International Journal of Microbiology and Biotechnology
2021; 6(2): 45-52
http://www.sciencepublishinggroup.com/j/ijmb
doi: 10.11648/j.ijmb.20210602.13
ISSN: 2578-9678 (Print); ISSN: 2578-9686 (Online)

Production of Microbiological Peptone from Hydrolysis of Slaughterhouse Offal Using Bacterial Protease

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To cite this article:
Medhanit Teshome, Eleni Belay. Production of Microbiological Peptone from Hydrolysis of Slaughterhouse Offal Using Bacterial Protease. *International Journal of Microbiology and Biotechnology*. Vol. 6, No. 2, 2021, pp. 45-52. doi: 10.11648/j.ijmb.20210602.13

Received: February 17, 2021; **Accepted**: March 29, 2021; **Published**: April 20, 2021

Abstract: Proteases are the most important class of industrial enzymes accounting for 60% of the global industrial enzyme market. Microorganisms are the major source of these enzymes. Production of hyrolysates from different protein sources is among the different application of proteases. Protein hydrolysates have a variety of food and non-food applications. Although different proteases are available in the market, there is always a need for the development of new enzymes from bacterial sources. This is especially important in countries like Ethiopia where there are no local enzyme producers. The aim of this study was, therefore, to isolate new protease producing bacterial isolates to be used for the hydrolysis of slaughterhouse offal, optimize enzyme production and hydrolysis conditions, and test hydrolysates as a microbiological growth media. Based on screening data on solid and liquid media, one bacterial isolate designated as aau_5 was selected for further study. The isolate grew under solid-state fermentation (SSF) and produced up to 5,773 U/g of enzyme. Enzyme production was optimal when the solid to moisture ratio was 1:2 (66.7% moisture content) and in the presence of organic nitrogen sources. Protease aau_5 was optimally active at pH 7.5 and temperature of 55°C. After one hour incubation, the enzyme retained up to 66% and 41% of its original activity at 50°C and 55°C, respectively. Protease aau_5 was used for the hydrolysis of slaughter house offal (lung and bone) and soybean protein. The hydrolysate (peptone) was then tested as a microbiological media for the growth of different bacterial species. Compared to commercial peptone, hydrolysate obtained from lung (LPA) and bone extracted protein (BPA) supported better growth of the test organisms. So, by using waste and by products of slaughter houses, beneficial hydrolysate like peptone can be produced through enzymatic hydrolysis.

Keywords: Protease, Offal, Hydrolysis, Peptone

1. Introduction

Proteases are the most important class of commercial enzymes accounting for 60% of the global industrial enzyme market [1]. They find huge application in detergent, food, pharmaceutical, silk and leather industries.

Although all organisms produce proteases, from a commercial perspective microorganisms are the most preferred sources of these enzymes. This is because microorganisms have fast growth rate, require limited space for their cultivation and the ease at which their enzymes can be genetically manipulated to generate new enzyme variants for specific applications [2].

Protease production by microorganisms is affected by different parameters, such as growth temperature, quantity of inoculum, medium pH, and type and composition of medium [3]. Once produced the activities of microbial protease can also be influenced by temperature and pH of the reaction medium. Thus based on the optimum reaction proteases are classified as acid, neutral or alkaline proteases [4].

One important application for microbial proteases is in the hydrolysis of proteins from different wastes, such as offal from slaughterhouses, feather from poultry processing industries and fish processing wastes [5]. Protein rich wastes released by slaughterhouses include meat attached to bone and animal viscera. The protein in these wastes can be enzymatically hydrolyzed in to peptides or amino acids and can be used for different applications. Some of the applications include use as food or feed supplements, leather tanning supplements, microbiological media and cosmetics

[6, 7].

Currently the non-edible part of the cattle like, lung, trachea, kidney, brains, spleen, intestine, bone and other Slaughterhouse offal are mainly used for wild and domestic animals feed [8]. Considering the high protein content of these wastes, such practices are wasteful, especially for developing countries like, Ethiopia where the hydrolysate could find huge application in different sectors of the economy. For example in Ethiopia, the only known use for cattle lung is as a feed to cats. But in the majority of cases it is disposed as waste at the slaughterhouse. Similarly meat attached to bones and the intestine parts of cattle is also discarded to the garbage. This in addition to losing valuable resource, it potentially causes environmental pollution. Development of methods for the enzymatic hydrolysis of such offal is expected to have significant economic returns and that requires development of appropriate enzymes for the hydrolysis and optimization of the reaction conditions.

The aim of this study was, therefore, to isolate a new protease producing bacteria, characterize the enzyme, optimize reaction conditions for the hydrolysis of slaughterhouse offal and test the hydrolysates as a microbiological media.

2. Materials and Methods

2.1. Isolation and Screening of Protease Producing Bacteria

Soil samples were collected from different area of Addis Ababa university plant gardens and it was dried by air. To isolate bacterial isolates 1 gram of soil was suspended in 9 ml of sterile distilled water and serially diluted in the range of 10^{-2} to 10^{-6}. Then 0.1 ml aliquot from each dilution was spread on skim milk agar plates containing (g/l): skim milk powder, 10; peptone, 3; yeast extract, 3; NaCl, 5; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.1; and K_2HPO_4, 0.2, finally the pH was adjusted by 0.1% of KH_2PO_4 at pH 7. After 48 hr incubation at 37℃, isolates that form clear hallo zone were considered protease positive. All positive isolates were purified through repeatedly streaking on agar plates. The pure isolates were preserved at 4℃ for further study.

2.2. Characterization of Selected Isolate

The identification of bacteria was carried out by microscopic, morphological and biochemical studies. The biochemical tests were Gram staining and catalase activity. Culture characterization on agar plate like colony morphology was also done. In addition, starch and gelatin hydrolysis were tested.

3. Enzyme Production

3.1. Submerged Fermentation

Sterile skim milk broth (25 ml) in a 250 ml conical flask was inoculated with a loop full of the culture from fresh slants and incubated at 30°C on a rotary shaker at 150 rpm. After 48 hr of incubation, 1 ml of the culture was transferred to 1.5 ml sterilized eppendorf tubes and centrifuged at 10,000 rpm for 5 min. The cell free culture supernatant was used as crude enzyme preparation.

3.2. Solid State Fermentation

Solid substrate (10 g wheat bran) was transferred to a 250 ml conical flask; 13 ml of salt solution with the composition of NaCl, 0.5; $MgSO_4.7H_2O$, 0.02; $CaCl_2$, 0.01; and K_2HPO_4, 0.02, was added and autoclaved at 121°C for 30 min. Each flask was inoculated with 2 ml of the selected bacterial broth culture grown at 37°C for 48 hr. At the end of fermentation period, the enzyme was extracted by adding 100 ml of distilled water followed by filtration through a muslin cloth.

3.3. Protease Activity Assay

Proteolytic activity was measured using casein as the substrate. To 450 µl of 1% (w/v) of casein in 50 mM phosphate buffer, pH 7 was mixed with 50 µl crude enzyme extract and incubated for 20 min in a water bath at 50°C. The reaction was stopped by adding 450 µl of 10% (w/v) trichloroacetic acid (TCA) and was kept at room temperature for 10 min. After centrifugation at 10,000 rpm for 5 min, 150 µl supernatant was mixed with 750 µl of 0.5 M Na_2CO_3 and 150 µl of 1 N Follin Ciocalteus phenol reagent. The mixture was incubated in the dark for 30 min and absorbance was measured at 660 nm against a reagent blank. One unit of protease activity was defined as the amount of enzyme that resulted in the release of 1 µg of amino acid equivalent to tyrosine per min.

4. Characterization and Optimization of Reaction Conditions

4.1. Effect of pH on Enzyme Activity

The effect of pH on activity of the enzyme was assayed in the pH range of 6.0 – 10.5. The buffers used include phosphate (pH 6.0 – 8.0), Tris HCl (pH 7.5 – 9.0), and glycine NaOH buffer (pH 8.5 – 10.5).

4.2. Effect of Temperature on Enzyme Activity

The effect of temperature on enzyme activity was determined by performing the standard assay procedure at pH 7 within a temperature range from 40°C to 65°C in a 5°C interval.

4.3. Temperature Stability

Thermo stability of enzyme was measured by incubating the protease alone in different eppendorf tube for different incubation time of 0, 10, 20, 30, 40, 50 and 60 min in a water bath at 50 and 55°C. Then each of the incubated enzymes was assayed in the standard assay procedure at 50°C. Finally the residual activity of protease enzyme was calculated as follow.

Relative residual activity (RRA)$=\frac{individual\ residual\ activity}{residual\ activity\ at\ time\ of\ 0} \times 100\%$

5. Optimization of Culture Conditions for Enzyme Production

The protease activity was determined by varying the pH, temperature and fermentation period to optimize the reaction condition in order to scale up the target product.

5.1. Time Course of Enzyme Production

The culture medium was incubated for varying periods of time, 24, 48, 72, 96 and 120 hr to find the optimum time required for maximum enzyme production by SSF media at 37°C. At the end of the incubation time, the enzyme was harvested and activities were determined by standard enzyme assay procedure.

5.2. Effect of Moisture Level

Optimum moisture content required for the growth of bacteria and for protease production was determined by growing the organism at 37°C in the SSF media at a moisture level (v/w) of 33.3%, 50%, 60%, 66.7%, and 75%. The enzyme was harvested after 72 hr and activity determined.

5.3. Effect of Nitrogen Sources on Protease Production

The effect of nitrogen sources for protease production was determined by organic and inorganic nitrogen source. This includes peptone, ammonium nitrate, yeast extract, casein and sodium nitrate. Each source was used at a concentration of 0.5% (w/v) to replace nitrogen sources. Protease yield was determined after 72 hr of incubation at 37°C in a SSF with the moisture content of 66.7%.

6. Collection and Preparation of Protein

6.1. Offal Collection (Animal Source)

Samples of slaughterhouse offal (lung and raw meaty bone waste) was collected from the local retail butcher shop in sterilized container and transported to the laboratory. The lung was chopped and the meat on the bone was detached by using NaOH to dissolve proteins on the meat. Then the dissolved protein was precipitated by HCl [9].

6.2. Soya Bean Protein Isolation

Isolation of soya protein was carried out following the methods of the previous study [10]. A 10% (w/v) of defatted soya flour was dissolved in distilled water and pH adjusted between pH 10-11 using 1N NaOH. After removing the insoluble fraction, the solution was precipitated by adjusting the pH in the range of 5 and 6 using 1N HCl. The protein precipitate was recovered, dried in oven over night at 40°C, and pulverized to a powder passing through a 250 µm sieve.

6.3. Deffatting Process

1) Soya flour defatting

Soya flour from plant source was defatted by using hexane. The flour was soaked for 2 hr and for this time solvent to flour ratio was 1:3. Then the defatted portion was separated through filtration and it was dried by atmospheric temperature (air- dried overnight).

2) offal defatting

First lung of the cattle was chopped by knife and soaked in hexane for 2 hr. the lung defatting process was repeated depending on the fat content with n-hexane for better defatting and the defatted portion was separated through filtration. Then it was dried overnight in oven at 50°C Finally it was milled to a powder form and then sieved through 250 µm sieves to remove all fibers and it was stored at 4°C for further analysis [11].

6.4. Proteins Hydrolysis

Defatted lung and bone protein was dissolved with distilled water by adjusting the pH to 7.5 using 1N NaOH or 1N HCl, mixed the enzyme and incubated at 55°C for 3 hr.

Followed to protein isolation (section 7.2.) soya bean hydrolysis is done by the same process to offal hydrolysis. Then, after 3 hr of incubation the inactivation of the enzyme took place by boiling the solution for 5 min. Finally, the hydrolysate solution was dried at 40°C overnight in oven. After drying, the hydrolysate was mashed until it became powder and sieved through 250 µm sieves to use as a peptone for microbiological media.

7. Test of Protein Hydrolysate as Microbiological Media Components

7.1. Media Preparation

Microbial media were prepared from the hydrolysate of defatted flour of soya bean, lung, bone and commercial peptone agar that has a composition of (0.5% peptone, 0.1% dextrose, 0.1% yeast extract and 1% agar) in distilled water. The pH was adjusted to 7.4 ± 0.2 at 25°C by 1N NaOH. Finally the media were sterilized by autoclaving at 121°C for 15 min [12]. From each culture broth dilution, 0.1 ml was spread on the agar medium. The plates were incubated at 37°C for 24 – 48 hr. For each culture duplicate plates were prepared [11].

7.2. Inoculum Preparation

From Ethiopian health and nutrition research institution (EHNRI) a pathogenic bacteria of *E. coli, Streptococcus agalaetiae, Staphylococcus aureus, Shigella flexneri, Salmonella thyphimerium* and *Klebsiella pneumonia* were obtained. Each bacterium was separately cultured on nutrient agar for 24 hr at 37°C to refresh the pathogens and for isolation of pure colony. Single colony was taken and inoculated to nutrient broth and incubated for 24 hr. Then 1 ml of cultured broth was serially diluted in sterile distilled

water (10^4-10^9 dilution) [11].

7.3. Growth Measurement

The numbers of colonies were directly counted from the culture. It was done in duplicate to increase the validity of number of colony counting.

8. Results

8.1. Isolation and Screening of Protease Producing Bacteria

Based on the formation of a clear zone around the colony on skim milk agar media (Figure 1) a total of 17 bacterial isolates were selected as protease producers.

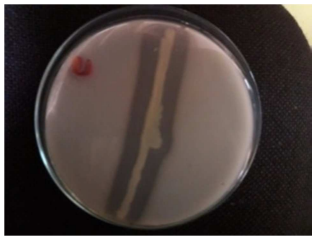


Figure 1. *Protease producing isolates on skim milk agar media.*

On the basis of enzyme production in liquid culture and their ability to grow in SSF, 4 isolates were selected for further screening. Out of the four isolates, one isolate that has relatively high enzyme production designated as *aau*$_5$, was selected for further study.

8.2. Characterization of Selected Isolate

Isolate *aau*$_5$ was a Gram-negative, rod shaped, catalase positive, and white creamy with irregular shape of colonies on skim milk agar medium (Table 1). This isolate was not able to hydrolyze starch and gelatin but can hydrolyze skim milk. Based on these morphological and biochemical features this isolate was tentatively grouped under the genus *Pseudomonas*.

Table 1. *Morphological and biochemical features of the bacterial isolate (aau*$_5$*).*

No.	Characterization	Result
1	Shape	Rod
2	Gram staining	-ve
3	Catalase	+ve
4	Casein hydrolysis	+ve
5	Starch hydrolysis	-ve
6	Gelatin hydrolysis	-ve
7	Skim milk hydrolysis	+ve

8.3. Enzyme Production Through Solid State Fermentation

8.3.1. Effect of Moisture Level on Protease Production

To determine the optimum moisture level for enzyme production isolate aau$_5$ was grown on wheat bran containing different moisture levels. Maximum enzyme production (5,031.93U/g) was observed at moisture level of 66.7%. With increasing moisture level above its optimum enzyme production sharply decreased (Figure 2).

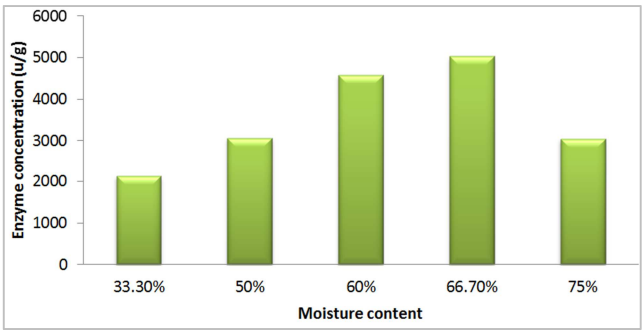


Figure 2. *Moisture levels of SSF medium and enzyme production by isolate aau*$_5$*.*

8.3.2. Effect of Incubation Period on Protease Production

Enzyme production increased as time of incubation increased from 24 hr – 72 hr (Figure 3) in the solid state fermentation. Then maximum protease production (5773 U/g) of *aau*$_5$ was observed at 72 hr of incubation. After 72 hr of incubation period a gradual reduction in the relative enzyme production was observed.

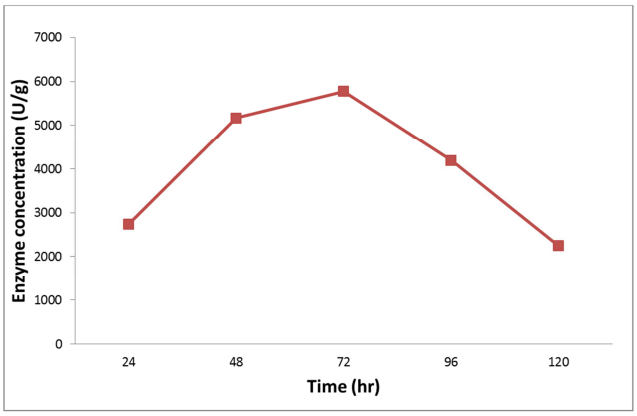


Figure 3. *Time course of protease production by isolate aau*$_5$*.*

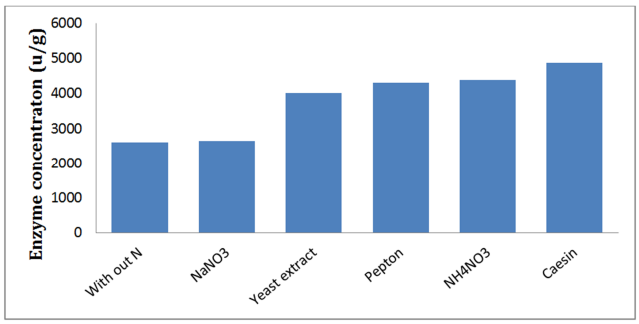


Figure 4. *Effects of different nitrogen sources on protease production by isolate aau*$_5$*.*

8.3.3. Effect of Nitrogen Source on Enzyme Production by aau$_5$

Best nitrogen source was determined in the presence and absence of different nitrogen source under a solid state fermentation in a wheat bran medium. Of the different

nitrogen supplements tested enzyme production was the highest (4870 U/g) in the presence of casein. On the other hand, using Sodium nitrate for aau_5 growth showed almost equal amount of protease compared to protease production in the absence of nitrogen supplement (Figure 4). Relatively, addition of organic nitrogen source (yeast extract, peptone and casein) supported higher enzyme production.

8.4. Characteristics of the Protease Enzyme

8.4.1. Effect of pH on the Activity of aau_5 Protease

The aau_5 protease has higher activity in wider pH range and the maximum activity was observed at pH 7.5. The enzyme maintained more than 75% of its activity in the pH range of 6 to 10. Relatively, the lowest activity was observed at pH 10.5 (Figure 5).

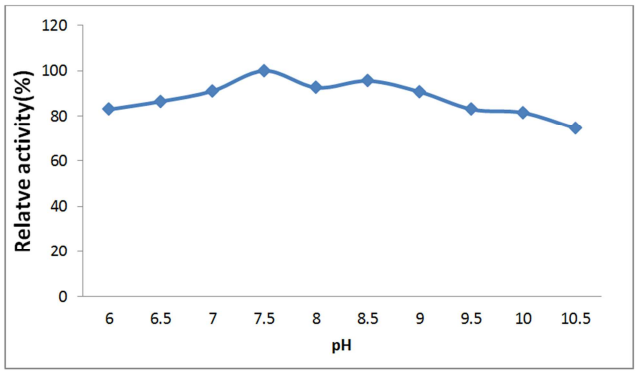


Figure 5. *Relative activity of aau_5 protease at different pH value.*

8.4.2. Effect of Temperature on the Activity of Protease

The optimum temperature for aau_5 *protease* was at 55°C and retained 90% of its maximum activity at 60°C (Figure 6).

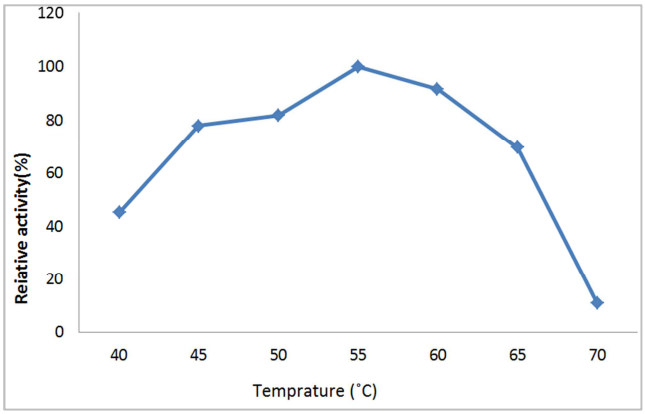


Figure 6. *The relative activity of aau_5 protease at different temperature values.*

8.4.3. Effect of Temperature on the Stability of Protease

The temperature stability of aau_5 protease was determined by assaying the pre-incubated enzyme. The relative activity of this enzyme decreased with increasing incubation temperature. At 55˚C about 41% of its activity was retained after 60 min. The enzyme also retained 60% of its activity after 1 hr incubation at 50˚C (Figure 7).

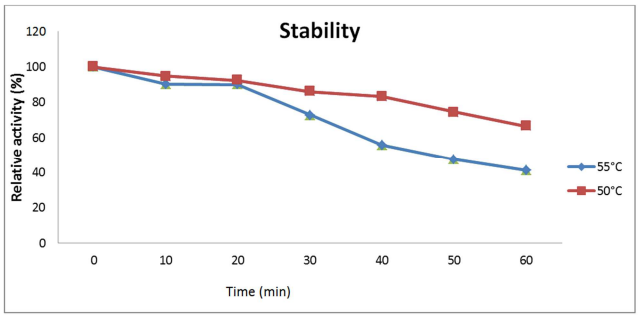
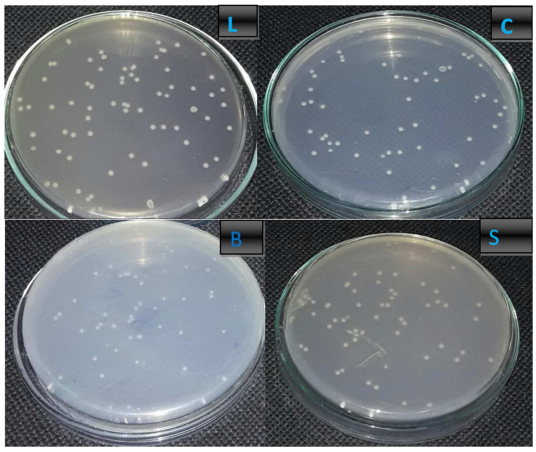


Figure 7. *Temperature stability of aau_5 protease at 50°C and 55°C.*

8.5. Test the Hydrolysate for Bacterial Growth as a Microbiological Media on the Laboratory Produced Peptone

The peptone produced from lung and bone was compared with the soya bean and commercial peptone as a microbial media for the growth of different pathogenic microorganisms. Media prepared from laboratory prepared peptone supported a good growth of the test bacteria and was better than media prepared from commercial peptone (Table 2).

Compared to the commercial peptone agar, *E.coli* and *Klebsiella pneumonia* showed better growth in media prepared from lung, bone, and soya peptone (Figure 8). LPA and BPA were better for microbial growth in almost all organisms except *Klebsiella pneumoniae* which was slightly higher CFU in commercial peptone agar than BPA and SPA. Relatively, *Staphylococcus aureus* showed the least growth in all media. In general the peptone from animal source (lung and bone) was better for bacterial growth than soya bean and commercial peptone agar media.



Note: - B, BPA; C, CPA; L, LPA and S, SPA

Figure 8. *Colony of Salmonella agalaetiae on lung peptone agar (LPA), bone peptone agar (BPA), soya peptone agar (SPA) and commercial peptone agar (CPA).*

Table 2. *Comparison of pathogenic bacterial colony count on lung and bone peptone agar against soya bean and commercial peptone agar.*

Test Bactria	*Cfu/ml*			
	LPA	**BPA**	**SPA**	**CPA**
E.coli	87×10^7	89×10^7	80.14×10^7	70.28×10^7
Shigella flexneri	37.14×10^7	42.14×10^7	29.57×10^7	32.21×10^7
Streptococcus agalactiae	11.14×10^7	13.43×10^7	7.57×10^7	7.86×10^7
Staphylococcus aureus	4.36×10^7	6.21×10^7	0.29×10^7	1.07×10^7
Salmonella thyphimerium	22.21×10^7	20.71×10^7	21.14×10^7	13.57×10^7
Klebsiella pneumoniae	42.57×10^7	39.35×10^7	40.21×10^7	41.21×10^7

Note: - BPA, bone peptone agar; CPA, Commercial peptone agar; LPA, lung peptone agar and SPA, soya bean peptone agar.

9. Discussion

Soil contains many nutrients and it is often considered as a major source of different useful microbes, including those producing proteases [13]. In this study the 75% of the bacterial isolates were shown a proteolysis activity on skim milk agar and they were isolated from soil sample. For the initial isolation of protease producers a simple and efficient screening procedure could have huge advantages. Based on its simplicity, rapidity, range of detection and sensitivity, the clear zone formation due to proteolytic activity of the microbe on the skim milk agar plate was used for the initial screening of protease producing microbes [14]. However, in this study the size of clear zone on skim milk agar was given ambiguous result in some selected isolate. Therefore, use of other screening method like enzyme production on SMF and SSF was necessary to select best protease producing isolate.

The selected isolate (isolate aau_5) grow under SSF using wheat bran shows its potential as a source of commercially important enzyme with low production cost. For the large-scale application of proteases reduction in the production cost of the enzyme (s) is very important. Currently large-scale production of proteases in developed countries is carried out in submerged fermentation. However, in developing countries enzyme production through solid state fermentation (SSF) offer several advantages. First, it uses cheap agro processing wastes that can help to greatly reduce the production cost. Secondly, growth under SSF is often associated with higher product yield [15]. Third, most of bacteria require higher water activity for growth, but SSF has a lower risk of contamination.

The growth and enzyme production of microorganisms under a SSF can be influenced by the moisture level [16]. The highest enzyme production for isolate aau_5 (reaching up to 5031 U/g) was observed in the presence of 66.7% moisture. Increasing the moisture content to75% resulted in reduction of enzyme production by more than 40%. Similarly, enzyme production below the optimum moisture level was also lower. Most bacterial species optimally grow in the presence of high water activity. But in SSF increasing moisture content above a critical value could affect porosity of the medium and limit gas transfer [17]. On the other hand low moisture content leads to reduction in nutrient diffusion and that can also affect the enzyme production [15]. The optimum moisture content required for high enzyme production is different based on the strains. For example maximum protease enzyme production by Penicillium sp. was observed at moisture content of 50% [18].

In most organisms, maximum extracellular enzyme production is often observed at or around the end of the exponential or beginning of the stationary phase where the concentration of one or more nutrients in the medium is depleted [19]. Isolate aau_5 showed a fast enzyme production on wheat bran under SSF (up to 2,740 U/g) after 24 hr of incubation. But, maximum enzyme production was observed after 72 hr incubation. After that enzyme production showed a sharp decrease. The end of the stationary phase of microbial growth cycle is associated with depletion of essential nutrients in the culture medium and accumulation of waste product in the medium that inhibit growth and enzyme production [20].

For maximum enzyme production the culture medium must contain the required carbon and nitrogen sources. For organisms that grow under SSF using wheat bran, the solid substrate could supply up to 75% of the required carbohydrate [21]. This shows that there is no need to supplement wheat bran with additional carbon sources. On the other hand the nitrogen content of wheat bran may not be sufficient to support microbial growth. Therefore, there is a need to add nitrogen supplements to bring about optimal growth under SSF. For isolate aau_5 protease enzyme production under SSF was higher in cultures supplemented with organic nitrogen than cultures grown without any nitrogen supplement and cultures supplemented with $NaNO_3$ as inorganic nitrogen source. Other reports also showed that supplementing wheat bran with organic nitrogen sources lead to better growth and protease production [22]. This shows wheat bran may not contain the required amount of nitrogen for the optimum growth of the organism. Some reports showed that supplementation of some strains with inorganic nitrogen source resulted in better growth and protease production [23]. This shows the requirement of specific nitrogen source differs from organism to organism [24].

Protease aau_5 was active in the pH range of 6.5-9.0, with an optimum at pH 7.5. Protease optimally active around neutrality are considered ideal for application in foods processing industries [25]. Moreover, for industrial application in food processing proteases active in the temperature range of 50- 60°C are considered ideal [26]. aau_5 protease showed very good stability and activity in the temperature range of 50°C to 55°C. This shows that the enzyme has a good potential for application in the protein hydrolysis and other food processing industries.

Hydrolysis by protease aau_5 was used for the production of peptone from slaughter house offal (lung and meat extracted from bone) and soybean protein used for the formulation of microbiological growth media. [27] Reported that peptone prepared from different sources show significant differences in supporting good microbial growth. In this study, compared to the commercial peptone, peptone produced in the laboratory from slaughterhouse offal (lung and bone) and from soybean protein supported better growth of the test bacterial species (Table 2). The difference in the different peptone preparations might be a result of differences in composition of essential amino acids, vitamins, and/or other growth factors [28]. To date media used for microbial growth in Ethiopia is imported from abroad. Given the large quantity of offal released each day in the country, the results of this study suggest the existence of a good potential to produce effective microbiological media from cheap local resources and help the country save in foreign currency.

In this study the growth of tested bacteria favorably well grow in animal source compared to that of plant and commercial source of peptone. Based on the source and hydrolysis process of proteins, the essential amino acids in the peptone are also different. For example the amino acids composition of plant and animal proteins is quite different [29]. In addition to that peptones that are produced from plants source have an antibacterial property over that of animal based peptone. Soya bean is one of the plant sources of protein that have an anti-microbial effect against *Staphylococcus aureus* [30].

Peptones derived from animal source have also an advantage by reducing the cost of raw material for peptone production. According to Durrani et al.'s study, most peptone from plant source is derived from edible source of cereal and leguminous plants like soya bean [31]. In contrast, peptone from slaughterhouse offal can be produced from inedible parts such as lung, tendons, intestine and bone allowing waste valorization. And these ultimately greatly reduce the production cost of the peptone while at the same time avoiding environmental pollution from the release of offal.

10. Conclusion and Recommendation

Protein hydrolysates prepared from slaughterhouse offal through enzymatic hydrolysis were tested as potential microbiological media. Peptone prepared in the lab supported the growth of different test microorganisms better than or equal to commercial peptone. Given the large number of animals slaughtered each year in Ethiopia and the amount of offal disposed as waste, protein recovery and enzymatic hydrolysis could lead to production of protein hydrolysates with huge economic benefit. This requires availability of efficient enzymes with affordable cost. The result of this study shows the potential of Ethiopia's bacterial diversity as a source of new enzymes for protein hydrolysis. The fact that the organism grew under SSF fermentation using cheap agricultural waste help to greatly reduces the cost of enzyme production.

For large-scale application it is recommended that enzyme production and hydrolysis reaction is scaled up. It is also recommended that the resulting protein hydrolysate to be tested or used in the food, animal feed, and cosmetics industries is studied.

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