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Isolation, Characterization and Optimization *of Saccharomyces Cerevisiae* Jssatpvr1 for Protease Production

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KEYWORDS

Soil, Protease, Isolation, bacterial strains.

ABSTRACT:

Soil is a rich reservoir of diverse microorganisms with immense biotechnological potential, including those capable of producing proteases, enzymes crucial for various industrial processes. This study aimed to isolate soil microorganisms and identify protease-producing strains for potential biotechnological applications. Soil samples were collected from diverse geographical locations, and serial dilution techniques were employed to isolate microbial colonies. The isolates were screened for protease production using skim milk agar plates supplemented with casein as a substrate. Positive isolates were further characterized biochemically and molecularly.

Results revealed a diverse microbial community in the soil samples. Among these, several strains exhibited significant protease activity, indicating their potential for industrial applications. Biochemical characterization revealed the proteases to be of various types, including serine, cysteine, and metalloproteases, suggesting diverse enzymatic capabilities within the isolated strains. Molecular identification using 16S rRNA sequencing and ITS region analysis identified the predominant protease producers as saccharomyces cerevisiae.

Furthermore, the protease-producing strains demonstrated robust enzymatic activity under a wide range of environmental conditions, including pH and temperature variations, highlighting their potential for industrial processes requiring enzyme stability under diverse conditions. Moreover, the production of proteases by indigenous soil microorganisms suggests their adaptation to local environmental conditions, making them promising candidates for biotechnological applications in waste management, bioremediation, and pharmaceutical industries.

In conclusion, this study underscores the importance of soil as a reservoir of diverse microorganisms with biotechnological significance. The isolated protease-producing strains exhibit promising enzymatic capabilities and environmental adaptability, laying the foundation for further exploration and utilization of soil microbial diversity for sustainable industrial processes

1. Introduction

Soil, one of the most complex and dynamic ecosystems on Earth, harbors an astonishing diversity of microorganisms. These microorganisms play pivotal roles in nutrient cycling, soil fertility, and ecosystem functioning [1]. Among the myriad of functions they perform, the production of enzymes, particularly

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proteases, holds significant biotechnological importance. Proteases are enzymes that catalyze the hydrolysis of peptide bonds in proteins, leading to their degradation into simpler compounds [2]. This capability renders proteases indispensable in various industrial processes, including detergent formulation, food processing, leather industry, and waste management.

The isolation and characterization of soil microorganisms, particularly those capable of producing proteases, have garnered considerable attention in recent years due to their potential applications in biotechnology [3]. Soil represents a vast and largely untapped resource for discovering novel microorganisms and enzymes with unique biochemical properties. By exploring the microbial diversity within soil ecosystems, researchers can uncover valuable enzymes with enhanced catalytic activities, stability, and specificity, addressing the demands of diverse industrial sectors [4].

2. Methods

Soil collection

Soil samples were gathered from various regions of South India, including beaches, agricultural lands, and lakes. Using a shovel, samples were collected from 10 to 15 locations in each area, reaching at least one foot below the surface. To ensure homogeneity after collection, the samples were meticulously mixed. Foreign materials were then removed which may include materials like roots, stones, pebbles, and gravel. By the process of quartering the bulk soil was reduced to 1-1.5 kilograms. Each of the mixed soil sample was divided into equal parts of four for quartering. After discarding two opposite quarters, the remaining two were combined and mixed. This process of quartering was repeated until sample of desired size was got. Then this soil samples were transferred to trays or sampling bags and labeled accordingly. To preserve the microbial community composition and minimize microbial activity, the samples of soil were stored at 4°C in containers. They were kept at this temperature for further analysis.

Soil Dilutions

For soil dilutions, 1 gram of soil was mixed with 100 ml of sterile water. Five drops of tween 80 were added to a sterile conical flask, which was then placed on a rotary shaker for 30 minutes. After the allotted time, the soil settled at the bottom of the flask. Serial dilutions were prepared to estimate the concentration of microorganisms in the sample. In this study, the tenfold serial dilution method was employed for this purpose**.**

Formula

For each sample, ten test tubes containing 9ml of distilled water were prepared. Using a sterile pipette, 1ml of supernatant from a sterile conical flask was added to the first test tube, was thoroughly mixed and labeled as the initial concentration 10-1. Subsequently, 1ml of the mixture from the 10-1 dilution was transferred to the second test tube, with the second tube then having a total dilution factor of 10-2.

The procedure was replicated for the remaining tubes, where 1ml from the preceding tube was transferred to the subsequent 9ml of diluent. With ten tubes utilized, the ultimate dilution reached 10-10. This methodology was applied to all three soil samples, facilitating the creation of serial dilutions [6].

Platting

The spread plate method was chosen over the pour plate method due to its various advantages, such as enhanced handling flexibility, reduced interference for temperature-sensitive organisms, prevention of aerobic organism entrapment in agar medium, surface enumeration of colony forming units, and straightforward identification of distinct colony types. Typically used to isolate microorganisms from small sample volumes dispersed across agar plates, this technique produces discrete colonies evenly distributed over the agar surface. Widely applied in enrichment, selection, screening experiments, and viable plate counts, spread-plating enables the quantification of cell concentrations based on colony forming units per plate.

Serial dilutions ranging from 10-6 to 10-8 were prepared for each sample. Actinomycetes isolation agar was employed for plating. Sterile petri plates were labelled on the backside with the date, growth medium type, sample identity, and serial dilution number.

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Plates were thoroughly dried and allowed to cool before the plating process. Samples, ranging from 0.1 to 0.2 ml, were evenly spread onto the agar surface at the center of each plate. After ethanol sterilization of the spreader, samples were spread evenly across the entire petri dish while rotating the turntable. Following a minimum five-minute absorption period, plates were incubated at 27 degrees Celsius for seven days. This procedure was repeated for all three samples. [7]

Simple continuous streak plate method- Obtaining pure culture bacterial colonies:

The straightforward continuous streaking method evenly distributes the inoculum across the agar plate, moving continuously from the edge to the centre. This method is quick and simple, especially suitable when the inoculum is greatly diluted or when the goal is to grow a pure culture rather than isolate a specific organism. Multiple specimens can be streaked in each of the several sections of a 10 cm petri plate.

In this study, the simple continuous streak method was employed to isolate individual colonies from a mixed population of cells. Following seven days of incubation, plates were examined for pinpoint colonies. Selected colonies of interest were then streaked onto sterile petri plates using the same method.

Actinomycetes isolation agar was used, with plates prepared and cooled before streaking. Using a sterile metal loop, colonies of interest were streaked onto fresh plates and incubated. This process was repeated iteratively until pure cultures were obtained. Upon achieving pure cultures, triplicates were prepared and stored at 4 degrees Celsius for future use.[8]

Testing for protease activity

Procedure:

An enzyme called protease was responsible for catalyzing the breakdown of peptide bonds in proteins. These enzymes played essential roles in various biological functions, including protein turnover, cellular signaling, and digestion. Proteases were crucial for breaking down proteins in food during digestion into smaller peptides and amino acids that the body could absorb. Additionally, proteases cleaved proteins important in gene expression, communication pathways, and cell cycle development, thereby regulating cellular processes. Various types of proteases, including serine, cysteine, aspartic, metalloprotease, and threonine proteases, each had unique mechanisms and sets of substrates. Soil microorganisms produced proteases, enzymes used to break down proteins in their environment. Soil, a complex ecosystem supporting a variety of microorganisms such as bacteria, fungi, and archaea, served as their habitat. Many of these bacteria generated proteases as part of their metabolic activities to obtain nutrients.

To assess proteolytic activity, Casein hydrolysis and Milk coagulation tests were employed.

Casein Hydrolysis test

For the Casein Hydrolysis test, a small well was made in sterile milk agar plates, and into the wells the culture broth of each isolate was placed and incubated at 27 degrees Celsius for 3 days. The hydrolysed zones were then measured.

Table 1 Chemicals needed for casein hydrolysis test. Agar and milk powder is sterilized separately and mixed while pouring

Components	Quantity
Agar	2g
Skimmed milk	2g
Water	100ml

Milk coagulation test

Additionally, to study proteolytic activity, inoculated sterile tubes containing pasteurized skimmed milk were with soil isolates, incubated at 27 degrees Celsius for 2 days. The tubes were observed for changes in medium colour. [9],[10].

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Strain improvement using UV

Procedure:

UV radiation was a common method used in microbiology to induce mutations in bacteria, resulting in improved features such as increased production, altered metabolic pathways, or enhanced resistance to external stressors. UV light induced mutations in microbial DNA by forming thymine dimers, covalent links between neighbouring thymine bases in the DNA strand. These mutations led to changes in genetic coding, thereby altering microbial characteristics. UV mutagenesis was often utilized to enhance strains through an iterative process, where selected mutants underwent additional rounds of mutagenesis and selection to accumulate beneficial mutations and improve desired features.

Mutation:

To develop hyperprotease-producing stable UV mutants, the microbial culture was plated on milk agar. Plates were exposed to UV irradiation at a distance of 12cm for 5, 60, 120, and 180 seconds in the dark before being incubated for 24 hours at 27 degrees Celsius.

Isolation of stable mutants:

Survivors were selected based on clearing zone diameter to colony diameter ratio and subsequently screened for protease activity after cultivation on milk agar. Liquid medium cultivation was performed to assess enzyme activity across various mutant strains. The strain was refrigerated and subjected to retesting for enzyme activity.[11]

Growth optimization.

Procedure:

Optimization of scale up parameters Optimization of temperature:

The optimal temperature for strain growth and productivity was evaluated by incubating inoculated fermentation media at various temperatures between 27ºC and 37ºC for a duration of two days on an orbital shaker. After incubation, microbial growth was assessed through optical density measurements and visual observation.

Optimization of pH:

By adjusting, the ideal pH of the fermentation medium that is conducive to maximal growth and productivity of the microorganism was within the range of 5 to 7. The cultures were then incubated for two days on an orbital shaker at 29ºC after that the cultures were evaluated for both growth and productivity.

Optimization of RPM:

The optimal temperature for achieving maximum growth and productivity of Pichia pastoris was determined by incubating the inoculated fermentation media at various RPM settings ranging from 0 to 50 for two days in an orbital shaker. After the incubation period, microbial growth was assessed through optical density measurements and physical observation.[12],[13]

Downstream processing

Centrifugation of broth was was done at 10,000 rpm at a temperature of 15 degrees Celsius for 30 minutes. Subsequently, resulting supernatant was taken separately in a sterile conical flask and preserved at -20 degrees Celsius for subsequent experiments.

Ammonium sulphate precipitation

Fresh ammonium sulphate was used to ensure uniform and rapid dissolution. The ammonium sulphate was heated in an oven at 120 degrees Celsius using a drying dish or beaker the day before use. It was then ground into a fine powder and promptly utilized. For achieving 70% saturation, 43.6 grams per 100 milliliters were employed. The concentrated solution of ammonium sulphate was added slowly to the protein solution while stirring constantly to avoid excessive localized increases in salt content, which could lead to protein denaturation or aggregation. The powder was added gradually and mixed thoroughly to prevent the formation of clumps. Allowed precipitate to form for 30 minutes at 4°C with continuous stirring. Centrifuged entire system was at 3000 RPMs for 40 minutes. Drained the supernatant, and rinsed and

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dissolved the pellets in a buffer of 50 mM Tris Cl, pH 8. If any undissolved particles were present, centrifugation was performed at 3000 rpm for approximately 15 minutes.[14]

Bradford assay

The Bradford assay depend on the the color shift that can be detected spectrophotometrically and is a result of Coomassie Brilliant Blue dye binding to proteins. The Coomassie dye (G250 grade) when it bound with arginine and aromatic amino acid residues. Maximum absorption spectrum at 595 nm was exhibited by the anionic form, while the cationic form absorbed at 470 nm. The amount of dye that was bound was correlated with the rise in absorbance at 595 nm, with the content of protein in the sample. It was preferred over the Lowry method due to its heightened sensitivity, broader linear range, and compatibility with a wide range of sample types.

The concentrate of Bradford dye was diluted to a ratio of 1:4 with distilled water (1ml dye + 4 ml water), and then the solution was filtered. The diluted reagent could be stored for about 2 weeks.

A standard curve was prepared by filling 6 cuvettes, each with a capacity of 1ml, with varying amounts of 0, 2, 5, 8, 10, and 15 µg of BSA.1ml of Bradford dye was added. The cuvettes were carefully vortexed and incubated for approximately 5 minutes at room temperature. Samples were consistently incubated before measuring absorbance with a spectrometer at 595nm. The OD of unknown samples and the OD of the extraction buffer (blank) were measured to adjust for its contribution to total protein. The results from BSA samples were plotted to create a standard curve which was used for determining the concentration of protein in sample[15].

SDS Polyacrylamide gel electrophoresis

Assembled gel plates with 0.1mm spacers and sealed sides with 1-inch cellotape to prevent leaks. Clamped the assembled set onto the gel casting stand securely. Marked the desired level for pouring the separating gel mixture below where the wells would form. Prepared the acrylamide separating gel mixture in a conical flasks. Poured the acrylamide mixture into the glass mould and overlaid with distilled water. After polymerization (20-30 minutes), washed the top of the gel by removing overlay. Prepared the acrylamide stacking gel mixture and poured it over the separating gel. Immediately inserted the comb and allowed the gel to polymerize (approximately 10 minutes). In the electrophoresis chamber placed the gel mould, electrophoresis buffer filled, and removed the comb. Prepared the protein sample by mixing with sample buffer and heating to denature the proteins. Loaded the samples onto the gel and applied appropriate voltage for stacking gel (70 volts) and separating gel (150-200 volts). Until the bromophenol blue dye reached the bottom the gel was ran. Turned off the power supply, removed the gel mould, and separated the plates. Marked the orientation of the gel and immersed it in staining solution for 4 hours at room temperature. Removed the stain and destained the gel as needed. Stored the gel in 7% acetic acid and visualized the bands under an illuminator[16]

rRNA Gene Sequencing

Genomic DNA Isolation:

The cells that are grown in monolayer were lysed initially on suspending 1-3 colonies in a 2 ml microcentrifuge tube containing of lysis buffer 500 µl, and then lysed by repeated pipetting. 4 µl of RNAse, 500 µl of neutralization buffer was added to the lysed cells. The contents were vortexed and then for 30 minutes incubated in a water bath at 65˚C, ensuring minimal DNA molecules shearing. The tubes were then centrifuged for 10 minutes at 10,000 rpm. The resulting supernatant was transferred without disturbing the pellet into a 2 ml microcentrifuge tube. Chloroform Isoamyl Alcohol 600 µl was added and centrifuged at 10,000 rpm for 10 minutes, and then carefully transferred into a new microcentrifuge tube 600 µl of the aqueous phase. For the binding step, added buffer solution 600 µl and mixed, then incubated at ambient temperature for a period of 5 minutes. Transferred 600 µl contents in a 2 ml collection tube and placed to a spin column placed. Then centrifuged at 10,000 rpm for 2 minutes. The remaining 600µl of the lysate was transferred. They were centrifuged again for 2 minutes at 10,000 rpm. For washing, the spin column added with 500 µL washing buffer I, centrifuged at 10,000 rpm for 2 minutes. Added 500µl washing buffer II, centrifuged at 10,000 rpm for 2

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minutes and tube was then dry spun for 5 minutes at 10,000 rpm. Transferred the spin column was to a sterile 1.5-ml microcentrifuge tube. For elution, added 100 µl of the elution, avoiding contact with the filtrate. The incubated the tubes for 2 minutes at room temperature and then centrifuged at 10,000 rpm for 2 minutes. The DNA was now present in the buffer in the microcentrifuge tube which was measured using a Qubit flurometer 3.0 or 1% Agarose Gel Electrophoresis [17].

PCR Protocol:

PCR amplified specific cloned or genomic DNA sequences using primers and a unique enzyme, DNA polymerase. DNA polymerase directed DNA synthesis

If a synthetic oligonucleotide primer was recombined to a single-stranded template containing a complementary region, DNA polymerase could extend its 3` end, generating double-stranded DNA. PCR involved multiple cycles of denaturation, annealing of primers to target DNA, and extension by DNA polymerase, resulting in exponential amplification of the target sequence.

Table 2: PRIMER DETAILS (for fungus)

Name	Sequence	No. of base
ITS1	5' TCCGTAGGTG AACCTGCGG 3'	19
ITS4	5' TCCTCCGCTTA TTGATATGC 3'	20

PCR Reaction Setup:

Isolated DNA was combined with PCR reaction solution of volume 25 µL of, containing 1.5 µL of Forward and 1.5 µL of Reverse Primers, Taq Master Mix of volume 12 µL and 5 µL of deionized water. The components were mixed thoroughly to ensure even distribution [18].

Bioinformatics protocol: The sequence underwent a similarity search with NCBI BLAST tool.

After results, a phylogenetic analysis was conducted comparing the query sequence and sequences that are closely related, after the alignment of multiple sequence using MUSCLE 3.7. The sequences aligned were refined by using of Gblocks 0.91b thereby alignment noise was also reduced. For conucting the phylogeny analysis, PhyML 3.0 aLR was used.[19].

3. Results

SOIL COLLECTION

Soil Sample	Sampled from
Sample -1	Agriculture land, Coimbatore, Tamilnadu.
Sample -2	Alappuzha lake, Alappuzha, Kerala
Sample-3	Varkala beach, Kerala
Sample-4	Kaveri river, Karnataka
Sample -5	Bhavanisagar, Tamilnadu,India

A total of five different types of soil from various habitats such as beaches, lakes and agricultural lands from various parts of southern parts of India were collected in sample bags, quartering was done and stored in 4°C for further analysis.

SOIL DILUTIONS

10-fold soil dilutions were done for each sample and the dilutions from 10^{-1} to 10^{-10} were made.

PLATTING

The dilutions from10^{-6} to 10^{-8} were taken from each sample and plated in nutrient agar and is incubated at 27 degree Celsius for 7 days. After 7 days the pinpoint colonies were observed from the crowded plate technique.10^{-6} to 10^{-8} were selected to cover a wide range

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of possible microbial concentrations in the samples. These dilutions allow for the creation of a dilution series, which is crucial for obtaining colony counts within a range suitable for accurate measurement.

Figure 1: Soil sample collected from Varkala beach Kerala

Figure 2: Soil sample collected from agriculture land Coimbatore

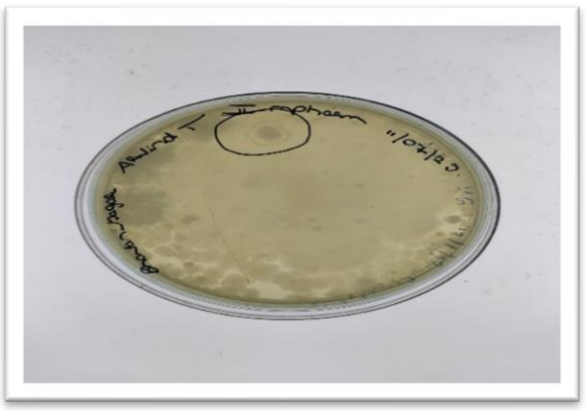

Figure 3: Soil sample collected from Bhavani Sagar

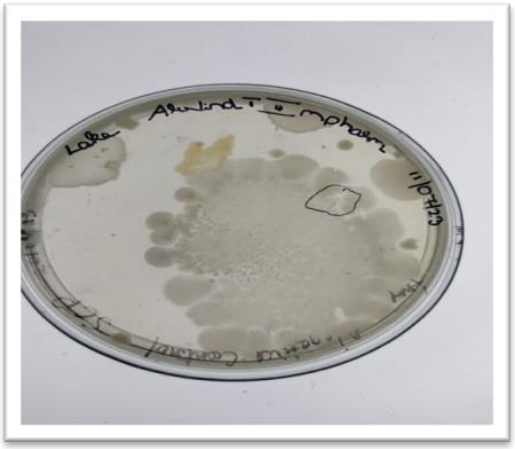

Figure 4: Soil sample isolated from Kaveri, Karnataka

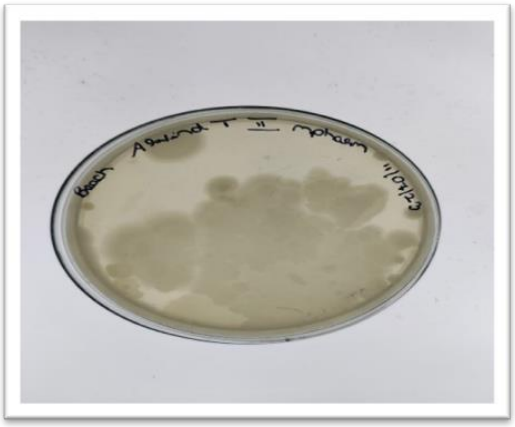

Figure 5: Soil sample collected from Alappuzha, Kerala

SIMPLE CONTINUOUS STREAK PLATE METHOD – OBTAINIG MICROBIAL PURE CULTURE

A total of two pin point colonies were streaked using simple continuous streaking method and made into pure cultures from the soil sample collected from agriculture land and kaveri lake respectively. These pinpoint colonies were grown in nutrient broth and was streaked in nutrient agar for obtaining pure culture. The pure cultures were triplicated and is stored and used for further analysis. The two pure cultures were named JSSCPOPVRAT1 and JSSCPOATPVR1.

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Table 4: Microbial strain isolated from solid samples.

STRAIN NAME	STRAIN COLOR	ISOLATED FROM
JSSCPOATPVR1	Yellow	Agriculture land, Coimbatore, Tamil Nadu
JSSCPOPVRAT1	Green	Kaveri river, Karnataka.

Figure 6: Pure culture – JSSCPOATPVR – 1

Figure 7: Pure culture – JSSCPOATPVR -1

TESTING FOR PROTEASE ACTIVITY

The proteolytic activity of isolated soil strains was analysed by conducting casein hydrolysis and milk coagulation test. It indicates that JSSCPOATPVR 1 shows proteolytic activity through this analysis. Whereas JSSCPOPVRAT does not show any proteolytic activity.

SOIL ISOLATE	CASINOLYTIC ACTIVITY	MILK COAGULATION
JSSCPOATPVR1	Shows zone of inhibition	Change in medium colour
JSSCPOPVRAT1	No zone of inhibition	No change in medium colour.

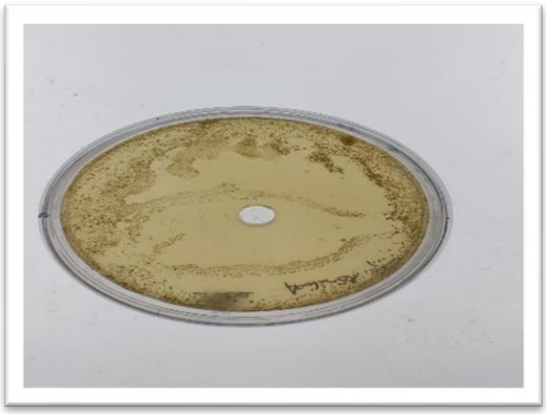

Figure 8: Protease activity observed in JSSCPOATPVR -1 (skimmed milk agar)

STRAIN IMPROVEMENT USING UV

The JSSATPVR 1 strain was genetically modified using UV irradiation. The studies were carried out by exposing isolated strain JSSATPVR 1 to ultraviolet radiation at various intervals. The strain was then incubated at 27 degrees Celsius for 24 hours. It was discovered that no zone of inhibition formed, hence the isolated wild strain was employed in subsequent tests rather than the modified.

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Figure 9 UV strain improvement done on JSSCPOATPVR 1 strai

SCALE UP AND GROWTH OPTIMIZATION

Optimization of temperature

To identify the optimal temperature for the strain's development and production, the strain JSSATPVR 1 was kept at 27ºC to 37ºC for two days in an orbital shaker. After incubation, microbial growth was measured using optical density and physical inspection.

Table 6: Represents OD values of optimization of temperature

DAY	AT 27^0C	AT 28^0C
Day 1	**0.186**	**1.491**
Day 2	**2.235**	**1.667**

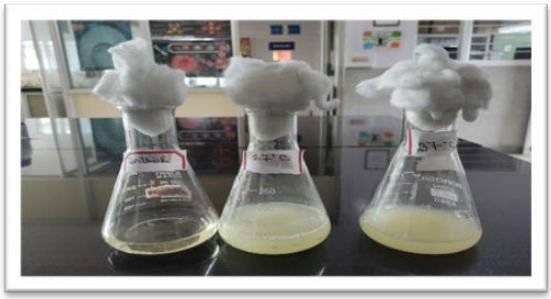

Figure 10: Optimization of temperature of JSSATPVR 1

To establish he optimal pH for microorganism growth and productivity, the pH of the medium JSSATPVR 1 was adjusted to a pH range of 5-7 and incubated at 29ºC for 2 days on an orbital shaker. Following the incubation period, cultures were assessed for growth and production.

Table 7: Represents OD values of optimization of pH of JSSATPVR 1

Day	At 5	At 6	At 7
Day 1	0.339	0.869	0.143
Day 2	0.574	1.234	0.152

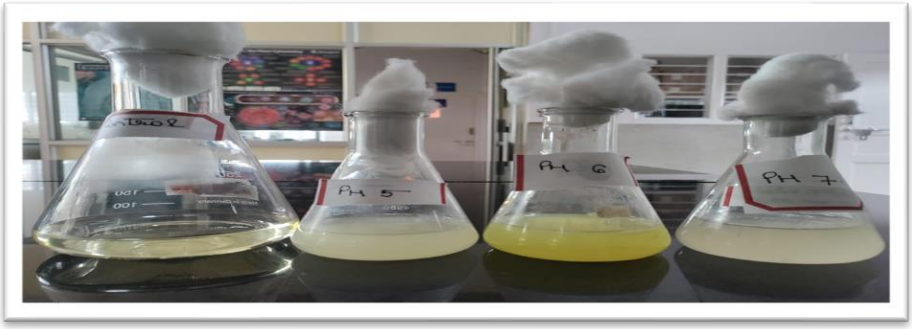

Figure 11: Optimization of pH of JSSATPVR

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Optimization of RPM:

Optimal temperature for growth maximum growth and productivity of JSSATPVR 1 strain was determined by keeping the inoculated fermentation media at different rpm ranging from 0 and 50 for two days in orbital shaker. After incubation time, microbial growth was determined by measuring optical density and physical observation. After optimization, favorable conditions were provided to JSSCPOATPVR 1 strain to yield better results.

Table 8: Represents OD values of optimization of RPM of JSSCPOATPVR 1

Day	**RPM 0**	**RPM 50**
Day 1	0.347	0.155
Day 2	0.163	0.062

Figure 12: Optimization of RPM of JSSCPOATPVR

AMMONIUM SULFATE PRECIPITATION

The JSSATPVR 1 strain was optimized and purification was done by ammonium sulphate precipitation at 70% saturation.

Calculation

Formula G = 533(S2-S1)/100-0.3(S2), where S1 is the initial concentration and S2 is the final concentration. Amount of Ammonium sulphate for 70%saturation:

G= 533(70)/100-21

= 472.27g for 1000 ml

=23.61g for 50 ml

After Ammonium sulphate precipitation, it was found that 50 ml of the JSSCPOATPVR 1 strain's broth contains 23.61grams of crude protease enzyme.

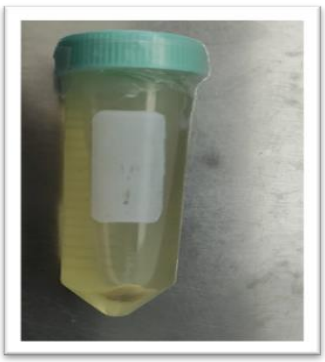

Figure 13: Ammonium sulphate precipitation of JSSCPOATPVR 1 strain

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BRADFORD ASSSAY

The protein concentration of unknown sample is determined by plotting a standard curve using BSA.

Table 9: BSA standard curve values

Concentration (µg/ml)	Absorbance
2	0.374
5	0.476
8	0.557
10	0.551

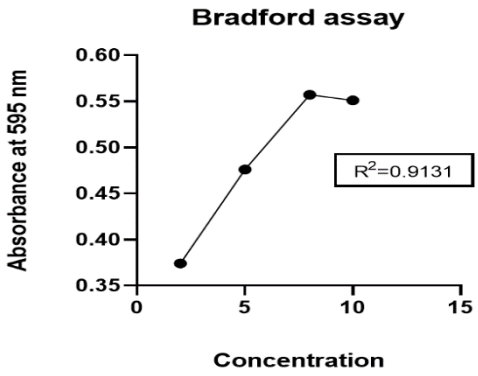

Table 10: Crude Protease protein JSSCPOATPVR 1 sample values

Sample amount	Absorbance	Concentration
1mg/ml	0.477	5.714 µg/ml

The 1mg/ ml of sample was taken and experiment was done. The enzyme concentration was determined by comparing with standard graph (BSA) by Bradford's assay. The result shows 1 mg of sample contains 5.714 µg/ml of enzyme.

SDS – PAGE

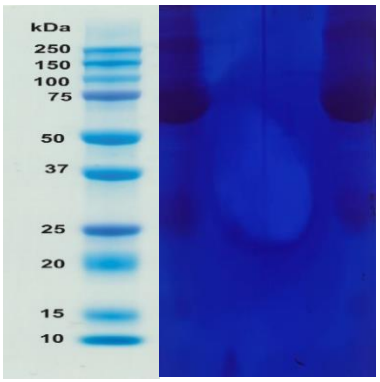

Figure 14 : SDS PAGE results of JSSCPOATPVR 1 strain

The crude protein extract from [JSSATPVR 1 strain.] was subjected to SDS-PAGE analysis to evaluate the molecular weight profile of the protease enzymes. After staining the gel with Coomassie Brilliant Blue, numerous protein bands werdemonstrating the presence of diverse proteins in the crude extract. Notably, multiple strong bands were found in the molecular weight range expected for protease enzymes (35-45 KDA).

Bioinformatics results

JSSCPOATPVR 1 strain has been published in NCBI.
Link: https://www.ncbi.nlm.nih.gov/nuccore/OR552410

JSSCPOPVRAT 1 strain has been published in NCBI
Link:
https://www.ncbi.nlm.nih.gov/nuccore/OR544081

Discussion &Conclusion

Soil samples were collected from in and around South India. The soil isolates were screened for pharmaceutically useful microorganisms. Two microbial strains were isolated and named JSSCPOATPVR 1 and JSSCPOPVRAT 1. The microbial strain JSSATPVR 1 was found to have protease activity. UV strain improvement was done hoping to increase the yield of the JSSATPVR1 strain. The optimal conditions such as temperature, pH and RPM were adjusted and favorable conditions for the growth of the JSSCPOATPVR 1 strain was incorporated. Downstream processing was done and

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the protein was purified using ammonium sulfate precipitation. Bradford's assay was performed to find the protein concentration of JSSCPOATPVR 1 strain. The molecular weight of the protein was found using SDS PAGE. Gene sequencing was done for both JSSCPOATPVR 1 strain and JSSCPOPVRAT strain and the type of microbial strain was interpreted. BLAST sequence and phylogenetic analysis was done to find the percentage similarity of the microorganisms.

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Conflict of Interest

Authors declare no conflicts

Reference

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