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Screening and Characterization of Proteolytic Bacteria and Its Application in Antibiofilm Activity

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Abstract

Proteases are the important compounds of all life on earth including fungi, plants, animals and prokaryotes. About 60% of the total enzyme market has been grabbed by protease. Protease may also be called as proteinase, peptidases or proteolytic enzyme that hydrolysis (Addition of water across the peptide bonds) the peptide bond in between the amino acids. Depending on their acid base behavior they are divided into 3 groups acid protease produced by mostly fungi, Neutral protease produced by plants sources and finally Alkaline protease. *Bacillus* species are well known for their extracellular protease enzyme production and industries majorly uses *Bacillus subtilis* for the production of various enzymes. *Bacillus* is rod shaped bacterium with protective endospore that can withstand extreme conditions. They are obligate aerobes or facultative anaerobes in which few are pathogenic and others are free living. The present study reports The Screening and characterization of proteolytic bacteria from various samples and its application in antibiofilm activity. 34 bacterial species were isolated and screened. Two bacterial species (*LSSP03* and *SCPW01)* were selected for the optimization of protease enzyme using various parameters such as carbon, nitrogen and pH. In order to identify the bacteria species using molecular methods two isolates (FS 1 & SP1) were selected for genome sequencing. The protease produced from the bacteria was checked for its antibacterial activity using well diffusion method and the antibiofilm activity was checked against the biofilm produced by *Pseudomonas aeruginosa* by Tube and Microtitre plate method.

Article Info

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Keywords

Protease, Hicrome Bacillus agar, *Bacillus* sps, Antibiofilm

Introduction

Micro-organisms has an incredible characteristics of producing a wide variety of extracellular and intracellular enzymes. These enzymes have many applications on in the industrial scale. The maximum yield can be achieved by selecting the appropriate micro-organism strain and culturing them at optimum conditions. By this way, Products such as beer, wine, cheese, bread can be made. These bacterial enzymes are used instead of chemicals and other constituents because the microbial enzymes are eco-friendly and harmless. The modern biotechnological industry plays a vital role in the production of enzymes like protease etc. This production may involve various disciplines such as microbiology, genetics, engineering, biochemistry (Gaurav Pant *et al.*,).

Materials and Methods

Isolation and identification

Various Samples like domestic and livestock waste soil, Agricultural soil, Frozen fish samples were collected from various sites and bought to the laboratory for processing. These samples were subjected for serial dilution technique and spread plate method was performed. Skim Milk agar medium used to isolate the proteolytic or protease producing bacteria and then further it was screened and its phenotypic character was studied by using Hicrome Bacillus agar for Identifying *Bacillus* species. The isolates was identified based on cellular morphology, Gram staining, endospore staining and biochemical tests, and further confirmed by molecular level taxonomy.

Screening of protease utilizers

Different types of bacteria were isolated from the various samples by serial dilution method and they were screened for the production of protease enzyme. Sterile production medium called Skim milk agar medium was inoculated with the organism. After incubation period, the clear zone was formed. Formation of zone around the organism was considered as positive [protease utilizers]

Optimization of protease effect of pH on protease production

The isolates such as *LSSP03 and SCPW01 were* inoculated in Tryptic soy broth at different pH ranges from pH 4 to 9 and incubated at 37℃ for 48 hours. The Protease production was observed in spectrophotometer at OD of 560nm.

Effect of various carbon source on protease production

The isolates such as *LSSP03 and SCPW01* were inoculated in the Tryptic soy broth supplemented with various carbon source such as Glucose, Sucrose, Lactose, Maltose Mannitol, Glycerol incubated at 37 °C for 48 hours. The protease activity was measured by spectrophotometry of 660nm

Effect of various nitrogen source on protease production

The isolates such as *LSSP03 and SCPW01* were inoculated in the Tryptic soy broth supplemented with various nitrogen source such as Casein, peptone, yeast extract, beef extract, ammonium chloride, 40%urea incubated at 37°C for 48 hours. The protease activity was observed in spectrophotometer at OD of 660nm.

Genome sequencing

The following cultures FS1 and SP1 were subjected for genome sequencing and the sequencing was done in PACE Microbial technology. Pondicherry.

16srRNA isolation, amplification, sequencing and treeing programme protocol

1. Preparation of template DNA It is important to use a pure cultured bacterium for identification. Colonies are picked up with a sterilized toothpick, and suspended in 0.5ml of sterilizes saline in a 1.5ml centrifuge tube. Centrifuged at 10,000 rpm for 10 min. After removal of supernatant, the pellet is suspended in 0.5ml of InstaGene Matrix (Bio-Rad, USA). Incubated 56°C for 30 min and then heated 100°C for 10 min. After heating, supernatant can be for PCR.
2. PCR Add 1µl of template DNA in 20µl of PCR reaction solution. Use 518 F/800 R primers for bacteria, and then perform 35 amplification cycles at 94°C for 45 sec, 55°C for 60 sec, and 72°Cfor 60 sec. DNA fragment are amplified about 1,400bp in the case of bacteria. Include a positive control (*E.coli* genomic DNA) and a negative control in the PCR.

Purification

Products remove unincorporated PCR primers and dNTPs from PCR product by using Montage PCR clean up kit (Millipore).

Sequencing

The purified PCR products of approximately 1,400bp were sequenced by using the primers (785 F 5' GGA TTA GAT ACC CTG GTA 3' and 907 R 5' CCG TCA ATT CCT TTR AGT TT 3'). Sequencing were performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA) . Sequencing product were resolved on an Applied BioSystems were model 3730XL automated DNA sequencing system (Applied BioSystems, USA).

Phylogenetic tree construction

The culture sequence obtained were subjected to BLAST analysis, the phylogenetically similar type strains sequences and other phylogenetic related sequence were

selected from the Gen Bank and they were subjected to multiple sequence alignment and then align sequences were trimmed to similar length in nucleotides and were subjected to phylogenetic tree (neighbour joining) construction using MEGA 6. In the tree the number at the nodes indicate levels of the bootstrap support [high bootstrap values (close to 100%) meaning uniform support] based on a neighbour- joining analysis of 1,000 re-sampled data sets. The bootstrap values below 50% were not indicated. Bar 0.005 substitutions per site.

Antibacterial activity of the protease against biofilm producing bacteria

50 ml Tryptic soy Broth was prepared and sterilized.,Biofilm producing bacteria *Pseudomonas aeruginosa*(ATCC 27853) was inoculated into the broth aseptically and allowed to incubate for about 3-4 hrsSterile Trytic Soy Agar Plates were prepared. After incubation, using a sterile swab the culture was spread on the TSA plates. Then the wells were cut and the enzyme extract was added to the wells and allowed to incubated for aft 24hrs.

Antibiofilm activity

Test tube method

10 ml of Tryptic soy broth with 0.2 grams of glucose was prepared and transferred 3 ml in 3 test tubes and sterilized using autoclave,3 sterilized test tubes with TSB was marked as Control, Biofilm and Antibiofilm. The Second and third test tube was inoculated with *Pseudomonas aeruginosa* (ATCC 27853)and incubated for 24 hrs. The uninoculated broth is kept as control.

Biofilm test

After 24 hrs, the second test tube was taken and the broth is poured out.5ml of Ethanol was added to the biofilm tube and incubated for 2 mins, Ethanol was discarded and 5 ml Phosphate buffered saline was added and washed for 3 times. Staining dye- 5 ml of 20% Crystal violet was added and left for 15 mins.

After 15 mins, The crystal violet was discarded and the destaining agent 95% ethanol was added and discarded, PBS Solution was added and washed 3 times and the tubes were inverted. Control was also treated the same way the biofilm tube was treated.

Antibiofilm test

The third test tube was taken and the broth was poured out.6 ml of the crude protease enzyme was added and incubated for 3 hrs. After incubation, the enzyme was removed and the biofilm test was performed.

Microtitre plate method

In the microtitre well plate, First well was marked as control second well as biofilm and third well as antibiofilm.100 µl of Uninoculated broth was added in the control well

100µl 4 hrs grown *Pseudomonas aeruginosa* (ATCC 27853)culture in TSB supplemented with glucose was added in the biofilm and antibiofilm well separately.

After 24 hrs, The plate was inverted and tapped so that the broth is completely removed.

In the control and biofilm wells, 200µl of ethanol was added and incubated for 2 mins.

Ethanol was discarded and 200µl of PBS Solution was added and washed thrice.

200µl 20% Crystal violet dye was added and kept for 15 mins. After 15 mins, The crystal violet solution was discarded and destained with 95% Ethanol and washed with PBS thrice.

In the biofilm well, After the broth removal 200 µl Ethanol was added and kept for 2 mins. After 2 mins, 200 µl of Crude protease enzyme was added and incubated for 3 hours. After 3 hours, The OD Value was observed for the 3 wells in spectrophotometer.

Results and Discussions

A total number of 10 different samples were collected from Cuddalore and processed in the lab , among 10 different samples 34 isolates were been obtained (Table 1).

Skim milk agar base medium

The protease producing bacteria were isolated from various samples using skim milk agar by the appearance of Zone of clearance around the bacterial colonies (Proteolysis).

Table.1

S.NO	Sample	Sampling area	Number of isolates	Total Number of Isolates
1	Milk Waste soil	Pathirikuppam, Cuddalore.	6	34
2	Domestic Waste soil	Pathirikuppam, Cuddalore	1	
3	Chicken Waste soil	Pathirikuppam Cuddalore.	2	
4	Canteen Waste water	St Joseph's College of Arts and Science, Cuddalore	4	
5	Slaughter Waste soil	oulgaret, Puducherry	3	
6	Maize Agricultural soil	Thottapettu	3	
7	Frozen fish	Puducherry	5	
8	Oil mill waste soil	SS Oil mill	5	
9	Shrimp pond waste water	Port novo	3	
10	Paddy Agricultural soil	Thavalakuppam	2	

Table.2 Morphological & biochemical characteristic results (5.2) gram staining and spore staining results

S.No	Isolate Name	Gram staining	Spore staining	Appearance on Hicrome Bacillus agar
1	LSSP01	Gram positive rods	Spore forming	Yellowish Green
2	LSSP02	Gram positive rods	Spore forming	Yellow Mucoid
3	LSSP03	Gram positive rods	Spore forming	Yellowish Green
4	LSSP04	Gram positive rods	Spore forming	Yellowish Green
5	LSSP05	Gram positive rods	Non-Spore forming	Yellow Mucoid
6	LSSP06	Gram positive rods	Spore forming	Yellow Mucoid
7	CMWW01	Gram positive rods	Spore forming	Light green
8	CMWW02	Gram negative rods	Non-Spore forming	Orange
9	CMWW03	Gram negative rods	Non-Spore forming	Pink
10	CMWW04	Gram positive rods	Spore forming	Yellowish Green
11	DWSS01	Gram positive rods	Spore forming	Yellowish Green
12	SHWS01	Gram positive rods	Spore forming	Yellowish Green
13	SHWS02	Gram positive long rods	Spore forming	Yellowish Green
14	SHWS03	Gram positive Short rods	Spore forming	Light Green
15	CWSS03	Gram positive rods	Spore forming	Light Green
16	CWSS04	Gram positive rods	Spore forming	Yellowish Green
17	ASSS01	Gram positive rods	Spore forming	Yellow Mucoid
18	ASSS02	Gram positive rods	Spore forming	Yellow Dry
19	ASSS03	Gram positive rods	Non-Spore forming	Light Green
20	FFSS01	Gram positive rods	Non-Spore forming	Yellow Mucoid
21	**FS1**	Gram positive rods	Non-Spore forming	Yellowish Green
22	FFSS03	Gram positive coccI	Non-Spore forming	Pale Yellow
23	FFSS04	Gram positive rods	Spore forming	Yellow Mucoid
24	FFSS05	Gram positive rods	Spore forming	Yellowish Green
25	OMSS01	Gram negative long rods	Non-Spore forming	Light Green
26	OMSS02	Gram positive rods	Spore forming	Light blue
27	OMSS03	Gram positive rods	Spore forming	Light Green
28	OMSS04	Gram positive rods	Spore forming	Light Green
29	OMSS05	Gram positive rods	Non-Spore forming	Blue
30	SCPW01	Gram positive rods	Spore forming	Yellow Mucoid
31	SCPW02	Gram positive rods	Spore forming	Yellow Mucoid
32	**SP1**	Gram positive coccobacilli	Non-Spore forming	Yellowish Green
33	PFSS01	Gram positive rods	Spore forming	Light Green
34	PFSS02	Gram positive rods	Non-Spore forming	White colonies

Table.3 Biochemical reactions

S.No	Isolate Name	Indole	MR	VP	CITRATE	UREASE	TSI	LIA	CATALASE	OXIDASE
1	LSSP01	-	-	-	+	-	A/A	Lysine decarboxylase	+	-
2	LSSP02	-	-	-	+	-	A/A	Lysine decarboxylase	+	-
3	LSSP03	-	-	-	+	-	A/A	Lysine decarboxylase	+	+
4	LSSP04	-	-	-	-	-	A/A	Lysine decarboxylase	+	-
5	LSSP05	-	-	-	-	-	A/A	Lysine decarboxylase	+	+
6	LSSP06	-	-	-	+	-	A/A	Lysine decarboxylase	+	-
7	CMWW01	-	-	-	-	-	A/A	Lysine decarboxylase	+	-
8	CMWW02	-	-	-	-	-	A/A	Lysine decarboxylase	+	-
9	CMWW03	-	-	-	-	-	A/A	Lysine decarboxylase	+	-
10	CMWW04	-	-	+	-	-	K/A	Lysine decarboxylase	-	-
11	DWSS01	-	-	-	+	-	K/A	Lysine decarboxylase	+	+
12	SHWS01	-	-	-	-	-	A/A	Lysine decarboxylase	+	+
13	SHWS02	-	-	-	-	-	A/A	Lysine decarboxylase	+	-
14	SHWS03	-	-	-	-	-	K/A	Lysine decarboxylase	-	-
15	CWSS03	-	-	-	-	-	A/A	Lysine decarboxylase	-	-
16	CWSS04	-	-	-	-	-	A/A	Lysine decarboxylase	+	-
17	ASSS01	-	-	-	+	-	K/A	Lysine decarboxylase	+	-
18	ASSS02	-	-	-	+	-	K/A	Lysine decarboxylase	+	-
19	ASSS03	-	+	+	-	-	K/A	Lysine decarboxylase	+	-
20	FFSS01	-	-	-	+	-	A/A	Lysine decarboxylase	+	-
21	**FS1**	-	-	-	+	-	K/A	Lysine decarboxylase	+	-
22	FFSS03	-	-	-	+	+	A/A	Lysine decarboxylase	+	-
23	FFSS04	-	-	-	+	+	K/A	Lysine decarboxylase	+	-
24	FFSS05	-	+	+	-	-	K/A	Lysine decarboxylase	+	-
25	OMSS01	-	-	-	-	-	A/A	Lysine decarboxylase	+	-
26	OMSS02	-	+	-	-	-	K/A	Lysine decarboxylase	+	-
27	OMSS03	-	+	+	-	-	K/A	Lysine decarboxylase	+	-
28	OMSS04	-	+	-	-	-	A/A	Lysine decarboxylase	+	-
29	OMSS05	-	+	-	-	-	A/A	Lysine decarboxylase	-	-
30	SCPW01	-	-	-	+	-	A/A	Lysine decarboxylase	-	-
31	SCPW02	-	-	-	+	+	K/A	Lysine decarboxylase	-	-
32	**SP1**	-	-	-	-	-	A/A	Lysine decarboxylase	-	+
33	PFSS01	-	+	+	-	-	K/A	Lysine decarboxylase	-	-
34	PFSS02	-	+	+	-	-	K/A	Lysine decarboxylase	-	-

Skim Milk Agar

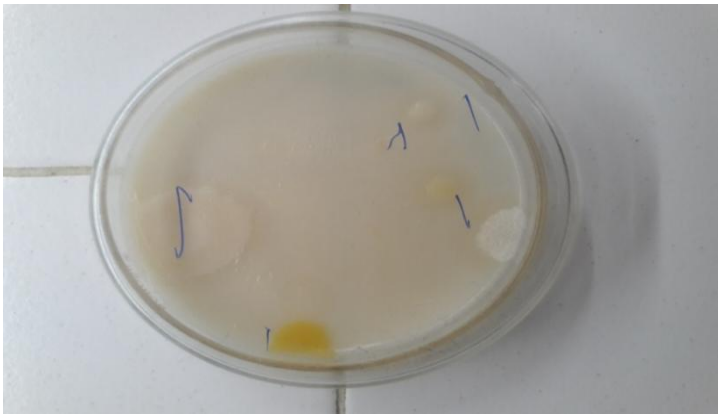


Plate.1 Showing the Zone of clearance around the colonies (Proteolysis)

Hichrome Bacillus Agar

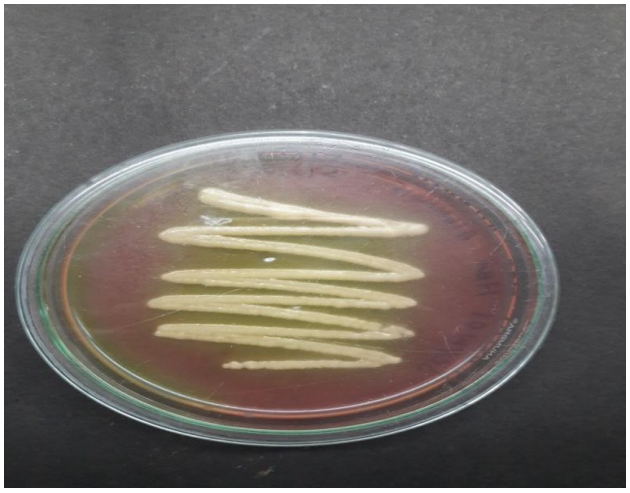


Plate.2 Shows the growth of different *Bacillus* species forming colourful colonies
Antibacterial activity of the Protease Enzyme against Biofilm Producing Bacteria

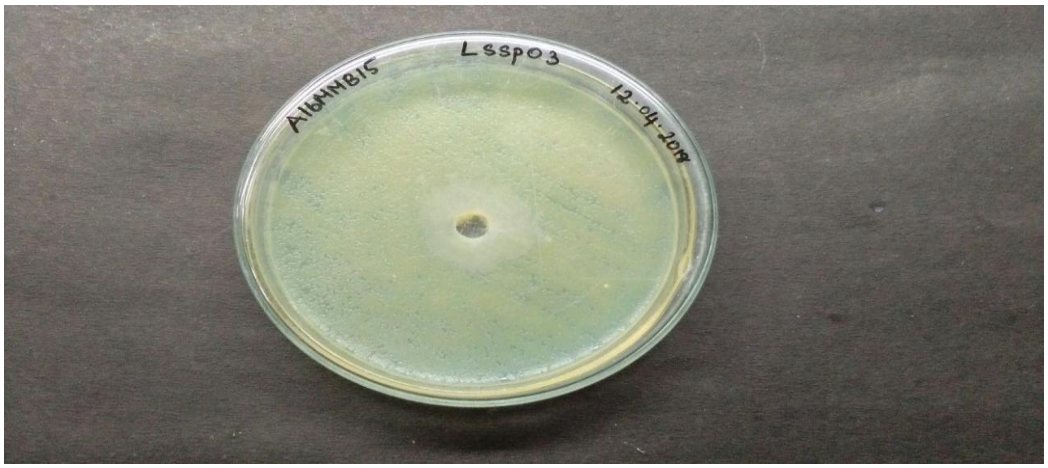


Plate.3 Showing the zone of inhibition against *Pseudomonas aeruginosa* by Protease enzyme

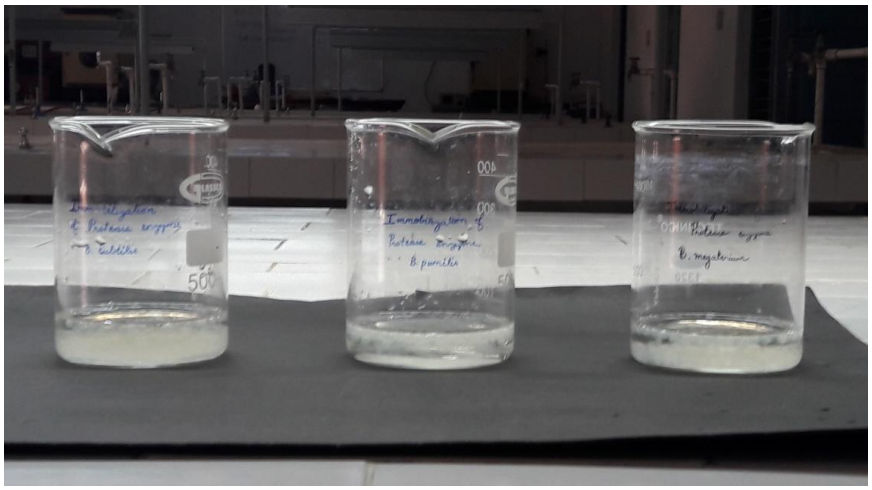


Plate.4 Showing the Immobilization of Enzymes of Isolates LSSP03, SCPW01, OMSS04

Optimization of protease enzyme at different parameters

Protease enzyme at different pH

The isolates such as LSSP03, SCPW01 were inoculated in tryptic soy broth broth at different pH 4to 9 and incubated at 37°C for 48 hours. The Protease production was observed in spectrophotometer at OD 660nm.

Table.4 Effect of various pH on protease production by isolate LSSP03

S.No	Tryptic Soy Broth in different pH levels	OD Value at 660nm
1	4	0.023
2	5	0.230
3	6	0.257
4	7	0.015
5	8	0.109
6	9	0.041

Graph.1 Effect of various ph on protease production by isolate LSSP03

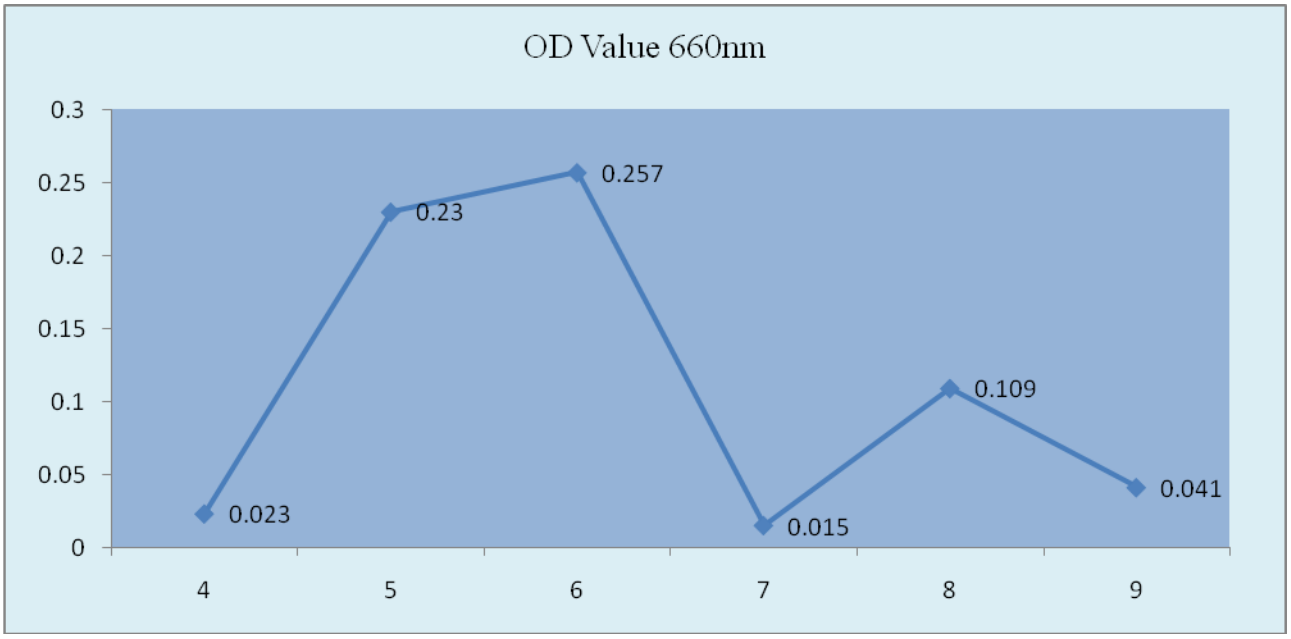
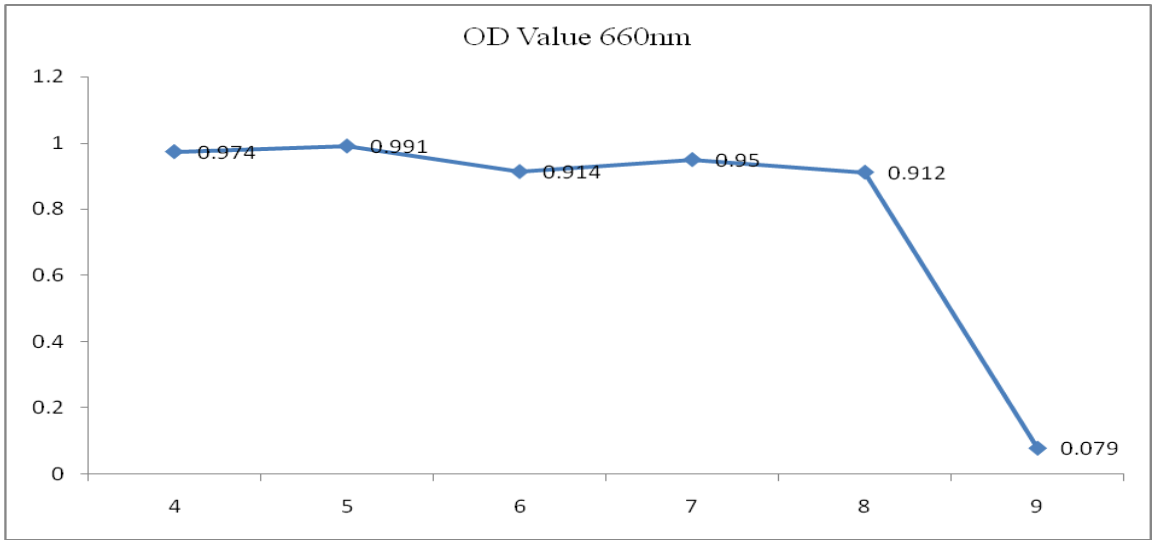


Table.5 Effect of various pH on protease production by isolate SCPW01

S.No	Tryptic Soy Broth in different pH levels	OD Value at 660nm
1	4	0.974
2	5	0.991
3	6	0.914
4	7	0.950
5	8	0.912
6	9	0.079

Graph.2 Effect of various pH on protease production by isolate SCPW01



Protease enzyme at different carbon source:

Table.6 Effect of various carbon source on protease production by isolate LSSP03

S.No	Tryptic Soy Broth in different Carbon sources	OD Value at 660nm
1	Glucose	1.312
2	Sucrose	1.111
3	Lactose	0.149
4	Maltose	1.164
5	Mannitol	1.315
6	Glycerol	0.013

Graph.3 Effect of various carbon source on protease production by isolate LSSP03

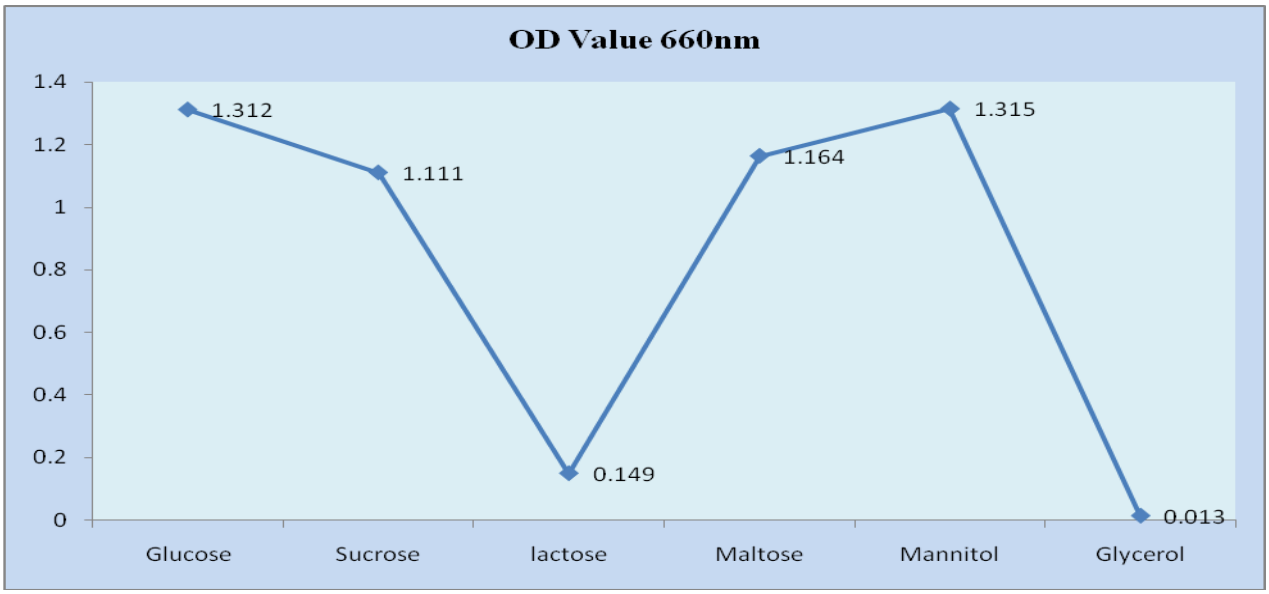
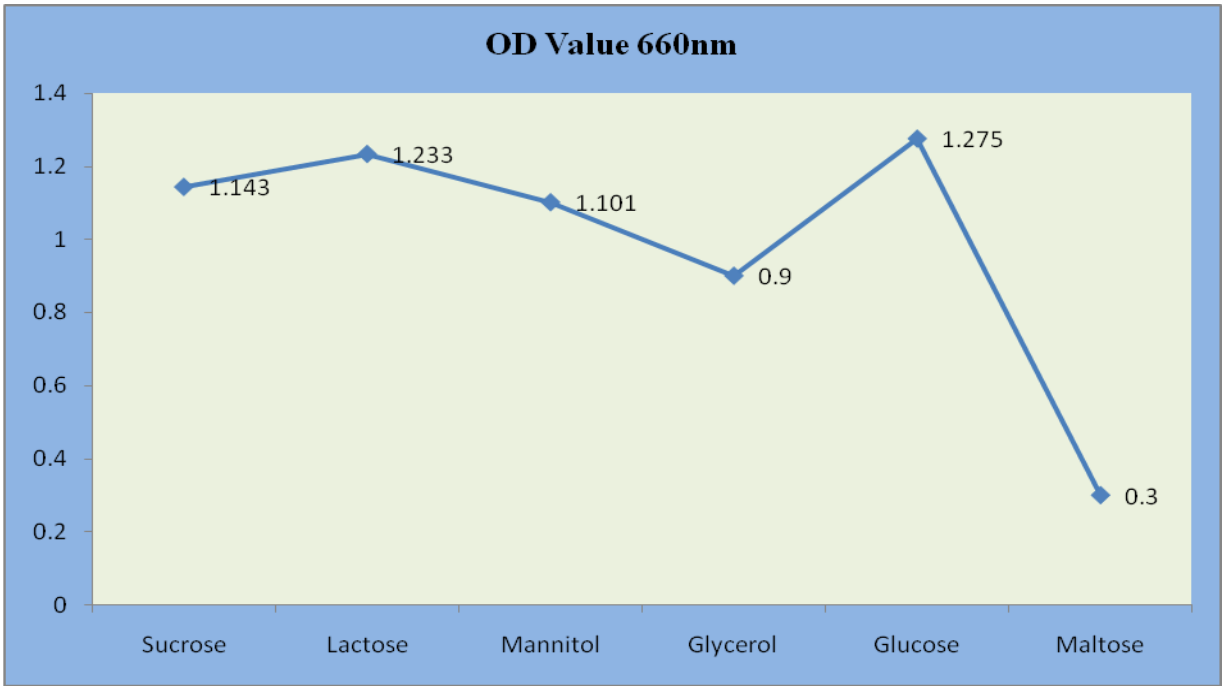


Table.7 Effect of various carbon source on protease production by isolate SCPW01

S.No	Tryptic Soy Broth in different Carbon sources	OD Value at 660nm
1	Sucrose	1.143
2	Lactose	1.233
3	Mannitol	1.101
4	Glycerol	0.900
5	Glucose	1.275
6	Maltose	0.300

Graph.4 Effect of various carbon on protease production by isolate SCPW01



Protease enzyme at different nitrogen source:

Table.8 Effect of various nitrogen source on protease production by isolate LSSP03

S.No	Tryptic Soy Broth in different Nitrogen source	OD Value at 660nm
1	Ammonium Chloride	1.093
2	Casein	1.434
3	Peptone	0.736
4	Beef extract	1.442
5	40% Urea	1.124
6	Yeast extract	0.916

Graph.5 Effect of various nitrogen on protease production by isolate LSSP03

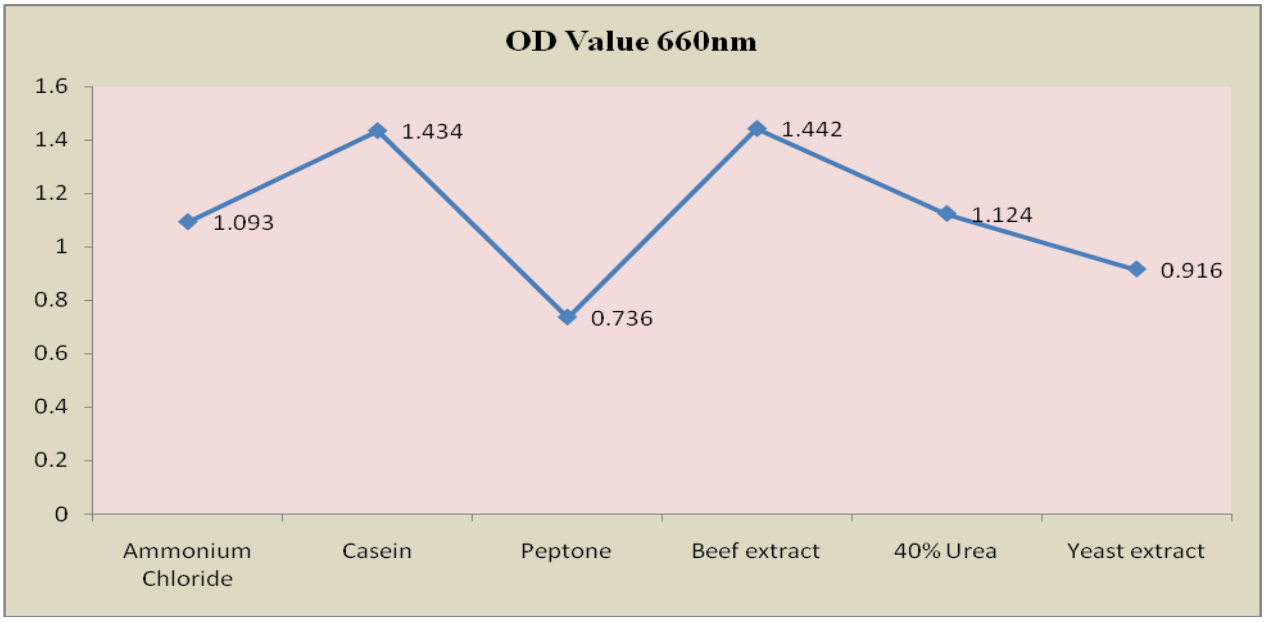
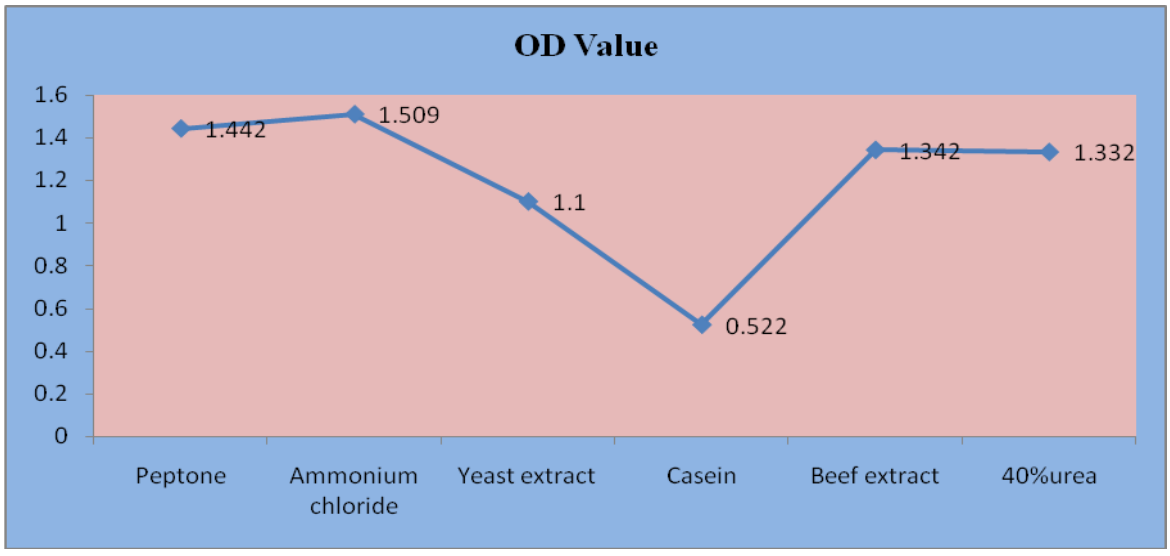


Table.9 Effect of various nitrogen source on protease production by isolate SCPW01

S.No	Tryptic Soy Broth in different Nitrogen sources	OD Value at 660nm
1	Peptone	1.442
2	Ammonium chloride	1.509
3	Yeast extract	1.100
4	Casein	0.522
5	Beef extract	1.342
6	40%urea	1.332

Graph.6 Effect of various nitrogen source on protease production by isolate SCPW01



Antibiofilm activtiy of protease enzyme by microtitre plate assay:

CONTROL: Sterile Tryptic Soy Broth
SAMPLE 1: Inoculated Biofilm Producing Bacteria (*Pseudomonas aeruginosa*. ATCC 27853)
SAMPLE 2: Inoculated Biofilm Bacteria with Protease Enzyme

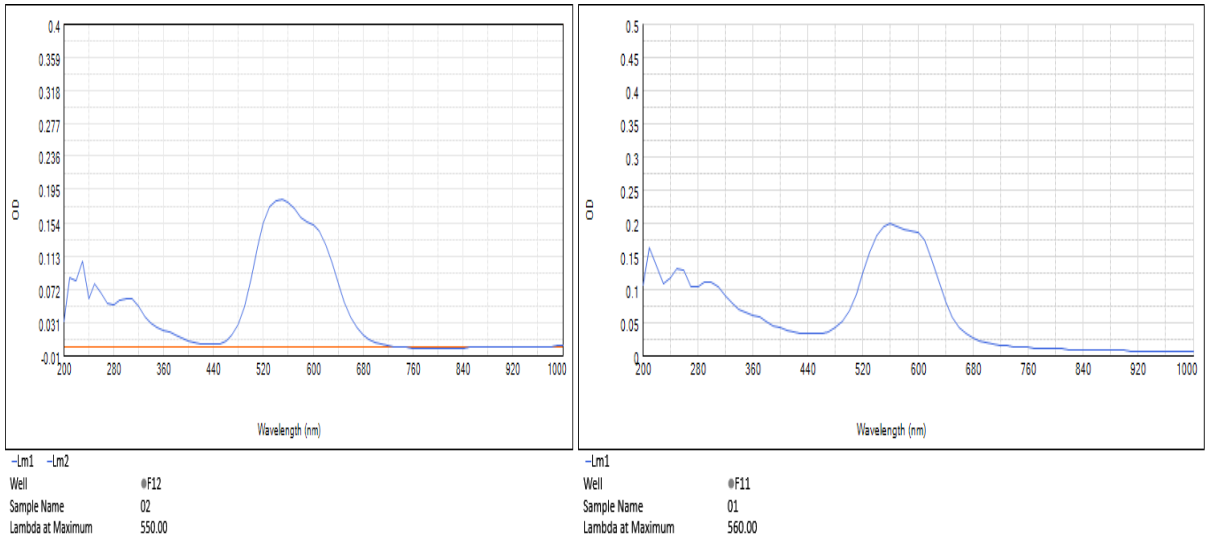
Table.10 Represents the OD Value of Biofilm and Antibiofilm Well in the Microtitre Plate

Control (OD Value)	Sample 1 (OD Value)	Sample 2 (OD Value)
0.000	0.189	0.175

Result: The OD value (560nm)and the spectrum(200-1000range) indicates that the protease enzyme has Antibiofilm Activity

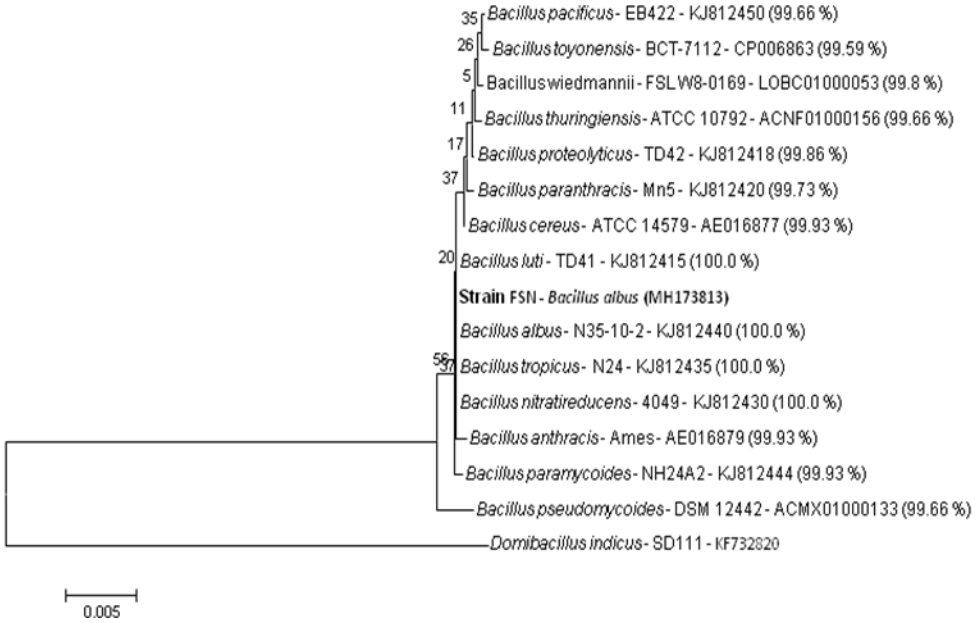
Spectrum

Graph.7 Sample-I- Biofilm **Graph.8** Sample-II-Antibiofilm

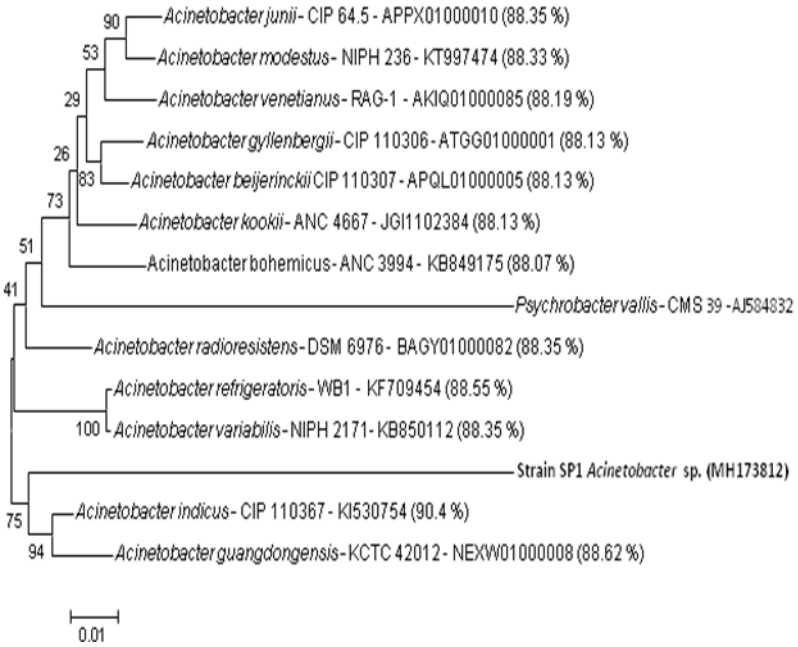


Results of genome sequencing

The following cultures FS1 and SP1 were subjected for genome sequencing and the results were identified by molecular method such as, FS1 – *Bacillus albus* and SP1- *Acinetobacter indicus.* The genome sequenced isolates were submitted to NCBI-GENBANK and the culture name and accession number of FS1and SP1 are MH173813 and MH173812 respectively.



53



The aim of the present study was Screening and Characterization of Proteolytic Bacteria and their Application in Antibiofilm Activity. For this study 10 different samples were collected from various sites in Cuddalore and Pondicherry. The soil and water samples were collected in the sterile container, bought to laboratory and then used for the further process. The present study was the isolation of protease producing bacterial species from various samples and Out of 10 different samples 34 different species of proteolytic bacteria were isolated and identified.

The samples were serially diluted and 0.1ml of diluted sample was transferred in the skim milk agar medium

and incubated the plates at 37°c for 24 hours. After incubation period, the colony morphology and the phenotypic characteristics were observed. The protease producing isolates were identified by skim milk agar medium. The isolates were streaked in the skim milk agar medium and incubate for 37 °C for 48 hours. After incubation period, the zone formation occurs around the organism which indicates the organism is protein utilizers.

The optimization of the protease enzyme was carried out by different pH (4,5,6,7,8,9), different carbon sources such as Glucose, Maltose, Mannitol, Sucrose, Lactose, Glycerol and different nitrogen source such as casein, peptone, yeast extract, beef extract, ammonium chlorite, 40% urea. Enzyme immobilization technique was done by using sodium alginate and calcium chloride with the following isolates such as LSSP03- *Bacillus subtilis*, SCPW01-*Bacillus megaterium*, OMSS04-*Bacillus pumilus*. The immobilized protease beads were formed.

The following cultures FS1, SP1 were subjected for genome sequencing and the results were identified by molecular method for 16srRNA by BLAST and found to be FS1 – *Bacillus albus* and SP1- *Acinetobacter indicus.*

Antibacterial activity of the protease enzyme against Biofilm producing organism such as *Pseudomonas aeruginosa* (ATCC 27853) were performed. The results show Zone of inhibition of 21mm size which clearly indicates the Antibacterial effect. Biofilm and Antibiofilm test was performed using TSB medium supplemented with glucose and Antibiofilm assay was performed using the crude enzyme on the biofilm formed and the OD value was noted using the spectrophotometer. So the study reveals that extracellular enzyme has wide application in controlling biofilm formation and it can be further studied analysed its mode of action against various clinical pathogens producing biofilms.

Protease is the commercially important enzyme and the protease can be extracted from the proteolytic bacteria by culturing them in skim milk agar. Proteolytic bacteria are wide in nature and screening can be done by the visualization of zone of clearance around the colonies. The crude enzymes can be purified by various techniques such as chromatography, ammonium sulphate precipitation etc. and characterized using SDS-PAGE (molecular weight) and other techniques. These enzymes are used in various industries pharmaceuticals etc. for their efficiency. Biofilms being the major threat to mankind in the hospitals as they cause nosocomial infections Antibiofilm assay is done using the protease enzyme as it is eco-friendly and not as harsh as chemicals disinfectants.

Proteases are one of the most important groups of industrial enzymes and account for nearly 60% of the total enzyme sale (Brown and Yada, 1991; Escobar and Barnett 1993; Manjeet Kaur *et al.*, 1998; Dutta and Banerjee, 2006; Shankar *et al.*, 2011). Most of the available proteases produced commercially are of microbial origin (Oskouie *et al.*, 2007).

Significant and impact of the study

The present study reports The Screening and characterization of proteolytic bacteria from various samples and its application in antibiofilm activity. 34 bacterial species were isolated and screened. Two bacterial species (LSSP03 and SCPW01) were selected for the optimization of protease enzyme using various parameters such as carbon, nitrogen and pH. In order to identify the bacteria species using molecular methods two isolates (FS 1 &SP1) were selected for genome sequencing. The protease produced from the bacteria was checked for its antibacterial activity using well diffusion method and the antibiofilm activity was checked against the biofilm produced by *Pseudomonas aeruginosa* by Tube and Microtitre plate method.

The culture (FS 1 & SP1) isolated from various samples was sequenced in PACE Microbial technology Puducherry. The sequencing was interpreted by performing BLAST and aligned data sequence was produced. The isolate **FS1** was identified as *Bacillus albus.* The isolate **SP1** was identified as *Acinetobacter indicus*. From the present investigation, we could conclude that protease producing bacteria are present enormous in the environment and other sources and working on their industrial aspects such as using them as an antibiofilm and antibacterial agents can be effectively done to eradicate biofilms and biofilm producing bacteria.

References

1.Ali jaffarsaleem, 2012. Relationship Study between the Alkaline Protease Production and the Growth Phases of *Pseudomonas aeruginosa* isolated from Patients Advances in Microbiology, 2012, 354-357.
2.Amrita raj., Nancy Khesh, Namratapujar, Sourav Bhattacharya, Arijit das,

SubbaramiahSundararajan.2012.Enhancement of protease production by *Pseudomonas aeruginosa* isolated from dairy effluent sludge and determination of its Fibrinolytic Potential. Asian Pacific Journal of Tropical Biomedicine 2012. S1845-S1851.

3.Ariole,. And E. Ilega., 2013.Alkaline Protease Production by *Pseudomonas aeruginosa* isolated from the Gut of *Pila ovata* Journal of Global Biosciences Vol. 2(5), 2013, pp. 126-131.

4. Charolette Johansen., Per Falholt., and Lone Gram. 1997. Enzymatic Removal and Disinfection of Bacterial Biofilms. Applied and Environmental Microbiology, Pg 0099-2240/97.

5. DalalRupali, 2015. Screening and Isolation of Protease Producing Bacteria From Soil Collected from Different Areas of Burhanpur Region (MP) India. Int Journal. Curr. Microbiol App. Sci2015 Pp. 597-606.

6. El-safey., and U. M. Abdul-Raouf.2004. Production, Purification and Characterization of Protease enzyme from *Bacillus subtilis*. International Conferences for Development And The Environment In The Arab World, Assit Univ., March 23-25, 2004. p 14.

7. Gaurav Panta., Anil prakasha, J.V.P. Pavania, Sayantan Beraa, G.v.N.s. Devirama, Ajay Kumara, Mitalipanchpurib, Ravi Gyanaprasunaa. 2015. Production, optimization and partial purification of protease from Bacillus subtilis. Journal of Taibah University for Science 2015 pg 50–55.

8. Gitishreedas., and M.P. Prasad. 2010.Isolation, purification & mass production of protease enzyme from *Bacillus subtilis*. International Research Journals of microbiology. Vol. 1(2) pp. 026-031 April 2010.

9. Gururaj Tennall, Basavaraj Uapudi, Praveen Kumar Naik. 2012 Isolation of Proteolytic Bacteria and Characterization of their Proteolytic Activity. Vol. 2 No. 3 Aug-Oct 2012 pg185.

10. Hanan S. Alnahdi., 2012. Isolation and screening of extracellular proteases produced by new Isolated *Bacillus* sps Journal of Applied Pharmaceutical Science Vol. 2 (9), pp. 071-074, September, 2012.

11. Kanupriya Miglani Sharma, Rajesh Kumar, Surbhi Panwar Ashwani Kumar. 2017 Microbial alkaline proteases - Optimization of production parameters and their properties Journal of Genetic Engineering and Biotechnology 2017 15, 115–126.

12. Kazuyuki Morihara and Hiroshige Tsuzuki 1977 Production of Protease and Elastase by *Pseudomonas aeruginosa* Strains Isolated from Patients infection and immunity, March. 1977, p. 679-685 American Society for Microbiology Vol. 15, No. 3.

13. Mukherji. R., Patil and Prabhune. 2015. Role of Extracellular Proteases in Biofilm Disruption of Gram Positive Bacteria with Special Emphasis on *Staphylococcus aureus* Biofilms Mukherji, *et al.*, EnzEng 2015, 4:1.

14. Narendra *et al.*, 2012, Isolation and Characterization of Protease Producing Bacteria from soil and Estimation of Protease using spectrophotometer July. 2012 Vol. 1(1), 1-7.

15. Nehakarn, Santoshkumarkarn. 2014. Evaluation and Characterization of Protease Production by Bacillus Sp. Induced By UV-Mutagenesis Karn and Karn, Eng 2014.

16. Perrin baker., Preston J. hill, Brendan D. Snarr, Noor Alnabelseya, Matthew J. pestrak, Mark J. Lee, Laura K. Jennings, John Tam, Roman A. Melnyk, Matthew R. Parsek, Donald C. Sheppard, Daniel J. Wozniak, P. Lynne Howell.2016Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent *Pseudomonas aeruginosa* biofilms Baker *et al.*, Sci. Adv. 2016; 2: e1501632 20 May 2016 1 of 9.

17. Phyllis Molobela, T., Eugene clete and Mervynbeukes.2010 Protease and amylase enzymes for biofilm removal and degradation of extracellular polymeric substances (EPS) produced by *Pseudomonas fluorescens* bacteria. African Journal of Microbiology Research Vol. 4(14), pp. 1515-1524, 18 July, 2010.

18. Philipstiefel., Stefanmauerhofer, Janaschneider, Katharinamaniura-Weber, Rosenberg, B. Qunrena. 2016. Enzymes Enhance Biofilm Removal Efficiency of Cleaners Volume 60 Number 6 Antimicrobial Agents and Chemotherapy 3647.

19. Qing liu., Shujing Sun., Meizi Piao, and Ji Young Yang. 2013 Purification and Characterization of a Protease Produced by a *Planomicrobium* sps from Gut of *Octopus vulgaris* Prev. Nutr. Food Sci. 2013; 18(4): 273-279.

20. Shailima RD vardhini., Mohammed irath. 2013 Isolation, Production, Purification and Applications of Proteases from *Pseudomonas aeruginosa* VRI Biol Med Chem, Volume 1, Issue 2, October, 2013.

21. Soundra Josephine. F., Ramya V.S, Neelamdevi, Suresh babuganapa, Siddalingeshwara K.G. Venugopal. N and Vishwanatha T. 2012. Isolation, production and characterization of protease from *Bacillus* sps isolated from soil sample. Microbiol. Biotech. Res., 2(1): 163-168.

22. Stanley J. Cryz., Barbara H.iglewski.1980, Production of Alkaline Protease by *Pseudomonas aeruginosa*. Journal of Clinical Microbiology July 1980, p. 131-133 Vol. 12, No1.

23. Sudhir K. Shuklaa, T. SubbaRao. 2017. An Improved Crystal Violet Assay for Biofilm Quantification in 2 96-Well Microtitre Plate. Jan. 13, 2017.

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