Industrial Microbiology

New Technical programme

1.	**Title**	**Optimization of physiochemical parameters for protease production by bacterial isolate under solid state fermentation (SSF) (NTP. Sr. No: 17.5.3.20)**
2.	**Background Information**	A broad array of proteases is produced from microorganisms either intracellular or extracellular. Extracellular proteases are significant for the hydrolysis of proteins in cell-free environments. Today, proteases characterize one of the prime industrial enzymes, and hold approximately 60% share of worldwide sales of enzymes. Protease possesses considerable industrial potential due to their biochemical diversity and is a highly exploitable enzyme in food, leather, detergents, pharmaceutical, diagnostics and waste management etc. Although, industrially applicable protease enzymes have been identified from different sources, most of them could not resist drastic environmental changes and most of the sources are incapable to produce required quantities to fulfil industrial demands. So, new bacterial strains that can withstand harsh environmental conditions should be isolated for the enhanced production of such enzymes. Solid State Fermentation has been adopted to fulfil the demands as it is more simple, requires lower capital, has superior productivity, simpler fermentation media and absence of rigorous control of fermentation parameters, uses less water and produces lower wastewater, has easier control of bacterial contamination and requires low cost for downstream processing as far as economic is concern. In the present study a statistical approach will be employed in which a Plackett-Burman design will be used for identifying significant variables influencing protease production by the screened isolate. The levels of the significant variables are further optimized using response surface methodology-CCD.
3.	**Objective**	i. Screening of potential protease producers. ii. Identification of the screened isolate(s) using molecular methodology. iii. Optimization of physical (pH, Temperature, moisture content etc.) and chemical (C source, N source and minerals) process parameters for the Protease production. iv. Identification of significant components using Plackett-Burman design for the optimum protease production. v. Level optimization of screened variables (components) on the protease production using response surface central composite design. vi. Validation of the level of selected components for the maximum protease production.
4.	**Principal Investigator & Associates**	1. Dr. Vimakumar Prajapati, Assistant Professor, (Industrial Microbiology) : PI 2. Dr. R. M. Patel (Principal & Dean) : Co-PI
5.	**Location and Agro-climatic zone:**	Aspee Shakilam Biotechnology Institute, NAU, Surat
6.	**Name of Res Scheme**	12248

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7.	**Year**	2021
8.	**Crop & variety**	-
9.	**Experiment detail**	Screening will be carried out using samples collected from municipal green waste management site (SMC, Surat) and dairy effluent (Sumul, Surat) on casein/skimmed milk agar plate. Protease production will be assessed using the standard assay procedure at every 24 h of interval up to 7 days. Wheat bran will be taken as solid substrate for the production of protease and different processes parameter will be optimized using One Factor At a Time Methodology (OFAT) followed by Plackett-Burman design and Response Surface Methodology-CCD. One gram of the collected sample will be suspended in 9 mL of sterile distilled water. After a serial dilution of this suspension with sterile distilled water, 100 µL from each diluted suspension will be spreaded on skim milk agar (SMA; 2% skim milk, 1%glucose, 3% agar and pH 9.0) plates followed by incubation at 37C for 24-48 h. Genomic DNA extraction and the polymerase chain reaction (PCR) for amplification of the 16S rDNA of the selected potential bacterial isolates will be carried out. PCR products will be purified followed by sequencing. The 16S rDNA sequences will be analyzed using a free computer Program. The similarities of the sequence will be searched with the BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search program. The sequence is aligned with the similar sequences by using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and a phylogenetic tree will be constructed using Molecular Evolution Genetic Analysis (MEGA). Protease activity will be determined by using azo/casein as a substrate with some modifications of the method described by Kreger and Loekwood (1981). In brief, the reaction mixture contains (500 ml of 1.0% azo/casein in 50 mM Tris HCI, pH 9.0 and 1 ml of enzyme solution) incubated for 30 min at 40C or optimum temperature. The reaction is stopped by adding 2.8 ml of 5.0% (w/v) trichloroacetic acid (TCA), and was kept at 4C for 15 min. The precipitate was removed by centrifugation at 8,000 rpm for 20 min. A 2 ml of the supernatant was added to 2 ml of 1.0M NaOH and the absorbance was measured at 440 nm using a UV-visible spectrophotometer. Or else supernatant will be subject for protein estimation using Follin Lowery methology. Different Physicochemical parameter including incubation time, pH (4 to 11), Temperature (30 to 70), moisture content as well effect of additional carbon source at 1% (Glucose, fructose, maltose, lactose) and various organic and inorganic nitrogen sources (Casein, YE, peptone, NH_4NO_3, NH_4CL, $(NH_4)_2SO_4$) at 1% and metal ions if any will be optimized using one factor at a time methodology via supplementing it to SSF. Selected parameters via OFAT method will be subjected to Plackett-Burman design to screen the significant components affecting the protease production followed Response Surface Methodology-CCD to achieve the level optimization.
10.	**Observation to be recorded**	1. Initial screening will be done based on the plate assay to check the zone of clearances (Skimmed milk/Casein Agar plate). 2. Identification of the potential isolate will be done using 16s rDNA universal primer. 3. Best carbon and nitrogen source will be screened out using OFAT

		and Plackett-Burman Design. 4. Level optimization of the screened components will be checked using RSM-CCD
11.	**Expected outcome**	• Potent protease producing microbial strain will be screened, and subjected for molecular identification. • Physicochemical parameters for the protease production will be optimized under SSF using PBD and RSM-CCD followed by validation of the model. • Economically sustainable medium for the protease production will be formulated using the adopted strategy.