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A STUDY ON THE PROTEOLYTIC ACTIVITY OF MICROBIAL ISOLATES FROM DIVERSE SAMPLES COLLECTED FROM SEMELING, KEDAH, MALAYSIA

P. Vasanth Raj*, S.A. Dhanaraj, Tan Hooi Kuan, Shivanashini Mathiyalagan, Sangidaa Raman, Tan Jia Jin

Faculty of Pharmacy, AIMST University, Semeling, Bedong, Malaysia 08100.

ABSTRACT

An attempt was made to isolate industrially important microorganisms from diverse samples collected from Semeling, Kedah, Malaysia. Samples were aseptically collected from cafeteria soil, lake water, rotten wood soil, mangrove swamp, plastic bag and sewage water. All the nine isolates were investigated for biochemical test like indole test, methyl red test, Macconkey test, Celluose utilization test. Morphological testing such as gram staining, motility test, Mannitol semi solid agar method were investigated. The aim of this work was to study the ability of the microorganism to produce enzyme protease. Proteases are one of the most important industrial enzymes with much potential application in the food, leather and detergent industries. To explain the enzymes activity, its hydrolytic action was tested on casein. This proteolytic activity was carried out at 37°c and analysed using UV spectroscopy which showed the hydrolytic activity of protease on casein.

Keywords: Microorganisms; Protease; Casein; Hydrolytic Activity.

INTRODUCTION

Proteases (peptidases) are enzymes that hydrolyze peptide bonds in proteins. Exopeptidases cleave a terminal amino acid residue at the end of a polypeptide endopeptidases cleave internal peptide bonds [1]. Protease are very important group of enzymes and represent nearly 60% of total enzyme sales, since they are used in detergent, beer, meat, leather and dairy industries. The possibility of using these enzymes at elevated temperatures, especially in the food industry, implies increased reaction rates and improved solubility of reagents. Themostable proteases, that acts in the temperature range 65-85°c for the bioconversions of proteins into amino acids and peptides. These properties make the bacterial proteases most suitable for a wider industrial application in baking, brewing, detergent and leather industry [2]. The pancreatic protease extracts for use in detergents and leather processing, proteases in rennet from unweaned calves to coagulate milk for cheese production, and papain from papayas to tenderize meat [3]. Most protease are produced from wheat bran, casein, soy bean and rice bran. Proteases produced by solid-state fermentation (SSF) processes have greater economic feasibility [4]. Proteases are one of the most important industrial enzymes produced by a wide range of microorganisms such as bacteria, yeasts, molds and are also found in plants and in various animal tissues. Bacterial proteases are mostly extracellular, easily produced in larger amounts, thermostable, and active at a wider pH range [4]. However, because of increasing environment and worldwide concerns, the potential of protease use is increasing. Therefore, the development of protease from various sources, such as wheat bran has become the focus of much research interest [5]. Proteases are widespread in nature, microbes serve as a preferred source of these enzymes because of their rapid growth, the limited space required for their cultivation and the ease with which they can be genetically manipulated to generate new enzymes

Corresponding Author

P. Vasanth Raj
Email id: vasanth1780@gmail.com

with altered properties that are desirable for their various applications [6]. As a result, the demand for highly active preparations of proteolytic enzymes with appropriate specificity and stability over a wide range of pH, temperature and retention of activity in the presence of ions and organic solvents continues to stimulate the search for new enzyme sources. The use of enzyme based products is currently being explored in many areas of leather making process, with increasing importance in the dehairing process, thus eliminating the use of hazardous sodium sulfide. Due to the increasing demand of enzyme in the leather industry, there arises a need for new proteases. The alkaline protease from this organism effectively dehaired the goat hide within 18 h of incubation [7].

Proteases are classified into six groups: aspartate, cysteine, glutamate, metallo, serine, and threonine based on characteristic mechanistic features consistent within each member of a group. Through structural and functional diversity, proteases carry out a vast array of critical functions ranging from intracellular protein recycling to nutrient digestion to immune system cascade amplification. In general, current protease products rely on naturally evolved cleavage specificities, although other properties such as solubility and overall stability have been effectively engineered. Nevertheless, there is an incredible landscape of potential uses for engineered next generation proteases once the power and specificity of their individual hydrolysis reactions can be tailored for specific needs.

The concept of using engineered proteases for therapeutic applications has long been a goal in the pharmaceutical industry. The catalytic activity of proteases makes them a potentially unique therapeutic, allowing for smaller dosages, the potential to inactivate numerous target proteins and higher efficacy. In combination, these effects can lead to lower and less frequent dosages and more importantly, lower cost [8].

MATERIALS AND METHODS

Collection of Samples

The samples were aseptically collected from cafeteria soil, AIMST lake water, cafeteria water, rotten wood soil, mangrove swamp, plastic bag, and sewage water. The microorganism was inoculated in petri dish with nutrient agar medium and incubated at 37°c for 2 days for complete growth. Other materials include casein powder, acetate buffer, trichloroacetic acid (TCA), sodium acetate, acetic acid, potassium phosphate buffer, distilled water.

Aseptic technique

All the materials were autoclaved at 120°c for 20 minutes. The solutions were cooled to about 45° C. It was the poured into the sterile petri dish [9].

Microorganism inoculation

Microorganisms that were isolated earlier were use in this study. Total of nine isolates were inoculated on nine different nutrient agar plates respectively using aspectic techniques. Then the agar plates were incubated at 37°c for 48 hours. Streaking method was used to streak the microorganisms on agar plate. Once the microbes were grown on the agar plate then the microbes were taken and inoculated in nutrient broth medium. The sample from each culture were innoculated into 9 nutrient broth medium placed in conical flask. The nutrient broth was incubated at 37°c for 11 days with mild shaking by using shaking incubator. On the each 2nd, 3rd, 6th,7th , 8th,9th and 11th day the sample were taken and assayed for its hydrolytic action to detect the presence of protease.

Assay of protease

Proteolytic activity was determined according to standard protocols with minor modification [10]. The reaction mixture was made up of 0.4ml of casein (sigma), 0.55 (w/v) in distilled water and 0.4ml 0.2M acetate buffer, pH 5.5, to which 0.2ml of the crude enzyme solution was added. The reaction was carried out at 37 °c and the action of the enzyme was stopped after 30min with 1ml of 10% TCA (trichloroacetic acid). Test tube were centrifuged at 5000 rpm/5min and the absorbance of the supernatant was measured at 280nm. An appropriate control was prepared, in which the TCA was added before the enzymatic solution. One unit of enzyme activity (U) was arbitrarily defined as the amount of enzyme required to cause an increase of 0.1 in absorbance at 280nm, under the assay conditions. Enzymatic activity was calculated as follows : U/ml = (Abs 280nm ×10×dilution factor/0.2).the specific activity was expressed as units of enzyme activity per mg of protein [11].

RESULTS AND DISCUSSIONS

Protease production

Different bacteria were isolated from the soil to determine if they produced protease. Figure 1 shows the production of protease from nine different micoorganisms. The microorganism were taken from natural environment such as different soils, river, ponds and tap water, were incubated with nutrient agar medium at 37°c for 48hours. Following growth, sample from each culture were innoculated on the nutrient broth medium at 37°c for 11 days with mild shaking by using shaking incubator.

A graph of optical density (at 280nm) versus time (days) were plotted, at 2nd, 3rd, 6th,7th ,8th, 9th and 11th day the protease production were assayed through Ultraviolet Spectrometry. At 2nd day, the optical density of isolate number 1 shows high protease production than other isolates. Where else, on 3rd day the optical density of isolate number 2 shows more protease production which is at 4.1350 (280nm). Furthermore, at day 6th the protease production decreases, especially in isolate number 6th. Moreover, at day 7th and 9th there is slight increase in protease production this can be seen in isolate number 1. Finally at day 9th and 11 th, the protease production

decreases in all isolates.

Analysis of degradation of casein by microorganism

To investigate the degradation of casein by microorganisms, the product formed after degradation of casein were analysed. Casein hydrolysis can be demonstrated by supplementing nutrient agar media with milk [12]. The casein was digested by the proteases from the isolates, and protein accumulated gradually in the medium. The isolate could hydrolyse casein [13]. It is the stage of increasing amounts of proteins corresponded to the formation of proteases by the microorganism. The concentration of tyrosine in the medium gradually decreases at 9th and 11th day.

Microorganisms have the ability to decrease the protein casein by producing proteolytic exo-enzymes called protease. The process break down the peptide bond by introducing water into the molecule liberating the soluble amino acid pool for use in the synthesis of structural and functional cellular protein [14].

In the screening of microbes producing proteases, 10% trichloroaceticacid (TCA) is flooded on the glass tube after isolate being added into the glass tube and required incubation to precipitate the protein [15].

Table 1. Source of microorganisms

Isolate number	Name	Sample code
1	AIMST Cafeteria soil antimicrobial	CSA 1
2	AIMST lake water near guardhouse	ALWG2
3	AIMST Cafeteria water	CW 3
4	Rotten wood soil	RWS 4
5	Mangrove soil	MS 5
6	Big plastic	BP 6
7	Sewage water	SW 7
8	Small plastic soil	SPS(A) 8
9	Small plastic soil	SPS(O) 9

Table 2. Biochemical testing on isolated microorganisms

Isolates	Gram Staining	Mac- Conkey Test	Motility Test	Indole Test	Methyl Red Test
1	+	-	+	-	+
2	+	-	+	-	-
3	-	+	+	-	-
4	-	-	+	-	+
5	+	+	+	-	-
6	+	+	+	-	-
7	+	+	+	-	-
8	-	-	+	-	+
9	+	+	+	-	+

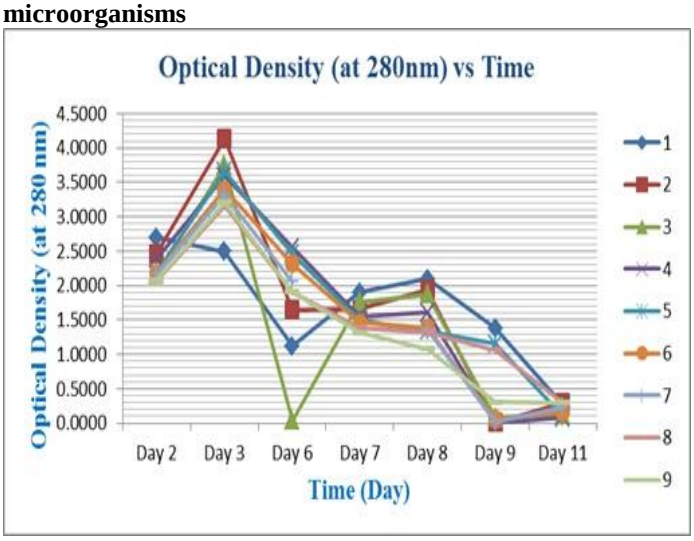
Table 3. Degradation of casein by isolated microorganisms

Sample	Optical Density at		(280nm)				
	Day 2	Day 3	Day 6	Day 7	Day 8	Day 9	Day 11
1	2.6970	2.4970	1.1170	1.9017	2.0924	1.3780	0.2614
2	2.4690	4.1350	1.6473	1.6587	1.9419	0.0034	0.3010
3	2.1580	3.7450	0.0378	1.7652	1.8794	0.0821	0.1259
4	2.3920	3.5950	2.5504	1.5495	1.6074	0.0044	0.0792
5	2.1960	3.6570	2.4624	1.5038	1.3353	1.1534	0.0966
6	2.1770	3.3960	2.3118	1.4624	1.3849	0.0576	0.1611
7	2.1580	3.3230	2.0568	1. 4041	1.3675	0.0102	0.2304
8	2.0620	3.1570	1.8971	1.3898	1.3204	1.0620	0.3000
9	2.0770	3.2060	1.9096	1.3137	1.0750	0.3050	0.3000

CONCLUSION

This Project has covered the major concerns about the nine different isolates, their morphology, biochemical characteristics, capability of producing protease. There are a large number of tests which are used to determine their morphology, namely gram staining and hanging drop method. These methods have brought good results as the morphology of all the isolates were identified clearly. Moving on to the biochemical tests, we adopted methods like Mac-conkey test, methyl red and Indole test which has

Figure 1. Degradation of casein by isolated microorganisms



given us a clearer picture on how the isolates react with different chemicals. Further we explored the proteolytic activity of the microbial isolates. The applications of proteases in industry and therapeutics have grown rapidly in the last two decades. Novel protein engineering strategies and techniques will continue to expand the commercial protease markets. In addition, taking advantage of the proteolytic activities of proteases in diseased tissues may also offer a new strategy for site-specific drug targeting and tumor imaging. This protease production research through casein hydrolysis by microorganism requires further investigation and further explore in future.

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