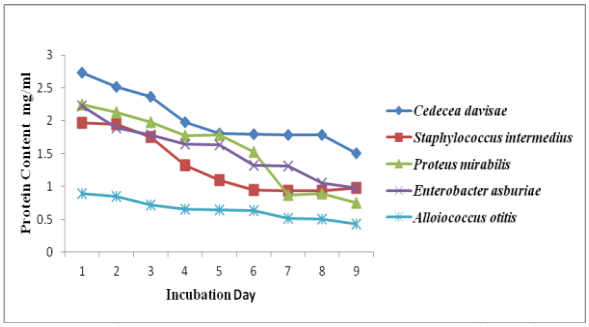

Fig.VI.Comparison of protein content in samples to detect the effect of protease activity

The results shown in Table. I revealed that all the bacterial isolates under study secreted protease enzyme at varied levels. The enzyme activity by *Cedecea davisae* (TKMFT8), *Staphylococcus intermedius* (TKMFT10) and *Enterobacter asburiae* (TKMFT25) was gradually increased from first day of incubation to sixth day of incubation and the activity were shown in Table. I. It was also clear from the results that the activity of enzyme gradually decreased from sixth day of incubation to tenth day of incubation. It was found that the highest protease activity by these isolates has been observed on sixth day of incubation and it was found to be 138.23±1.50U/mL, 125.66±1.98 U/mL and 61.53±1.98 U/mL respectively. The enzyme activity exhibited by *Proteus mirabilis* (TKMFT19) was gradually increased from first day of incubation to seventh day of incubation as shown in Table and after seventh day it started decreasing. The highest activity by *Proteus mirabilis* (TKMFT19) was found to be 126.96±1.98 U/mL and it was observed on seventh day of incubation. The activity of enzyme by *Alloiococcus otitis* (TKMFT61) has been increased from first day of incubation to fifth day of incubation and after that it started decreasing and the maximum activity was found on fifth day of incubation and it was found to be 103.56±1.98 U/mL. Protease activity and Protein content are related. It was clear from the data presented in Table. I and Fig. and graphical representation of protein Content in Fig.VI that increased protease activity decreased the protein content in the inoculated media. The decrease in the total protein content was resulted from the degradation of protein due to

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 10, October-2016

the action of proteolysis enzymes secreted by the selected bacterial isolates. Among all studied bacterial isolates, the maximum protease activity (138.23±1.50) was recorded in *Cedecea davisae* (TKMFT8). In a similar screening method in which a total of 118 bacterial strains were isolated from soil and *Bacillus* sp. predominant was used for the production of protease with activity (380U/ml) (Mohsin *et al* (2011). Sharma et al. (2015) reported the maximum protease activity (37.94 U/ml) by the bacterial isolate No. 2 at 37ºC after incubation time of 72 h. The highest extracellular protease production (243 U/ml) by *Bacillus sp* without optimization of culture conditions was reported by Alnahdi (2012). In another study, Sinha et al. (2013) reported maximum protease activity (124.2 U/ml) exhibited by *Bacillus sp* .Sp-5after 24 h of incubation at 25 ºC using different nitrogen and Carbon sources like yeast extract and sucrose respectively. The study conducted by Smita et al (2012) reported highest protease activity (66.23 U/ml) showed by the bacterial species *Serratia liquiefaciens* at 37 ºC after 48 h of incubation. In a similar study, Tiwari et al (2015) studied the enzyme activity of the bacterial isolate B2 in a medium (P^H 7.0) without optimization at 37 ºC for 48h in a shaker. It was found that the isolate produced 68.05IU/ml enzyme and also checked the bacterial isolates in an alkaline medium for quantitative test of extracellular protease in a liquid medium and were found the production of protease at varying levels. Other works reported that *Bacillus cereus* S-98 and *Bacillus anthracis* S-44 showed their maximum activity for the biosynthesis of proteases within 60h incubation period since the productivity of protease reached up to 240.45U/ml for *Bacillus cereus* S-98 and 126.09U/ml for *Bacillus anthracis* S-44 (Johnvesly B and Naik G R, 2001). These data are in agreement with the present findings and revealed that protease producing organisms are widespread in environment and can be able to produce protease enzyme at varied levels without optimization of the culture conditions.

4.5 Statistical Analysis

Statistical analysis was carried out using a SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. 17, 2008). The Pearson correlation analysis was performed between protein content and incubation days for selected five bacterial isolates. The statistical analysis shown in Table.4.5 revealed that organisms showed significant positive correlation at different incubation days.

Table. II Pearson's Correlation coefficients between the variables

		Cedecea davisae	*Staphylococcus intermedius*	*Proteus mirabilis*	*Enterobacter asburiae*	*Alloiococcus otitis*
Cedecea davisae	Pearson Correlation	1	.971**	.851**	.950**	.865**
	Sig. (2-tailed)		0	0	0	0
	N	30	30	30	30	30
Staphylococcus intermedius	Pearson Correlation	.971**	1	.856**	.953**	.828**
	Sig. (2-tailed)	0		0	0	0
	N	30	30	30	30	30
Proteus mirabilis	Pearson Correlation	.851**	.856**	1	.750**	.645**
	Sig. (2-tailed)	0	0		0	0
	N	30	30	30	30	30
Enterobacter asburiae	Pearson Correlation	.950**	.953**	.750**	1	.874**
	Sig. (2-tailed)	0	0	0		0
	N	30	30	30	30	30
Alloiococcus otitis	Pearson Correlation	.865**	.828**	.645**	.874**	1
	Sig. (2-tailed)	0	0	0	0	
	N	30	30	30	30	30

** Correlation is significant at the 0.01 level (2-tailed).

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 10, October-2016

4. CONCLUSION

Among the five selected isolates, the maximum protease activity (138.23±1.50) was exhibited by *Cedecea davisae* (TKMFT8) followed by *Proteus mirabilis* (TKMFT19) , *Staphylococcus intermedius* (TKMFT10), *Alloiococcus otitis* (TKMFT61) and *Enterobacter asburiae* (TKMFT25) and the activity was found to be 126.96±1.98, 125.66±1.98, 103.56±1.98 and 61.53±1.98 respectively. The highest protease producing organism (*Cedecea davisae*) can be used for the degradation of proteinaceous waste material from food manufacturing units leading to recycling of food industry waste.

ACKNOWLEDGEMENTS

The author sincerely thanks Cashew Export Promotion Council of India (CEPCI), Mundakkal, Kollam, and Kerala, India for providing experimental facilities to carry out the work.

REFERENCES

[1] Abdel, Galil, O.A. 1992. Formation of proteases by *Aspergillus fumigates* and *pencillium* sp. *J. King. Saud. Univ.*, Vol.4 (2): 127–136.

[2] Adulyatham, P. and Owusu-Apenten, R. (2005). *J Food Sci*, 70: C231-C234.24.Shafee, N., Aris, S. N., Rahman, R.Z.A., Basri, M. and Salleh, A.B. (2005) Optimization of Environmental and Nutritional Conditions for the Production of Alkaline Protease by a Newly Isolated Bacterium *Bacillus cereus* Strain 146. *Journal of Applied Sciences Research*, 1, 1-8.

[3] Ahmad, S.M. (2011) Production of thermostable alkaline protease from an alkaline-resistant *Streptomyces* isolate EGS-5. *International Journal of Academic Research*, 3, 393

[4] Alnahdi, H.S (2012). Isolation and screening of extracellular proteases produced by new isolated *Bacillus* sp. *J. Appl. Pharma. Sci.*, Vol.2(9): 071–074

[5] Anderson, J.K., Grimble, G.K. and Cowan, D.A. (1997) A process for producing a thermostable proteolytic enzyme from *Thermoactinomyces thalpophilus* THM1. PCT Pa- tent Application, WO 23605

[6] Anita,A and Rabeeth,M(2010) Isolation, purification and characterization of protease enzyme from Bacillus sp.,possible use in biodiesel production. Asian Jr of Microbiolo Biotech Env Csi 12, 71-77

[7] Arun Kumar Sharma, Vinay Sharma, Jyoti Saxena, Bindu Yadav, Afroz Alam, Anand Prakash(2015). Isolation and Screening of Extracellular Protease Enzyme from Bacterial and Fungal Isolates of Soil. *Int. J. Sci. Res. Environ. Sci.*, Vol.3(9): 0334–0340.

[8] Barett, A. J (1994). Proteolytic enzymes: serine and cysteine peptidases. Methods Enzymol, 244, p: 1–15.

[9] Barett, A. J (1995). Proteolytic enzymes: aspartic and metallopeptidases. Methods Enzymol. 248, p: 183.

[10] Beheshti Maal K, Emtiazi G, Nahvi I (2009). Production of alkaline protease by *Bacillus cereus* and *Bacillus polymyxa* in new industrial culture mediums and its immobilization. African J. Microbiology Research, 3(9): 491-497.

[11] Bhatnagar, D., Joseph, I., and Paul Raj, R (2010) .Amylase and acid protease production by solid state fermentation using *A. niger* from mangrove swamp. Indian J. Fish., 57: 45-51.

[12] Chu W.H. (2007). Optimization of extracellular alkaline protease production from species of *Bacillus*. Journal of Industrial Microbiology Biotechnology, 34(3): 241-245.

[13] Cupp C, Enyard (2008). Sigma's Nonspecific Protease Activity Assay-Casein as Substrate. J. Vis. Exp., 19: 899

[14] Emtiazi G, Nahvi I, Beheshti Maal K (2005). Production and immobilization of
alkaline protease by *Bacillus polymyxa* which degrades various proteins. Int. J. Environ. Stds. 62(1): 101-107.

[15] Folasade M. and Joshua O. Production dynamics of extracellular protease from bacillus species (2005).African J. Biotech ; 4 :776-779

[16] Gessesse, A. and Gashe, B.A. (1997) Production of alka- line protease by an alkalophilic bacterium isolated from an alkaline soda lake. *Biotechnology Letters*, 19, 479-481.

[17] Heidari, H.R.K., Ziaee, A.A., Schaller, J. and Amoozegar, M.A. (2007) Purification and characterization of an extra-cellular haloalkalineprotese produced by the moderately halophylic bacterium, *Salinivibrio* sp. strain AF-2004. *Enzyme and Microbial Technology*, 40, 266-272.

[18] Johnvesly B, Naik GR.(2001). Study on production of thermostable alkaline protease from thermophilic and alkaliphilic *Bacillus* spp. JB-99 in a chemically defined medium. Process Biochemistry ; 37: 139-144

[19] Kunamneni, A., Ellaiah, P. and Prasad, S. D. (2003). AAPS *Pharmatech*, 4 Article 56

[20] Louwrens W. Theron & Benoit Divol (2014). Microbial aspartic proteases: current and potential applications in industry. Appl Microbiol Biotechnol, 98:8853–8868

[21] Leng, X. W., and Yan Xu (2011). Improvement of acid protease production by a mixed culture of *Aspergillus niger* and *Aspergillus oryzae* using solid-state fermentation technique. Afr. J. Biotechnol., 10, p: 6824-6829

[22] Lee, J.K., Kim, Y.O., Kim, H.K., Park, Y.S. and Oh, T.K. (1996) Purification and characterization of a thermostable alkaline protease from *Thermoactinomyces* sp. E79 and the DNA sequence of the encoding gene. *Bioscience, Biotechnology, and Biochemistry*, **60**, 840-846.

[23] Lowry O H, Rosebrough N J, Farr A L & Randall R J.(1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265

[24] Mohsin A. K., Nadeem A., Ahmad U.Z., Idrees A. N. And Muhammad A. Q (2011) Isolation and screening of alkaline protease producing bacteria and physio-chemical characterization of the enzyme African Journal of Biotechnology ; 10:(33) 6203-6212

[25] Mehreb-Dini, C., Cabral, H., Leite, R. S. R., Zanphorlin, L. M., Okamoto, D. N., Rodriguez, G. O. B., Juliano, L., Arantes, E. C., Domes, E. and Da Silva,
R. (2009). *J Agric Food Chem*, 57: 9210-9217.

[26] Onkar Nath Tiwari, Thiyam Bidyababy Devi, Kangjam Sarabati Devi, Gunapati Oinam, Thingujam Indrama, Keithellakpam Ojit, Oinam Avijeet, Lakreiphy Ningshen(2015) . Isolation and optimization of alkaline protease producing Bacteria from undisturbed soil of NE-region of India falling under Indo-Burmabiodiversity hotspots Journal of Applied Biology & Biotechnology Vol. 3 (04), pp. 025-031

[27] Oyama, H., Kinjoh, M., Watari, M. and Murao, S. (1997) Purification and characterization of an alkaline protease produced by *Pimelobacter* sp. Z-483. *Journal of Fermen-tation and Bioengineering*, 84, 351-353

[28] Rao, M.B., Tanksale, A.M., Ghatge, M.S. and Deshpande, V.V. (1998) Molecular and biotechnological aspects of microbial proteases. *Microbiology and Molecular Biology Reviews*, 62, 597-635

[29] Rattray, F.P., Bockelmann, W. and Fox, P.F. (1995) Puri-fication and characterization of an extracellular proteinase from *Brevibacterium linens* ATCC 9174. *Applied Envi-ronmental Microbiology*, 61, 3454-345.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 10, October-2016

[30] Rifaat, H.M., Hassanein, S.M., El-Said, O.H., Saleh, S.M. and Selim, M.S.M. (2006) Purification and characterisa- tion of extracellular neutral protease from *Streptomyces microflavus*. *Arab Journal of Biotechnology*, 9, 51-60.

[31] Sankeerthana, C., Shabana Pinjar, Rizwana, T. J., Shamala Bhavimani, Anupama, S, Sarovar, B. and Shashikala R. I (2013). Production and Partial Characterization of Protease from *Aspergillus Flavus* using Rice Mill Waste as a Substrate and its Comparison with *Aspergillus Niger* Protease. International Journal of Current Engineering and Technology. 1

[32] Shafee, N., Aris, S. N., Rahman, R.Z.A., Basri, M. and Salleh, A.B. (2005) Optimization of Environmental and Nutritional Conditions for the Production of Alkaline Protease by a Newly Isolated Bacterium *Bacillus cereus* Strain 146. *Journal of Applied Sciences Research*, 1, 1-8.

[33] Shi, J., Coyne, V.E. and Weiner, R.M. (1997) Identification of an alkaline metalloprotease produced by the hy- drothermal vent bacterium *Hyphomonas jannaschiana* VP3. *Microbios*, 91, 15-26.

[34] Sinha P, Singh RK, Srivastva R, Sharma R, Tiwari P (2013). Characterization and optimization of alkaline protease enzyme produced by soil borne bacteria. Trends Life Sci., 2(2): 2319–4731.

[35] Sjodahl, J., Emmer, A., Vincent, J., Roeraade, J (2002). Characterization of proteinases from *Antarctic krill* (Euphausia superba). *Protein Expression and Purification*, Vol.26: 153–161

[36] Smita GS, Ray P, Mohapatra S (2012). Quantification and Optimisation of Bacterial Isolates for Production of Alkaline Protease. Asian J. Exp. Biol. Sci., 3(1): 180-186. 48.

[37] Soumya Thrivikraman Vasantha, Abhilash Thankappan Subramanian (2012). Optimization of cultural conditions for the production of an extracellular protease by Pseudomonas species, International Current Pharmaceutical Journal, 2(1): 1-6

[38] Sumantha, A., Larroche C., and Pandey, A (2005) .Microbiology and industrial biotechnology of food - grade proteases: A Perspective.

[39] Sumantha A, Larroche C, Pandey A (2006) Microbiology and industrial biotechnology of food-grade proteases: a perspective. Food Technol Biotechnol 44:221–20

[40] Tsuchida, O., Yamagata, Y., Ishizuka, T., Arai, T., Yamada, J., Takeuchi, M. ad Ichishima, E.(1986). An alkaline proteinase of an alkalophilic Bacillus sp., *Curr. Microbiol.* 14**:**7.

[41] Valasaki, K., Staikou, A., Theodorou, L.G., Charamopou- lou, V., Zacharaki, P. and Papamichael, E.M. (2008) Puri- fication and kinetics of two novel thermophilic extracel- lular proteases from *Lactobacillus helveticus*, from kefir with possible biotechnological interest. *Bioresource Tech- nology*, 99, 5804-5813.

[42] Verma OP, Prashansa K, Shruti S, Abha S (2011). Production of Alkaline Protease by *Bacillus subtilis* (MTCC7312) using Submerged Fermentation and Optimization of Process Parameters. European Journal of Experimental Biology; 1: 124-129.

[43] Ward OP, Rao MB, Kulkarni A (2009) Proteases, Production. In: Schaechter M (ed) Encylopedia of microbiology. Elsevier, USA, pp 495–511

[44] Yadav, S.K., Deepali, B., Shikha and Nandan, S.D (2011). Oxidant and solvent stable alkaline protease from *Aspergillus flavus* and its characterization. African J. Biotechnol., 10: 8630-8640.

[45] Yang, S.S. and Wang, J.Y. (1999) Protease and amylase production of *Steptomyces rimosus* in submerged and so- lid state cultivations. *Botanical Bulletin of Academia Si- nica*, 40, 259-265

[46] Yeo, I.O., Choi, S.H., Lee, J.S. and Kim, C.J. (1995) Characteristics of an alkaline protease from *Alteromonas* sp. *Agricultural Chemistry and Biotechnology*, 38, 106- 110.

[47] Younes Ghasemia, Sara Rsoul-Aminib, Alireza Ebrahiminezhada, Aboozar Kazemia, Maryam Shahbazia, Najme Talebniaa (2011).Screening and Isolation of Extracellular Protease Producing Bacteria from the Maharloo Salt Lake. Iranian Journal of Pharmaceutical Sciences, 7(3): 175-180