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Isolation and Identification of Proteolytic Microorganisms from Soil

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Abstract:- The enzyme protease hydrolyses protein into peptides and amino acids. One of the major sources of protease producing bacterial strains is soil. A total of 30 soil samples were collected aseptically from Changanacherry, and brought to the laboratory. The samples were serially diluted and isolated using Nutrient Agar plates. Skim Agar media was used for the enumeration of protease producing microorganisms. The plates were incubated at 37^0C for 24-48 hours. The pure culture of 8 colonies exhibiting highest proteolytic activity was obtained by identifying the clear zones. After analysing the optimal growth conditions and antagonistic activities within the strains, 2 isolates were subjected to antibiotic sensitivity test towards twelve different antibiotics, microbiological characteristics, biochemical analysis and extracellular protease enzyme production. Among the 2 isolates, the one which exhibited highest protease production was identified as *Bacillus thuringiensis* using molecular methods. The results have increased the scope of finding industrially important bacteria from soil which can be used as monoculture or consortium for the degradation of waste on a large scale.

***Keywords*:-** *Soil, Bacterial Strains, Antagonism, Extracellular Protease.*

I. INTRODUCTION

The production of waste and the need to control it have taken an important role in our environment. The amount of waste generated has been increasing due to the rise in population, development related activities, and change in the lifestyle pattern of the inhabitants. The ways in which waste should be handled and disposed is an expanding issue as the amount of waste being generated is increasing day by day. By the application of microorganisms, eco- friendly waste management can be achieved which has tremendous acceptance. Bacteria consumes organic waste for their nutrition thereby prevents the production of odours, pollution, sludge etc. When bacteria consume waste, they convert the waste products into safe by-products and in due course of this conversion they actually produce several metabolites to break down the complex waste into simple compounds. Thus, the soil microorganisms are increasingly becoming an important source in the search for industrially important enzymes [[1]]. Isolation and selection of the desirable microorganism plays an important role in production of desirable enzymes. Proteases are enzymes that have an important role in all life forms on Earth. In industries protease enzyme produced by microorganisms are greatly welcomed due to its stability and its property to induce changes through various biotechnological ways. Proteases constitute one of the most important groups of industrial enzymes, accounting for more than 65 percent of the total worldwide production of enzymes [[2]]. Proteases are enzymes that catalyzes cleavage of the peptide bonds between amino acid in proteins.

Microbial enzymes are relatively more active and stable than the corresponding enzymes derived from animals or plants [3,4]. *Bacillus* species are the potent producers of protease enzyme although many other microorganisms are able to do so. They are preferred more as they need much less space, grows rapidly, easily maintained and are suitable for gene manipulation.

Protease enzyme has a wide variety of functions and wide applications in the field of leather industry, food industry, pharmaceutical industry, bio-remediation process etc. They are ubiquitous in nature and identification and isolation of good strains of proteolytic microorganisms is necessary for the production of the enzyme protease. This study was conducted to isolate bacterial strains from soil having proteolytic activity with the ultimate objective of waste degradation.

II. MATERIALS AND METHODS

- *Sample Collection*

Soil samples for screening proteolytic bacteria were collected from Changanacherry ($9^0$26'47.4"N$76^0$32'24.8"E) superficial layers of soil and aseptically transferred into sterile polythene bags and labelled with date of collection. These were stored in low temperature (refrigerator).

- *Isolation of Bacteria from Soil Sample*

Serial dilution technique was used for the isolation of bacteria. In this technique sample suspension was prepared by adding soil (1g) to 99ml of sterile water (the stock) and

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shaken vigorously for atleast 1 minute. The dilute was then sedimented for a short period. Sterile dilution blanks were marked sequentially starting from stock (10^{-2}) and 10^{-3} to 10^{-6}. One ml from the stock was transferred to the 10^{-3} dilution blank. One ml from the 10^{-3} dilution was transferred to the 10^{-4} tube then repeating this until 10^{-6} dilution. From each dilution tube 0.1ml of dilution fluid was transferred into Skim milk agar and incubated at room temperature for 24 hours. After successful growth of microorganisms, the pure cultures of bacteria were sub-cultured in NA slants; incubated at 28°C to achieve vigorous growth and then preserved in 20% glycerol vials at -23°C [5].

➢ *Determination of Moisture Content (%) and pH of Soil Samples*

Petriplates were weighed and approximately 40g of moist soil were added to each petriplates. After re-weighing the petriplates, these were kept inside a hot air oven at 106°C and dried overnight. After removing the plates from the oven, they were allowed to cool. The petriplates with the oven dried soil was re-weighed. The soil moisture content for each replicate samples were calculated using the equation,

$$\% \text{ Moisture Content (MC)} = \frac{\text{Weight of moist soil (M) - weight of dry soil (D)}}{\text{Weight of dry soil (D)}} \times 100$$

To test the pH of the soil samples, soil testing kit was employed. 5 g of each soil sample was taken into tube number 1 and transferred to tube number 2. 2 ml of pH reagent (pH-1) was added to it and shaken for 5-10 minutes. One drop of decolourizer (D-1) was added and mixed well. The sample solution was filtered into bottle 3 and two drops of pH reagent (pH-2) was added and shaken for 1-2 minutes until the colour develops.The colour developed was compared with standard pH chart.

➢ *Analysis of Physico-Chemical Properties*

The analysis of Nitrogen, Phosphorus and Potassium content were analyzedusing soil testing kit.

For Nitrogen analysis of soil samples, 5 g of each soil sample was taken into tube number 1 and transferred to tube number 2. 2 ml of Nitrogen reagent (N-1) was added to it and shaken for 5-10 minutes. One drop of decolourizer (D-1)was added and mixed well. The sample solution was filtered into bottle 3 and two drops of Nitrogen reagent (N-2) was added and shaken for 1-2 minutes until the colour develops. The colour developed was compared with standard Nitrogen chart. The same was repeated for Phosphorus and Potassium using their respective reagents.

➢ *Antagonism of Bacterial Strains*

Each strain was grown at room temperature and subsequently tested by the cross-streaking method at room temperature and at 37°C. The cross-streaking method was performed as described by [6] with two modifications. The strain to be tested was inoculated as a 1.5 cm wide streak (instead of 1cm) diametrically across duplicate Nutrient agar plates. The plates were incubated overnight at room temperature and 37°C. A wider streak of the original inoculum was used because the inhibitory zones produced were larger and clearer. The indicator strains were streaked singly at right angles to the original inoculum by using a wire loop (8 strains per plate). The plates were incubated at room temperature and 37°C overnight, and inhibition was recorded where the indicator strains crossed the original inoculum. This procedure was followed until each of the strains had been tested against each other.

➢ *Optimization of Growth Conditions*

The pH of the culture media was adjusted to 2.2, 5.5, 7 & 9 in Nutrient Broth medium. For optimization of incubation period and temperature, the culture plates were incubated at 8^0, 21^0, 32^0, 37^0 & 60°C for 6-72 hours.

➢ *Antibiotic Sensitivity Assay*

The disc diffusion method [7] was used for antibiotic sensitivity testing. The test was performed by applying a sample inoculum to the surface of a Mueller-Hinton agar plate. Commercially prepared, fixed concentration, paper antibiotic discs of Gentamycin, Tetracycline, Streptomycin, Penicillin, Norfloxacin, Ceftriaxone, Ciprofloxacin, Co-trimoxazole, Mezlocillin, Amikacin, Carbemicillin and Tobramycin were placed on the inoculated agar surface and incubated for 24 hours at room temperature and zone formation was observed.

The MAR index was determined for each isolates using the formula

$$\text{MAR Index} = \frac{\text{Antibiotic resistance shown}}{\text{Total antibiotic used}}$$

➢ *Antimicrobial Activity*

Well Diffusion method was employed for the analysis of antimicrobial activity as described by [8]. The Agar plates were inoculated with the pathogenic strains (*Klebsiella pneumoniae, Staphylococcus aureus, Vibrio cholerae, Vibrio parahaemolyticus, Pseudomonas sp., E. coli* and *Salmonella sp.*) using a sterile swab on the entire agar surface. Holes of diameter 6-8 mm were punctured on the surface of agar using sterile cork borer. 10 µl of isolated bacterial sample was inoculated into the well using micropipette. The samples were incubated at 37^0 C for 24 hours and the result was obtained.

➢ *Microbiological and Biochemical Analysis of Isolated Bacteria*

Gram stain was performed to observe the cellular morphology and gram nature of the bacteria and biochemical characterization of the strains were also carried out. The bacterial strains were identified by biochemical tests suchas, fermentation of Carbohydrates, Citrate test, Oxidase test, Urease test, Methyl red test, Indole Test, Voges Proskauer test, Nitrate test and Catalase test.

➢ *Enzyme Assay*

Protease activity was determined by Caseinolytic method [[9]]. One unit (U) of enzyme is defined as the amount of protease that liberates peptide fragments

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equivalent to one mg of bovine serum albumin (BSA) per unit time (min-1) under the assay conditions .

➢ *Identification*

Genomic DNA was isolated from the samples and the quality was evaluated on 0.8% Agarose Gel. The 16s gene fragment was amplified by polymerase chain reaction (PCR) from the above isolated genomic DNA. The size and quality were evaluated on 1.5% Agarose Gel. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing of PCR amplicon was performed. The sequences were then used for similarity searches using the Basic Local Alignment Search Tool for identifying the sample.

III. RESULTS & DISCUSSION

➢ *Physical and Chemical Characteristics of Bacterial Isolates*

A total of 30 soil samples were taken. These samples were serially diluted (upto 10^{-6} dilutions) and plated on Nutrient agar plates. Skim agar media was used for the enumeration of protease producing microorganisms from the soil samples. After an incubation of about 24- 48 hours, the proteolytic (protein degrading) activities of bacterial isolates were evaluated using Skim agar media and was expressed as the appearance of a clear zone around the streaked lines (Figure 1). The best isolates with highest protease enzyme activity and those which expressed different colony morphology were selected (8 isolates viz; S1, S2, S3, M1, M2, M3, MS_2 & VC).

The sample, from which M1 was isolated, had a moisture content of 90% and pH 6.

A pH of 6 and moisture content of 94% was exhibited by the soil from which MS_2 was isolated. The remaining soil samples had varying pH ranging from 4-9 and moisture content less than 40%. The physico-chemical properties like Nitrogen, Phosphorus and Potassium content of isolated soil samples were analysed. The soil from which the isolate M1 obtained, the concentration of Nitrogen was 151-200 kg/Acre, that of Phosphorus was 8-10 kg/Acre and Potassium had a concentration of >150kg /Acre. The soil from which the isolate MS_2 obtained, the concentration of Nitrogen was 151-200 kg/Acre, that of Phosphorus was > 15 kg/Acre and Potassium had a concentration of 50-80 kg/Acre.

Fig 1 The Growth of Isolated Bacteria on Skim Milk Agar (Clear Zone Indicates Proteolytic Activity)

➢ *Antagonism Assay*

Cross streaking method was employed to determine the antagonism among the bacterial strains (Figure 2). Each isolates were tested by streaking on Nutrient agar plates with allother isolates and out of the 8 isolates, 2 isolates viz; S3 & M2 showed antagonistic activity against each other (Table 1). These two isolates were separated and the remaining 6 isolates were subjected to further studies.

Table 1 Antagonistic Activity of the Isolated Proteolytic Bacteria

Producing Strain		**Target Strain**							
Sl No		**S1**	**S2**	**S3**	**M1**	**M2**	**M3**	**MS_2**	**VC**
1.	S1	-	-	-	-	-	-	-	-
2.	S2	-	-	-	-	-	-	-	-
3.	S3	-	-	+	-	+	-	+	+
4.	M1	-	-	-	-	-	-	-	-
5.	M2	+	-	+	-	-	-	+	-
6.	M3	-	-	-	-	-	-	-	-
7.	MS_2	-	-	-	-	-	-	-	-
8.	VC	-	-	-	-	-	-	-	-

Fig 2 Antagonistic Activity Exhibited by Isolated Proteolytic Bacteria

➢ *Optimization of Growth Conditions*

The 6 isolates viz; S1, S2, M1, M3, MS_2 &VC were then optimized under different growth conditions viz; pH and temperature. Each of the 6 isolates was inoculated into tubes containing Nutrient Broth of different pH such as 9, 7, 5.5, and 2.2. Each isolate was transferred to the tube containing Nutrient broth of respective pH values. After inoculation, they were kept in different temperatures; 8^0C (refrigerator), 21^0C (BOD incubator), 32^0C (room temperature), 37^0C (Incubator) and 60^0C (Hot Air Oven). Out of 6 isolates, 2 of them were able to grow at high temperature (60^0C). The bacterial strains which showed enzyme production at high temperature can be used for industrial application as well as large-scale production [[10]]. As our aim is to identify microorganisms that can be used for waste degradation, these 2 isolates viz; M1 and MS_2, which were able to withstand the high temperature condition that arise during waste degradation is selected for further studies.

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➢ *Antibiotic Sensitivity Assay*

The Antibiotic susceptibility test of the two selected isolates to various antibiotics viz; Gentamicin, Penicillin, Streptomycin, Tetracycline, Norfloxacin, Ceftriaxone, Ciprofloxacin, Co-trimoxazole, Mezlocillin, Amikacin, Carbenicillin & Tobramycin were tested using Agar disc diffusion method. The result revealed the resistance shown by M1 to Gentamicin, Penicillin, Streptomycin, Tetracycline, Ceftriaxone, Mezlocillin, Amikacin, Carbenicillin & Tobramycin. MS_2 showed resistance towards Penicillin, Ceftriaxone, Co-trimoxazole, Mezlocillin, Amikacin, Carbenicillin & Tobramycin (Table 2). Out of the 12 antibiotics used, M1 was sensitive to Norfloxacin, Ciprofloxacin and Co-Trimoxazole while MS_2 was sensitive to Gentamicin, Streptomycin, Tetracycline, Norfloxacin, and Ciprofloxacin (Figure 3). The acquisition of an antibiotic resistance genotype may actually increase the fitness of certain bacteria in the absence of antibiotic selective pressure, possibly allowing rapid emergence and dissemination of antibiotic resistance on a world-wide scale [[11]]. The sample MS_2 and M1 revealed a MAR index of 0.75 and 0.58 respectively. The MAR index is effective, valid and cost-effective method that is used in source tracking of antibiotic resistant organisms [[12]].

Table 2 Susceptibility of Selected Isolates to Various Antibiotics

Sl No	Antibiotic	M1	MS_2
1.	Gentamycin	Resistant	Sensitive
2.	Penicillin	Resistant	Resistant
3.	Streptomycin	Resistant	Sensistive
4.	Tetracycline	Resistant	Sensitive
5.	Ceftriaxone	Resistant	Resistant
6.	Mezlocillin	Resistant	Resistant
7.	Amikacin	Resistant	Resistant
8.	Carbemicillin	Resistant	Resistant
9.	Tobramycin	Resistant	Resistant
10.	Norfloxacin	Sensitive	Sensitive
11.	Ciprofloxacin	Sensitive	Sensitive
12.	Co-Trimoxazole	Sensitive	Resistant

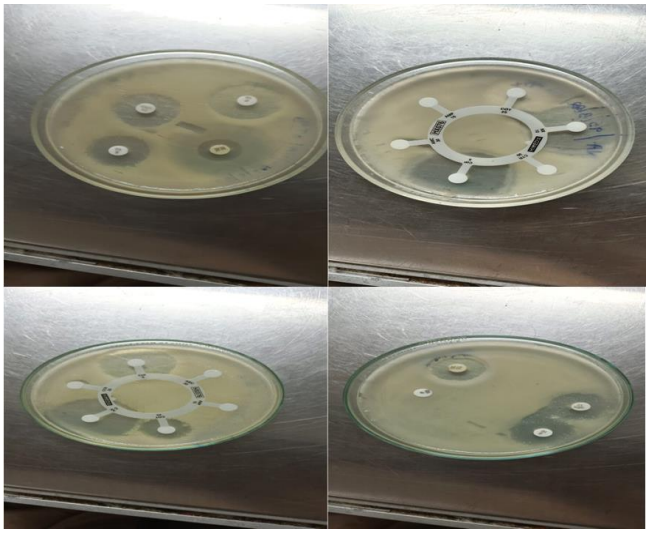

Fig 3 Susceptibility Shown by the Isolated Proteolytic Bacteria Towards Various Antibiotics

➢ *Antimicrobial Assay*

The Antimicrobial activity of the selected isolates to various pathogenic strains such as *Klebsiella pneumoniae, Staphylococcus aureus, Vibrio cholerae, Vibrio parahaemolyticus, Pseudomonas sp, E.coli and Salmonella sp.* revealed the antimicrobial property of sample MS_2 towards *Klebsiella pneumoniae* (Table 3 & Figure 4).

Table 3 Antimicrobial Activity Exhibited by Isolated Proteolytic Bacteria

Sl No	Pathogenic Strain	MS_2	M1
1.	Klebsiella pneumoniae	+	-
2.	Staphylococcus aureus	-	-
3.	Vibrio cholarae	-	-
4.	Vibrio parahaemolyticus	-	-
5.	Pseudomonas sp	-	-
6.	E.coli	-	-
7.	Salmonella sp	-	-

Fig 4 Antimicrobial Activity Shown by the Isolated Proteolytic Bacteria Towards Various Pathogenic Strains

➢ *Microbiological and Biochemical Characteristics*

Visual and microscopic observation was used to characterize the selected strains. Gram staining is an old and reliable method for observing the bacteria. Gram negative bacteria were decolourized by alcohol, losing the purple colour of crystal violet. Gram positive bacteria did not get decolourized and remained purple. In the study, sample MS_2 was identified as Gram-positive Bacilli and M1 as Gram negative Bacilli (Table 4). The biochemical analysis revealed positive results for Glucose fermentation, Lactose fermentation, Methyl Red, Citrate and Catalase test for the sample M1 and MS_2 showed positive results for, Methyl Red and Citrate test. Both isolates produced acid slant and acid butt for Triple Sugar Iron Agar test (Table 5).

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Table 4 Morphological Characteristics of Isolated Proteolytic Bacteria

Sl No	Sample	Colour of colony	Nature of colony	Gram nature	Shape
1.	MS_2	White	Irregular slimy	Gram positive	Bacilli
2.	M1	Cream	Irregular opaque colonies	Gram negative	Bacilli

Table 5 Biochemical Characteristics of Isolated Proteolytic Isolates

Sl No	Test	MS_2	M1
1	Glucose fermentation	-	+
2	Lactose fermentation	-	+
3	Sucrose fermentation	-	-
4	Indole test	-	-
5	Methyl red	+	+
6	Voges Proskauer	-	-
7	Citrate	+	+
8	Urease	-	-
9	Catalase	-	+
10	Oxidase	-	-
11	Triple Sugar Iron Agar	A/A	A/A

* A/A: Acid slant & Acid butt

➢ *Enzyme Assay*

The quantitative assay of the enzyme production of each strain was carried out using Caseinolytic method (Table 6). The absorbance of coloured complex was measured using colorimeter. MS_2 had an enzyme activity of 127.82 Units/ml and M1 had an activity of 65.06 Units/ml (Table). The quantitative assay helped in the identification of more efficient protease producing strains which can be used for industrial purposes.

Table 6 Enzyme Activity of Proteolytic Isolates

	Test	Blank
	5.00	5.00
Casein		
Enzyme solution	1.00	-----
Incubate at 37^0C for 30 minutes		
TCA	5.00	5.00
Enzyme solution	-----	1.00
Incubate at 37^0C for 30 minutes		
Filter the contents		
Filtrate	2.00	2.00
Na_2CO_3	5.00	5.00
F-C Reagent	1.00	1.00
Incubate at 37^0C for 30 minutes and record the absorbance at 600 nm		

Table 7 Enzyme Activity of Proteolytic Isolates

Sample	Enzyme activity (Units/ml)
M1	65.06
MS_2	127.82

➢ *Molecular Identification*

The strain showing maximum protease activity was identified using molecular methods. After purification and PCR of the genomic DNA and sequencing of 16s gene, the isolate was found to be *Bacillus thuringiensis*. The sequence was searched in BLAST programme in NCBI database to identify the sample (Figure 5). Based on the BLAST analysis done for the sequencing data, the sample MS_2 showed a 100% identity with *Bacillus thuringiensis* (NCBI Accession No: CP050183.1) with a query coverage of 100%.

- *Sequence Data of Sample (MS_2): (862bps)*

>TGCGTTAACTTCAGCACTAAAGGGCGGAAAC
CCTCTAACACTTAGCACTCATCGTTTACGGCGTGGA
CTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCT
TTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTC
GCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCA
TTTCACCGCTACACATGGAATTCCACTTTCCTCTTC
TGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCAC
GGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAA
ACCACCTGCGCGCGCTTTACGCCCAATAATTCCGG
ATAACGCTTGCCACCTACGTATTACCGCGGCTGCTG
GCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCG
TCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTT
CCCTAACAACAGAGTTTTACGACCCGAAAGCCTTC
ATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTC
CATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGG
AGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATC
ACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTG
AGCCGTTACCCACCAACTAGCTAATGCGACGCGGG
TCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAA
TTTCGAACCATGCAGTTCAAAATGTTATCCGGTATT
AGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGG
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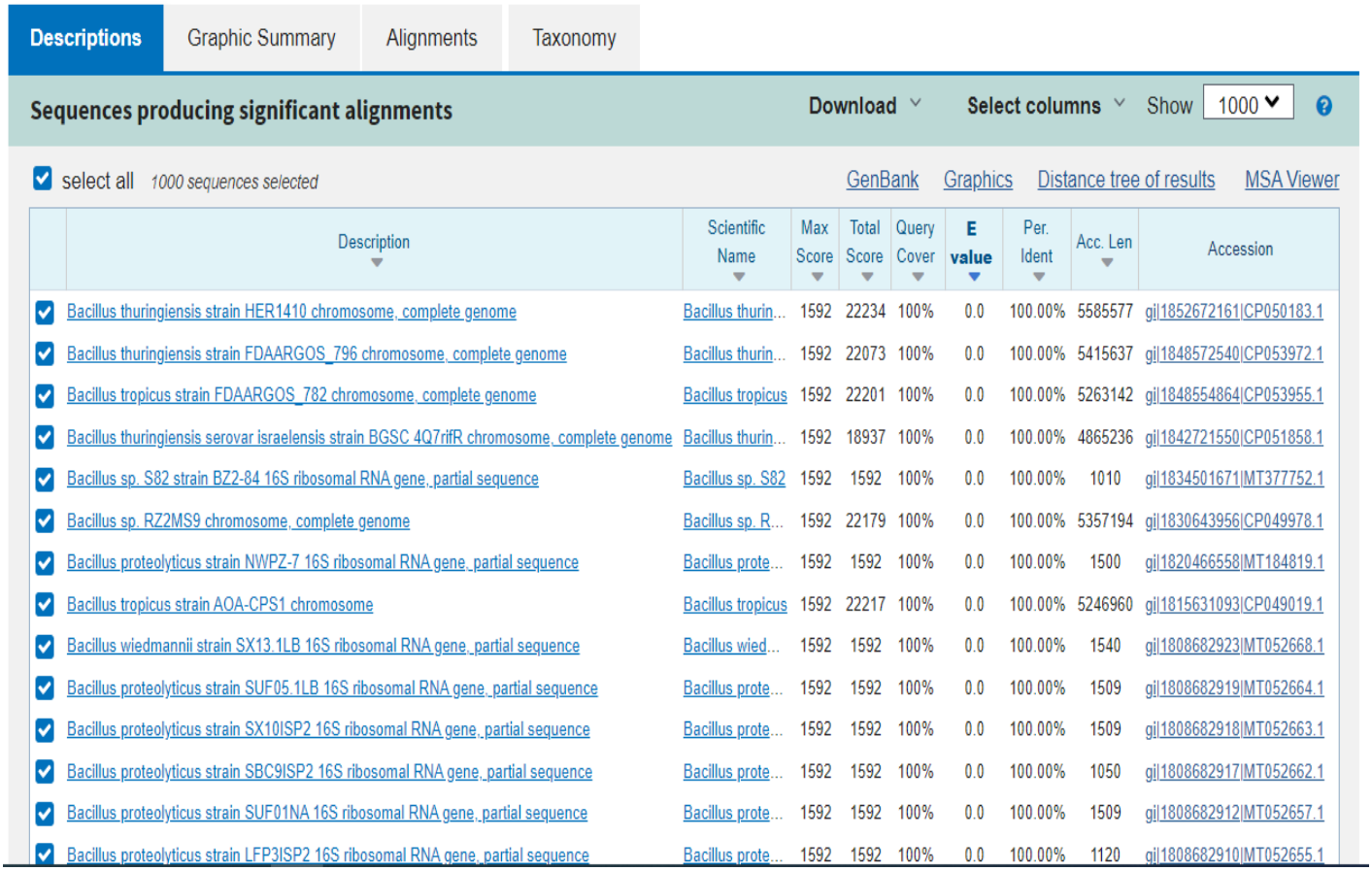
CTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCT
GAGCCAGG



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 1000

select all 1000 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus thuringiensis strain HER1410 chromosome, complete genome	Bacillus thurin...	1592	22234	100%	0.0	100.00%	5585577	gi|1852672161|CP050183.1
Bacillus thuringiensis strain FDAARGOS_796 chromosome, complete genome	Bacillus thurin...	1592	22073	100%	0.0	100.00%	5415637	gi|1848572540|CP053972.1
Bacillus tropicus strain FDAARGOS_782 chromosome, complete genome	Bacillus tropicus	1592	22201	100%	0.0	100.00%	5263142	gi|1848554864|CP053955.1
Bacillus thuringiensis serovar israelensis strain BGSC 4Q7rifR chromosome, complete genome	Bacillus thurin...	1592	18937	100%	0.0	100.00%	4865236	gi|1842721550|CP051858.1
Bacillus sp. S82 strain BZ2-84 16S ribosomal RNA gene, partial sequence	Bacillus sp. S82	1592	1592	100%	0.0	100.00%	1010	gi|1834501671|MT377752.1
Bacillus sp. RZ2MS9 chromosome, complete genome	Bacillus sp. R...	1592	22179	100%	0.0	100.00%	5357194	gi|1830643956|CP049978.1
Bacillus proteolyticus strain NWPZ-7 16S ribosomal RNA gene, partial sequence	Bacillus prote...	1592	1592	100%	0.0	100.00%	1500	gi|1820466558|MT184819.1
Bacillus tropicus strain AOA-CPS1 chromosome	Bacillus tropicus	1592	22217	100%	0.0	100.00%	5246960	gi|1815631093|CP049019.1
Bacillus wiedmannii strain SX13.1LB 16S ribosomal RNA gene, partial sequence	Bacillus wied...	1592	1592	100%	0.0	100.00%	1540	gi|1808682923|MT052668.1
Bacillus proteolyticus strain SUF05.1LB 16S ribosomal RNA gene, partial sequence	Bacillus prote...	1592	1592	100%	0.0	100.00%	1509	gi|1808682919|MT052664.1
Bacillus proteolyticus strain SX10ISP2 16S ribosomal RNA gene, partial sequence	Bacillus prote...	1592	1592	100%	0.0	100.00%	1509	gi|1808682918|MT052663.1
Bacillus proteolyticus strain SBC9ISP2 16S ribosomal RNA gene, partial sequence	Bacillus prote...	1592	1592	100%	0.0	100.00%	1050	gi|1808682917|MT052662.1
Bacillus proteolyticus strain SUF01NA 16S ribosomal RNA gene, partial sequence	Bacillus prote...	1592	1592	100%	0.0	100.00%	1509	gi|1808682912|MT052657.1
Bacillus proteolyticus strain LFP3ISP2 16S ribosomal RNA gene, partial sequence	Bacillus prote...	1592	1592	100%	0.0	100.00%	1120	gi|1808682910|MT052655.1

Fig 5 BLAST Result for the Sample (MS_2)

IV. CONCLUSIONS

The present study was conducted to isolate and identify the potential microorganisms that produce extracellular protease enzyme. Out of the 30 soil samples screened, 8 isolates producing extracellular protease were isolated from which two of the thermophilic strains which was able to survive at high pH were selected. Based on physico-chemical properties (pH, moisture, nitrogen, phosphorus and potassium content), proteolytic activity, antimicrobial activity, tolerance towards different pH (2.2 – 9) and temperature (37^0C – 60^0C) two strains were selected. They were identified using morphological, biochemical and molecular characteristics. The strain MS_2 showed maximum enzyme production.

Due to growing population, disposal of waste has become a great concern. Thus there arises a need to invent economically feasible ways for the disposal of waste. Naturally occurring soil microorganisms can be used to rectify this issue. Soil carries an abundance of microorganisms which can be screened for extracellular enzyme producing bacteria. The isolated strains can further be used to degrade waste on a large scale. They can be either used as monoculture or in consortium to make the process more efficient. This provides an easy and economical way to handle the biodegradable waste such as kitchen waste and litter.

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