

Isolation and Characterization of Amylolytic Bacteria in the Feces of Senduro Lamb and Adult Goat

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Abstract

Amylase enzyme can hydrolyze starch into simpler sugars and can be isolated from amylolytic bacteria, which can be sourced from animal feces. This study aims to isolate amylolytic bacteria from feces of Senduro goats aged 4-5 years and lamb aged 1-2 months, and to determine the amylase enzyme activity produced by the isolates. Isolation and purification of bacteria were carried out using serial dilution, pour plate, and quadrant streak techniques. Qualitative tests were conducted to calculate the Amylase Index (AI) produced and continued with quantitative tests using the DNS (*Dinitrosalicylic acid*) method on three isolates with high AI values from each sample. Amylolytic bacterial isolates with the highest amylase activity in order are isolates BAD4 (0.3683 U/mL), BAA5 (0.3547 U/mL), BAA6 (0.3153 U/mL), BAD6 (0.2744 U/mL), BAD11 (0.2275 U/mL), and BAA3 (0.067 U/mL). The six bacterial isolates were then characterized by bacterial cell morphology which showed cocci-shaped bacteria (isolates BAD4, BAD6, and BAA5), bacillus-shaped bacteria (isolates BAD11, BAA6, and BAA3), positive motile (isolates BAD6, BAA6, and BAA3), non-motile (isolates BAD4, BAD11, and BAA5) with Gram positive and positive catalase as a whole. These findings indicate the potential of Senduro goat feces as a source of amylolytic bacteria for enzyme production.

Keywords: amylolytic bacteria; characterization; feces; amylase enzyme; natural resources

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INTRODUCTION

Enzymes are biocatalysts required to improve chemical reactions in biological function by accelerating the reaction rate up to 10^8 to 10^{11} times faster when compared to a reaction that runs without a catalyst (Putra *et al.*, 2021). Enzymes are widely used in various chemical processes across different industries, particularly those derived from microbes, as they can be easily regulated through genetic manipulation and controlled by their growth environment (Deckers *et al.*, 2020; Kartika and Ibrahim, 2021). Enzymes used in industry in Indonesia 99% are still imported from China, India, Japan, and some European countries (BRIN, 2024). The volume of enzyme imports in Indonesia from 2019 to 2022 was 26,206.58 tons, 5.93 tons, and 5.88 tons, respectively (BPS, 2024). One of them is amylase enzyme, which can hydrolyze starch and can be mobilized into organic, inorganic, non-toxic, and biodegradable matrices, thereby expanding its applications in industries such as leather, paper, chemical, and pharmaceutical (Shanmugasundaram, 2021).

Amylolytic bacteria are bacteria that produce the enzyme amylase, enabling them to break down starch into glucose (Murtius, 2016). Amylolytic bacteria can be isolated from several sample sources. Wahyuni *et al.* (2021) isolated seven isolates of amylolytic bacteria with different characteristics from tilapia digestive tract samples. The Bifidobacterium group of bacteria capable of hydrolyzing starch is found in the human colon (Ji *et al.*, 1992). *Bacillus amyloliquefaciens* can be found in various sources such as soil, plants, animal feces, and aquatic environments and can produce various enzymes, one of which is amylase (Ningtyas *et al.*, 2023). In addition, in the research of Li *et al.* (2019), amylolytic bacterial genera were found in the duodenum and jejunum of goats. Feces can be used as a source of representation for the isolation of microbiota in the gastrointestinal tract (GIT) of ruminants, although in some other studies the rumen or fecal microbiota cannot represent all other GIT compartments.

According to the studies mentioned above, amylolytic bacteria can be isolated from the digestive system of ruminants. This is because the unabsorbed organic matter will be fermented in the rumen by gut microbiota, including amylolytic bacteria (Zubaidah *et al.*, 2019; Ramadhan and Wikandari, 2021). The population of amylolytic bacteria in ruminants varies depending on the type of feed given to

livestock. According to Cao *et al.* (2023) the microbial composition of the GIT varies depending on the age of the ruminant, geographical location, feeding style, and host species.

Lumajang district is one of the districts in East Java province. In 2022, the goat population in Lumajang district reached 132,109 (Dinas Peternakan Provinsi Jawa Timur, 2022). Although it is not among the areas with the highest goat production such as Trenggalek, Lumajang District has a flagship livestock, which is Senduro goats. Based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 1055/Kpts/SR.120/10/2014, Senduro Goat is designated as protected and preserved Indonesian local livestock genetic resources. Senduro goats cultivated in goat farm in Wonorejo, Kandangtepus, Senduro District, Lumajang Regency are given different types of feed between adult goats (4-5 years old) and lamb (1-2 months old). In Senduro goats, feed given to lamb are forage, concentrates, cassava pulp, and cow's milk, while in adult Senduro goats feed given are forage, concentrates, and cassava pulp. According to Xie *et al.* (2017) cassava pulp contains 40% starch and 11% cellulose. The amylose and amylopectin levels contained in cassava starch are 17% and 83%, respectively (Morgan and Choct, 2016).

Based on the description above, various studies related to the isolation of amylolytic bacteria have been carried out from various sources, but research on the isolation of amylolytic bacteria in Senduro goat feces has never been done. Therefore, this study focused on the isolation of amylolytic bacteria from two fecal samples of Senduro lamb and adult goat.

MATERIALS AND METHODS

This study was a descriptive observational study conducted for six months from September 2024-March 2025. Sampling was conducted in a goat farm located in Wonorejo, Kandangtepus, Senduro District, Lumajang Regency, East Java. Isolation, characterization, and ability test of bacterial isolates from the feces of adult Senduro goats and young as amylolytic bacteria were carried out at the Molecular Biology Laboratory and Microbiology Laboratory, Biology Study Program, Faculty Of Mathematics and Natural Sciences, State University of Surabaya.

Fecal sampling of adult female Senduro Goats and lamb was conducted in the morning from 07.00 - 09.00 WIB. Sample were taken from female goat aged 4-5 years and female lamb aged 1-2 months. The feces samples were put into a sterile centrifuge tube and stored in the refrigerator for about one month for further isolation.

Soluble Starch Agar (SSA) media was made by dissolving 10 g of Soluble Starch (SS), 12 g agar powder, and 3 g beef extract into 1 L distilled water (Lal and Cheeptham, 2012). Media was sterilized in autoclave for 15 minutes at 121°C. Oblique SSA media was made by putting 5 ml SSA media into a test tube and then sterilized in the same way. The test tube containing SSA media then tilted until it solidified. The starch agar media was used to inoculate the purified bacteria (Win *et al.*, 2017).

Bacteria isolated from feces sample by weighing 1 g of each fecal sample, then diluted in stages up to a dilution of 10^{-8} in 1 ml 0.85% NaCl solution. About 1 ml of each dilution from 10^{-1} to 10^{-8} was cultured in petri dishes in SSA media with pour plate method. After that, incubation was carried out for 24 hours at 37°C for bacteria growth (Silaban and Simamora, 2018).

Grown bacteria in the SSA media were then characterized based on morphology, including shape, color, elevation, and shape of colony edge (Isnawati and Trimulyono, 2018). Each isolate with different colony characteristics was then purified using quadrant scratch or streak plate method to obtain pure colonies (Arini, 2016).

Qualitative tests of bacterial amylase activity were carried out using the disc diffusion method. Starch agar medium was thawed and sterilized then poured into sterile petri dishes until it solidified. Bacterial isolates were first grown on the liquid media. Then, paper discs were dipped in bacterial suspension and planted on starch agar media (Stark *et al.*, 1953). Incubation was carried out for 24 hours at 37°C (Silaban *et al.*, 2020). After incubation, each Petri dish containing bacterial culture was inundated with iodine for 5 minutes (Hastuti *et al.*, 2015; Sundarapandiyan and Jayalakshmi, 2017). The formation of clear zone indicates activity of amylase enzyme because disaccharides and monosaccharides from starch hydrolysis do not bind to iodine (Nursanti *et al.*, 2022). Each of the three isolates with the highest Amylolytic Index (AI) was selected for further testing. The following formula was used to calculate the amylolytic index of isolates (Wulandari *et al.*, 2017):

$$AI = \frac{\text{Clear Zone Diameter}}{\text{Colony Diameter}}$$

Quantitative tests were carried out through three stages, which were production of crude amylase extract, preparation of glucose standard curve, and bacterial amylase activity test. Crude amylase extract was obtained by growing selected bacterial isolates on 20 mL liquid Yeast Peptone Starch (YPSs) liquid medium containing (g/L): 0.1 yeast extract; 0.25 peptone; 0.15 KH_2PO_4 ; 0.025 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.005 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; and 1 starch. Isolates were incubated for 3x24 hours at room temperature in an orbital shaker at 130 rpm. About 5 mL culture of each bacterial isolate with Optical Density (OD) value of 0.5 was transferred to 20 mL new YPSs liquid media and incubated in the same way (Nisa *et al.*, 2021; Swandi, 2020). Then liquid culture was centrifugated for 5 min at 10,000 rpm at 4°C (Nisa *et al.*, 2021). The resulting supernatant containing crude enzyme amylase was used for amylase activity testing (Supriyanti and Heryanto, 2013).

Glucose standard curve was prepared by preparing glucose stock with a concentration of 100 ppm which was then diluted to 0, 20, 30, 40, 50, and 60 ppm. A total of 1 mL of Dinitrosalicylic acid (DNS) reagent was added to each glucose solution and heated for 5 minutes until it turned brownish red. Next, 1 mL of 40% K-Na-Tartrate solution was added and cooled. Then, distilled water was added until the final volume reached 10 mL and homogenized. The absorbance value was measured using a UV-Vis spectrophotometer with a wavelength of 540 nm (Nisa *et al.*, 2021).

Amylase activity test was performed based on (Nimisha *et al.*, 2019) was carried out by mixing 1 mL crude enzyme and 1 mL of 1% starch solution in 0.05M phosphate buffer at pH 6.9, then incubated at 37°C for 10 minutes. The reaction was controlled by adding 1 mL of DNS reagent. Next, the test tube was placed in boiling water for 10 minutes and cooled. After cooling, absorbance readings were taken using a spectrophotometer with a wavelength of 540 nm against the blank control consisted of consists of 1 mL of 1% starch in phosphate buffer and 1 mL of DNS reagent (Lumba *et al.*, 2017). Bacterial isolates that have been quantitatively tested were then characterized by the nature of Gram and bacterial cell shape, motility test (Yulfizar, 2013), and catalase test (Aisyah *et al.*, 2014).

RESULTS

Bacterial isolates obtained from fecal sample amounted to 19 isolates with 13 isolates derived from Senduro adult goat and 6 bacterial isolates from Senduro lamb. The isolate codes obtained from Senduro Adult Goat feces samples include BAD1, BAD2, BAD3, BAD4, BAD5, BAD6, BAD7, BAD8, BAD9, BAD10, BAD 11, BAD12, and BAD13. Isolate codes obtained from Senduro Goat feces samples include BAA1, BAA2, BAA3, BAA4, BAA5, and BAA6. The morphological characteristics of amylolytic bacterial isolates from Senduro adult goats are shown in Table 1, while those from lambs are presented in Table 2.

Table 1. Colony morphology characteristics of isolates of amylolytic bacteria in the feces of Senduro Adult Goat

Isolate	Shape	Color	Elevation	Margin
BAD1	Circular	White	Flat	Undulate
BAD2	Circular	White	Convex	Entire
BAD3	Irregular	White	Flat	Lobate
BAD4	Rhizoid	White	Flat	Lobate
BAD5	Circular	White	Raised	Erose
BAD6	Irregular	White	Raised	Filamentous
BAD7	Filamentous	White	Flat	Lobate
BAD8	Irregular	White	Flat	Erose
BAD9	Filamentous	White	Flat	Filamentous
BAD10	Irregular	White	Raised	Curled
BAD11	Irregular	White	Flat	Curled
BAD12	Circular	White	Raised	Entire
BAD13	Circular	White	Flat	Curled

Table 2. Colony morphology characteristics of isolates of amylolytic bacteria in the feces of Senduro lamb

Isolate	Shape	Color	Elevation	Margin
BAA1	Filamentous	White	Flat	Filamentous
BAA2	Circular	White	Flat	Entire
BAA3	Circular	White	Convex	Entire
BAA4	Punctiform	White	Flat	Entire
BAA5	Spindle	White	Flat	Entire
BAA6	Irregular	White	Flat	Lobate

All bacterial isolates were then tested qualitatively for amylase activity to determine the Amylolytic Index (AI), with results presented in Table 3 for adult goat and Table 4 for lamb. From a

total of 13 bacterial isolates that have been qualitatively tested in adult goat sample, three bacterial isolates with the highest amylase activity index were selected for further quantitative testing: isolate BAD6 with AI of 1.017, BAD11 with AI of 0.988, and BAD4 with AI of 0.956. While from lamb feces samples, from six bacterial isolates that have been tested qualitatively, three bacterial isolates with highest amylase activity index were used for further quantitative testing, which were isolate BAA6 with AI of 0.941, BAA3 with AI of 0.564, and BAA5 with AI of 0.537.

Table 3. Qualitative test results of bacterial amylase activity from Senduro adult goat feces

Isolate	Average Colony Diameter (mm)	Average Total Diameter (mm)	Average Clear zone diameter (mm)	Amylolytic Index
BAD6	11.3	22.8	11.5	1.017
BAD11	14.08	28	13.92	0.988
BAD4	13.73	26.88	13.135	0.956
BAD9	6.3	11.15	4.85	0.769
BAD5	8.45	14.73	6.28	0.743
BAD13	11.08	18.715	7.165	0.646
BAD10	9.5	15.33	6.05	0.636
BAD1	9.665	15.715	6.05	0.625
BAD3	10.25	16.08	5.815	0.567
BAD12	14.015	20.88	6.235	0.444
BAD2	14.495	17.58	3.085	0.212
BAD7	14.165	16.05	1.885	0.133
BAD8	19.75	22.6	2.63	0.133

Table 4. Qualitative test results of bacterial amylase activity from Senduro lamb feces

Isolate	Average Colony Diameter (mm)	Average Total Diameter (mm)	Average Clear Zone Diameter (mm)	Amylolytic Index
BAA6	14.65	28.45	13.8	0.941
BAA3	18.95	29.65	10.7	0.564
BAA5	15.15	23.3	8.15	0.537
BAA4	11.83	18.065	6.235	0.527
BAA2	14.23	21.66	7.43	0.522
BAA1	14.3	19.165	4.86	0.339

Figure 1 presents clear zone in iodine-inundated media of the total of the six isolates selected for further quantitative testing, each three isolates from adult Senduro goats and lambs.



Figure 1. Clear zones of bacterial isolates in iodine-inundated media. a) BAD6; b) BAD11; c) BAD4; d) BAA3; e) BAA6; and f) BAA5

A quantitative test of bacterial amylase activity was conducted on selected bacterial isolates obtained from the feces of adult Senduro Goats and young goats, based on the highest amylase index value from the qualitative test results. Table 5 presents the result of quantitative amylase activity from select isolates.

Table 5. Quantitative test results of amylase activity of bacterial isolates from the feces of Senduro adult goat and lamb

Isolate	Absorbance (540 nm)	Reducing Sugar (mg/L)	Amylase activity (U/mL)
BAD4	0.262	331.48	0.3683
BAD6	0.200	246.98	0.2744
BAD11	0.169	204.73	0.2275
BAA5	0.253	319.21	0.3547
BAA6	0.227	283.78	0.3153
BAA3	0.063	60.26	0.067

The six isolates, designated as BAD4, BAD6, BAD11, BAA5, BAA6, and BAA3, were characterised by their colony morphology, followed by further characterisation of bacterial cell morphology, Gram characteristics, motility, and the ability to produce the catalase enzyme with results presented in Figure 3, 4, and 5. Table 6 compared the result of these tests from the select isolates.

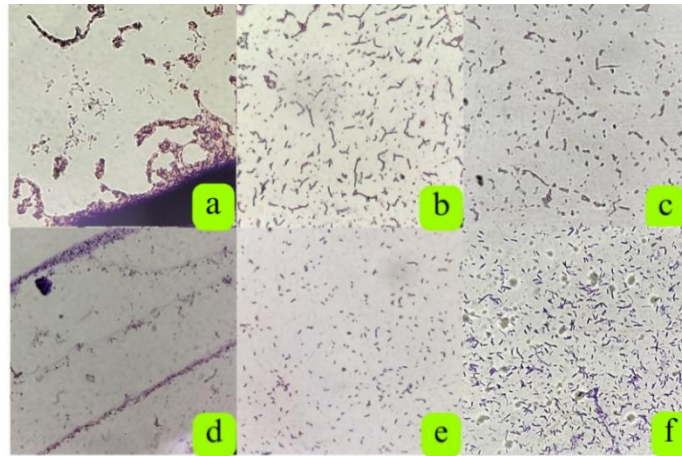


Figure 3. Gram staining results of amylytic bacterial isolates: a) Isolate BAD4; b) Isolate BAD6; c) Isolate BAD11; d) Isolate BAA5; e) Isolate BAA6, and f) Isolate BAA3.

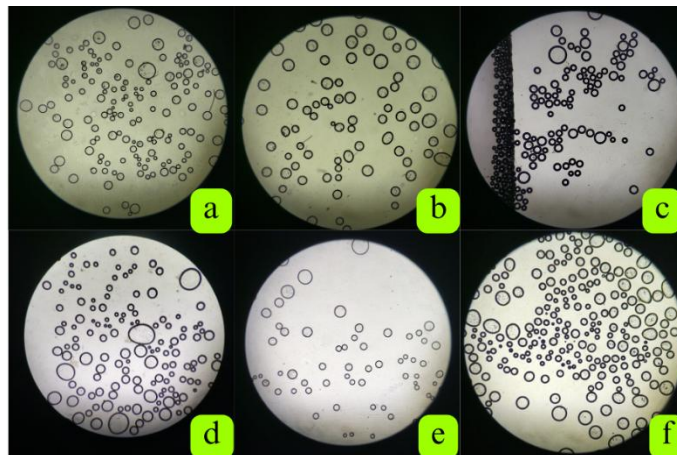


Figure 4. Catalase test results of amylytic bacterial isolates: a) Isolate BAD4; b) Isolate BAD6; c) Isolate BAD11; d) Isolate BAA5; e) Isolate BAA6, and f) Isolate BAA3.

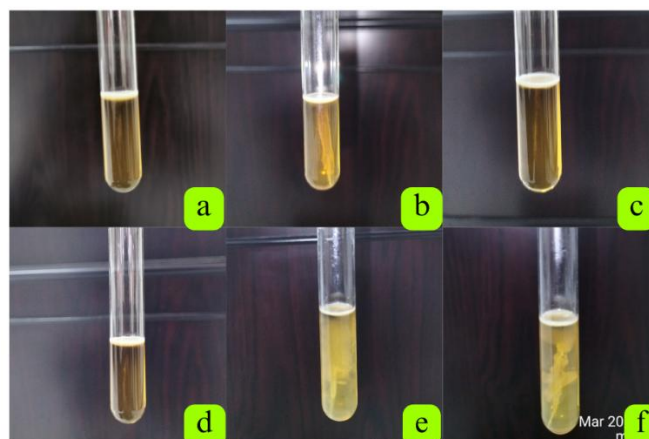


Figure 5. Motility test results of amylytic bacterial isolates: a) Isolate BAD4; b) Isolate BAD6; c) Isolate BAD11; d) Isolate BAA5; e) Isolate BAA6; and f) Isolate BAA3.

Table 6. Morphological characteristics of bacterial isolates

Characteristics	Isolate*					
	BAD4	BAD6	BAD11	BAA5	BAA6	BAA3
Cell shapes	Coccus	Coccus	Bacil	Coccus	Bacil	Bacil
Gram	+	+	+	+	+	+
Motility	-	+	-	-	+	+
Catalase	+	+	+	+	+	+

DISCUSSION

Amylolytic bacteria isolated from the feces of adult Senduro goats and lambs have the same white color characteristics, with varying elevation, colony shape, and margin characteristics (Table 1 and Table 2). In comparison, Wahyuni *et al.* (2021) isolated amylyolytic bacteria from the digestive tract of tilapia and produced six isolates with different morphological characteristics, which were then tested to determine their ability to produce the enzyme amylase. Other studies, such as Alif *et al.* (2025) obtained five isolates of amylyolytic bacteria with varying characteristics in the digestive tract of coconut caterpillars, while Yulianti *et al.* (2023) obtained 11 isolates of amylyolytic bacteria with diverse characteristics from digestive tract of the American cockroach (*Perilaneta americana*).

Based on Table 3 and Table 4, all bacteria isolated from the feces of Senduro goats has different amylyolytic index, but with the number isolates positively producing amylase enzymes were found more in adult Senduro goats compared to lambs. One of the factors that cause this to happen is due to the maturation of the digestive tract. A mature digestive tract will provide a more stable and diverse environment that supports a more varied microbial community (Estrada *et al.*, 2024). According to Li *et al.* (2019), increasing age of ruminants accompanied by changes in feed to concentrates and fiber leading to increase in saccharolytic and amylyolytic genera in the GIT in the cecum and colon.

Quantitative testing of amylase activity of amylyolytic bacterial isolates is done by the DNS (Dinitrosalicylic acid) method, which measures the reducing sugar resulting from amylase enzyme activity to break down or hydrolyze soluble starch (Sharma *et al.*, 2015). DNS is an aromatic compound that reacts with reducing sugars. The aldehyde group of sugar is reduced by DNS to form 3-amino-5-nitrosalicylic acid (Keharom *et al.*, 2016). Table 5 shows that the more reducing sugar produced, the higher the amylase activity produced. Nisa *et al.*, (2021) states that the more reducing sugar that is formed, the higher the absorbance value and amylase activity.

Based on Table 5, bacterial isolates with highest amylase activity were BAD4 (0.3683 U/mL), followed by BAA5 (0.3547 U/mL), BAA6 (0.3153 U/mL), BAD6 (0.2744 U/mL), BAD11 (0.2275 U/mL), and the smallest BAA3 (0.067 U/mL). Study by Nimisha *et al.* (2019) which isolated bacteria from soil resulted in highest amylase activity of 4.55 U/mL. The results of bacterial amylase activity isolated from the intestine of *Bombyx mori* averaged at $6.12 \pm 0.14 \times 10^5$ CFU/mL (Anand *et al.*, 2010). The results of amylase activity in these studies were lower, possibly due to the influence of incubation temperature during production of crude enzyme extracts, where the studies mentioned above incubated at 37°C. Temperature is one of the factors that affect the growth and production of metabolic substances by microorganisms, so that the optimum temperature will produce high amylase activity (Deb *et al.*, 2013).

The results of qualitative tests (Table 3 and Table 4) are not directly proportional to the results of quantitative tests (Table 4), as indicated by differences in the order of isolates with the highest amylase enzyme activity. This is thought to be because differences in the composition of the media used during qualitative test (soluble starch agar) and quantitative test (YPSs liquid containing yeast extract and peptone). Research by Swandi (2020) found that two of the three isolates of amylyolytic bacteria had amylyolytic index values that were not directly proportional to amylase activity (quantitative test results). According to Tanyildizi *et al.* (2007), water limitation can inhibit diffusion, cell metabolism will slow down due to lack of substrate. Although the clear zone produced is wider on solid media, the amount of amylase enzyme activity that plays a role in breaking down starch in the media is unknown (Melisha *et al.*, 2016).

Based on Table 6, the six isolates of amylyolytic bacteria were categorized as Gram-positive with varying shapes, motility test results, and catalase tests. Gram staining is one method of bacterial characterisation to determine cell morphology, such as Gram characteristics, cell shape, and cell arrangement (Yuniarty and Misbach, 2016). Bacteria with Gram-positive characteristics will have a purple color after Gram staining. Gram positive bacteria have a peptidoglycan layer thickness of 20 and 100 nm or even thicker than Gram negative bacteria and have lipoteichoic acid and teichoic acid located in the cell wall (Manfred, 2019). According to Hamidah *et al.* (2019), thick peptidoglycan is more capable of maintaining crystal violet color substances despite decolorization.

The characteristics of cell morphology in three isolates were coccus with Gram-positive characteristics and three other isolates were bacil with Gram-negative characteristics (Table 6). This is similar to Irsyadah and Santosa (2024), who found five out of the eight amylyolytic bacteria isolated had coccus and bacil cell shape with Gram-positive characteristics. Similar research by Novitasari *et al.* (2021) showed that amylyolytic bacteria characterized had morphological characteristics of coccus and bacil cell shapes with Gram-positive characteristics.

Catalase test in this study (Table 6 and Figure 4) shows that all positive isolates was able to produce catalase enzyme, characterised bubbles. The catalase enzyme functions to break down hydrogen peroxide into harmless compounds so that bacteria do not die (Zulfarina *et al.*, 2022). These results are in line with the research of Putri *et al.* (2023), which states that both isolates of selected amylyolytic bacteria positively produce catalase enzymes characterised by the formation of gas bubbles resulting from the reaction of bacteria that have been dripped by 3% H₂O₂ solution.

Motility test (Table 6 and Figure 5) shows that three isolates were motile (BAD6, BAA6, and BAA3), while three other isolates were non-motile (BAD4, BAD11, and BAA5). According to Yulvizar (2013) a positive motile test can be known by the growth of bacteria that spread around the isolate puncture marks, while a negative motile can be known from the bacteria that do not spread or only bring up one straight line of isolate puncture marks. The motility of bacteria can be characterized by the presence of flagella (Panjaitan *et al.*, 2020). The six selected bacteria in this study have motile and non-motile positive motility properties. Similarly, out of 14 amylyolytic isolates from Artha *et al.* (2019), six isolates were non-motile and eight others were motile.

Overall, the study's results indicate that the feces of Senduro adult goat and lamb contain 19 amylyolytic bacteria with varying enzymatic capabilities. The higher number and activity of isolates in adult goats indicate that age and diet composition influence the diversity and metabolic activity of the gut microbiota. Furthermore, the results of this study also show that the six selected bacterial isolates are all Gram-positive and catalase-positive, indicating their ability to adapt to environmental conditions and supporting bacterial metabolic activity.

CONCLUSION

Based on results of current research, it can be concluded that amylyolytic bacteria isolated from the feces of adult Senduro Goats and lambs have diverse results with as many as 13 isolates of amylyolytic bacteria originating from the feces of adult Senduro Goats (isolate BAD1, BAD2, BAD3, BAD4, BAD5, BAD6, BAD7, BAD8, BAD9, BAD10, BAD11, BAD12, and BAD13) and 6 isolates were lamb feces= (isolates BAA1, BAA2, BAA3, BAA4, BAA5). Quantitative test results using DNS method showed that the highest amylase activity was produced by isolate BAD4 (0.3683 U/mL) from adult goat, while BAA5 (0.3547 U/mL) from lamb.

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CONFLICT OF INTEREST

There is no conflict of interest.

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