CHARACTERIZATION OF CRUDE THERMOSTABLE CELLULASE FROM *GEOBACILLUS KAUSTOPHILUS* STRAIN SBKS0103 AND ITS APPLICATION AS AN ANIMAL FEED ADDITIVE

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Abstract

A thermophilic bacterium strain SBKS0103 was isolated from sediments of the Boeklueng hot spring in Thailand and identified as *Geobacillus kaustophilus* based on 16S rRNA and confirmed by phylogenetic analysis. The result showed that crude cellulase production was highest after 2 days on carboxymethyl cellulose (CMC) medium at 55°C. The most favorable conditions of the crude enzyme were found to be at pH 6 and a temperature of 70°C. The protein profile of crude enzyme exhibited the endoglucanase activity at a molecular weight of around 63 kDa as determined by SDS-PAGE and zymogram analysis. For stability, crude cellulase retained 75% of its activity at 40°C and 20% at 70°C after 2 h of incubation. Enzymatic digestion of animal feed ingredients (untreated cassava pulp or sorghum seed) was performed at 37°C for 24 h. At pH 3, the dry weight reductions of cassava pulp and sorghum seed were 7.10±0.21% and 10.34±0.17%, respectively, and at pH 6, they were 11.63±4.32% and 12.71±1.44%, respectively. Moreover, reducing sugar concentration increased gradually since 2 h of incubation which confirmed the enzymatic degradation. In conclusion, crude thermostable cellulase from *Geobacillus* sp. has the potential to be developed as an animal feed additive to facilitate lignocellulose digestion.

Keywords: **Animal feed, Cellulase, *Geobacillus kaustophilus*, Thermophilic Bacteria**

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Introduction

The global population reached approximately 8 billion in 2023 and is projected to reach close to 11 billion by the year 2100 (Worldometers, 2023), and as such, the efficiency of food production is crucial. According to Our World in Data (Ritchie *et al*., 2023), global meat production for human food has increased from 225 million tons to 331 million tons from 2000 to 2020. In the production chain, the animal feed industry, situated upstream, is important for meat and food quality. Various techniques such as utilizing enzymes and microbes (Gupta *et al*., 2021) or encapsulation of bioactive metabolites (Tolve *et al*., 2021) have been employed to enhance the nutritional content of livestock feed, aiming to optimize production yield.

Feed origins tend to exhibit remarkable diversities, with their composition subject to variation based on the particular species and age of livestock (Tolosa *et al*., 2021). Agricultural lignocellulosic products (e.g., wheat, corn, barley, sorghum, and vegetables) together with co-products (e.g., animal protein, brewer's yeast, molasses, and soybean meal) are examples of animal feed components. Most of them contain complex carbohydrates, including cellulose, hemicellulose, and pectin. Cellulose and hemicellulose, constituting around 70 %, represent the principal constituents of plant cell walls (Saini *et al*., 2015). Cellulose, a plentiful organic compound found on Earth, is a chain-like polymer made up of glucose units linked by β-1,4-glycosidic bonds. It is also a renewable substrate for food production, animal feed, textiles and nutrients source for herbivores (Patel *et al*., 2019). However, the pronouncedly structures crystalline arrangement of cellulose renders it resistant to digestion and hydrolysis (Haki and Rakshit, 2003). As a result, cellulose increases the viscosity of intestinal contents and has anti-nutritional effects in many animals. It adversely affects their health and, in turn, their performance (Pinotti and Dell'Orto, 2011), as their gastrointestinal tracts lack the appropriate enzymes to aid in the digestive process. Hence, the application of crude enzymes, including cellulases, β-glucanases, and xylanases, along with complimentary enzymes like phytases, proteases, and lipases in formulations, improves feed digestibility by hydrolyzing fiber that is not usually broken down by natural enzymes (Ojha *et al*., 2019). Cellulases hold significant importance both in industrial processes and the natural environment, as they play a pivotal role in the carbon recycling. Their primary function involves breaking down insoluble cellulose into soluble sugars by specifically targeting β-1,4-glycosidic bonds (Wilson, 2009). Various microorganisms, encompassing fungi and bacteria, synthesize cellulases during their growth on surface of the cellulosic materials. Additionally, thermophilic bacteria from such as *Acidothermus, Bacillus, Caldibacillus, Caldocellum, Clostridium* and *Geobacillus*, are cellulase producers that exhibited thermostability (Bhalla *et al*., 2013). In the production processes of animal feed, heat treatments are necessary to deactivate potential viral and microbial contaminants. One common method is steaming autoclaving at 121°C for 15-20 min. However, certain diet formulations may suffer negative effects at this temperature. Consequently, pasteurization, rather than sterilization, is frequently employed. Pasteurization typically entails subjecting the feed to a temperature of 107°C for 15-20 min in pasteurizer tank (Caulfield *et al*., 2008). As such, it is necessary to use thermostable cellulase to enhance the digestibility and nutrition of feed (Kuhad *et al*., 2011). From the previous report, a ruminant animal possesses multi-chambered stomach consisted of rumen, reticulum, omasum and abomasum diverticula. The physical variable of the rumen, the largest stomach with housing variety of microoraganisms, are pH at range of 6.0-6.4 and temperature at range of 37.5-42°C (Pérez-Barbería, 2020). To enhance enzyme cocktails for improved properties such as heightened activity, lower product inhibition and pH stability improvement, techniques like protein engineering and directed evolution can be employed (Bommarius *et al*., 2014; Contreras *et al*., 2020). Therefore, studying this cellulase structure and adopting these techniques to enhance enzyme thermostability is very promising.

The aim of the present is to determine the favorable conditions for the production of crude cellulase by strain SBKS0103, which was isolated from sediment in the Boeklueng hot spring (Phichitchaiphan and Euanorasetr, 2021). The investigation also includes an and examination of the optimal pH and temperature for the activity of the crude cellulase. Furthermore, the study involves conducting enzymatic degradation of animal feed components under simulated pH and temperature conditions resembling those found in the ruminant rumen, utilizing the crude enzyme.

Materials and Methods

Microorganisms and culture conditions

The strain SBKS0103 employed in this investigation was obtained from a sediment sample collected from the Boeklueng hot spring, Ratchaburi province. The bacteria were screened by spot on carboxymethyl cellulose agar (2 g/l carboxymethylcellulose sodium salt (Sigma), 2 g/l ammonium sulfate, 0.5 g/l magnesium sulfate heptahydrate, 1 g/l di-potassium hydrogen phosphate, and 17 g/l agar), The plates were flooded with gram's iodine and the calculated hydrolysis capacity (HC) as described in a previous study (Phichitchaiphan and Euanorasetr, 2021). The bacteria were enriched by growing in tryptic soy agar (TSA) (HiMedia) (17 g/l tryptone, 3 g/l soya peptone, 5 g/l sodium chloride, 2.5 g/l dextrose (glucose), 2.5 g/l di-potassium hydrogen phosphate, and 17 g/l agar; unadjusted pH) at 55°C for 24 h, aerobic, and were maintained in TSA at 4°C for morphological examination, DNA extraction and the preparation of crude cellulase.

Genomic DNA extraction and molecular identification

Genomic DNA extraction of strain SBKS0103 was performed using the heat treatment method (Dashti *et al*., 2009). The 16S rRNA gene sequence was amplified by polymerase chain reaction (PCR) with the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). A PCR reaction mixture with a volume of 20 µl was prepared. The components included 6 µl of distilled water, 10 µl of AccuPower PCR mastermix (Bioneer), 1 µl each of forward and reverse primers, 1 µl of dimethyl sulfoxide (DMSO), and 1 µl of DNA template. The PCR amplification involved an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 40 sec, annealing at 55°C for 1 min, extension at 72°C for 1 min 10 sec, and a final extension step at 72°C for 10 min. The PCR products, approximately 1500-bp in size, were visualized through agarose gel electrophoresis (1.5%) at 100 V for 30 min. Subsequently, the 1500-bp PCR products were purified using the AccuPrep PCR purification kit (Bioneer). The purified products were then submitted to U2Bio (Thailand) for DNA sequencing. The obtained 16S rRNA sequences were compared with those in the EzBioCloud databases (Yoon *et al*., 2017), and a phylogenetic tree was constructed using the neighbor-joining method in MEGA software version 10, with 500 bootstrap replicates (Kumar *et al*., 2018). The nucleotide sequence was subsequently deposited in GenBank under the accession number ON909194.

Preparation of crude enzyme

To prepare the inoculum, a pure culture of strain SBKS0103 was grown in tryptic soy broth (TSB) medium at 55°C for 18 h. One ml TSB culture was then inoculated into 100 ml CMC medium (2 g/l carboxymethylcellulose sodium salt (Sigma), 2 g/l ammonium sulfate, 0.5 g/l magnesium sulfate heptahydrate, and 1 g/l di-potassium hydrogen phosphate; sterilization at 121°C for 15 min) and incubated at 55°C for 5 days. The crude enzyme was prepared by centrifugation of whole culture broth at 5020g for 20 min at 4°C, followed by 0.2 µm Whatman cellulose acetate filtration. The cell-free crude enzyme for 2 days, kept at 4°C, was utilized for accessing enzymatic activity and conducting experiments on the degradation of animal feed in subsequent steps.

Measurement of cellulase activity

Cellulase activity, involving the hydrolysis of CMC, was assessed using the dinitrosalicylic acid (DNS) method developed by Miller (1959). Five hundred µl of crude enzyme was combined with 500 µl of 1% (w/v) CMC in a 100 mM sodium phosphate buffer at pH 7 and incubated at 50°C for 30 min (Ghose, 1987; Potprommanee *et al*., 2017). After the incubation, the reaction was terminated by the addition of DNS (10 g/l 3,5-dinitrosalicylic acid, 10 g/l sodium hydroxide (NaOH), and 192 g/l sodium potassium tartrate tetrahydrate) in a 1:1 (v/v) ratio, followed by boiling the mixture in a water bath for 5 min. Blank is in the same reaction without crude enzyme. The amount of reducing sugar released after cooling was measured by observing the optical density at 540 nm. Under the assay conditions, one unit (U) of cellulase activity was defined as the amount of enzyme-liberating 1 µmol of reducing sugar (glucose) per ml per minute. Glucose was employed as a standard, and all assays were conducted in triplicate.

SDS-PAGE and Zymogram analysis

The determination of the molecular weight of the crude cellulase was conducted through 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmil, 1970). Following electrophoresis, the gel was stained with Coomassie Brilliant Blue and subsequently de-stained using a mixture of glacial acetic acid, methanol, and water (1:1:8). For zymogram analysis, the polyacrylamide gel was mixed with 0.1 (w/v) CMC. Post-electrophoresis, the gel was

immersed in 100 mM sodium citrate buffer at pH 6 and incubated at 50°C for 10 min. The gel was then transferred to a 0.1% Congo red solution and stained with 1% sodium chloride until a clear zone became visible against a red background (Malik and Javed, 2021).

Effect of pH on cellulase activity of the crude enzyme

The optimum pH for crude cellulase activity was determined by incubating 500 µl of crude enzyme with 500 µl of the following buffers containing 1% (w/v) CMC: 100 mM sodium citrate buffer (pH 3-6), 100 mM sodium phosphate buffer (pH 7-8), and 100 mM glycine-NaOH buffer (pH 9). The reaction mixtures were subjected to incubation at 50°C for 30 min, and the reaction was stopped by boiling for 5 min. The quantification of the released reducing sugar was performed using the DNS method, as mentioned earlier. This was performed as three independent experiments, with all assays conducted in triplicate.

Effect of temperature on cellulase activity and thermostability of the crude enzyme

The optimal temperature for residual activity was determined by DNS method as already described in the previous section. Five hundred µl of crude enzyme was incubated with 500 µl of 1% (w/v) CMC in 100 mM sodium citrate buffer (pH 6) at different temperatures, increasing from 30 to 90°C in 10°C increments for 30 min. This was performed as three independent experiments, with all assays conducted in triplicate. For the evaluation of thermal stability, the crude enzyme underwent preincubation at different temperatures within the range of 30 to 90°C and was maintained in buffer pH 6 for 2 h. The activity of the crude enzyme was measured with the DNS method as previous mentioned. This was performed as three independent experiments, and all assays were conducted in triplicate. The percentage of relative activity was calculated as follow:

$$\text{Relative activity (\%)} = \frac{Reducing\ sugar\ of\ residual\ activity\ at\ each\ temperature}{Maximum\ reducing\ sugar\ of\ residual\ activity\ (30°C)} \times 100$$

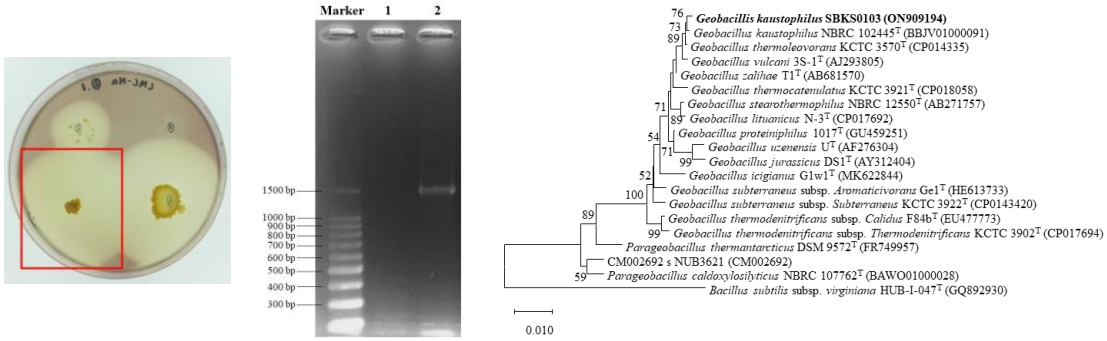
Enzymatic digestion of animal feed ingredients

Enzymatic digestion of the animal feed ingredients, cassava pulp (50 mesh) and sorghum seed (70 mesh), was studied. The ingredients were ground and dried at 105°C for 3 h. Twenty-five ml of crude cellulase, combined with 25 ml of 100 mM sodium citrate buffer (pH 3 or pH 6) containing 2.5 g of each substrate, was included in the reaction mixture. Buffer with no crude enzymes was used as a control. The reaction was performed at 37°C (reported temperature of remnant rumen) (Abdisa, 2017; Pérez-Barbería, 2020) for 24 h. The mixture was vacuum filtered and then dried at 60°C overnight prior to dry weight measurements. The dry weight reduction was quantified with deduction from that of control. To determine the releasing product in the mixture, reducing sugars concentration was measured using the DNS method every 2 h for 12 h comparing to that of the glucose standard. All assays were conducted in triplicate, and three independent experiments were performed.

Results and Discussion

Identification of thermophilic bacteria

Strain SBKS0103, isolated from sediments of the Boeklueng hot spring in Thailand, was chosen for further study to analyze cellulase activity as it had the highest HC on CMC agar in the previous study (Phichitchaiphan and Euanorasetr, 2021) (Figure 1(a)). From morphological investigation after incubation on TSA plates for 24 h at 55°C, SBKS0103 colonies had a circular form with yellow pigmentation, entire margins, and convex elevation and were approximately 1-2 mm in diameter. Moreover, the genus *Geobacillus* is characterized as thermophilic, neutrophilic, aerobic, or facultatively anaerobic. It grows at optimum temperatures between 55°C and 65°C, with an optimum pH ranging from 6.2 to 7.5 (Nazina *et al*., 2001). 16S PCR of strain SBKS0103 was successfully amplified with approximately 1500 bp (Figure 1(b)). The molecular identification of the SBKS0103 isolate using 16S rRNA sequence analysis showed that it was closely related to *Geobacillus kaustophilus* NBRC 102445^{T} (accession no. BBJV01000091) with 99.73% similarity by EzBioCloud analysis. The 16S rRNA sequence from this study was submitted to the GenBank database under accession number ON909194. Following phylogenetic analysis (Figure 1(c)), SBKS0103 was assigned to the same clade as *G. kaustophilus* NBRC 102445 and grouped with other members of *Geobacillus* sp. Based on these results, the isolate was designated as *G. kaustophilus* strain SBKS0103. A closely related strain, *G. kaustophilus* NBRC 102445, has been isolated from pasteurized milk (Priest *et al*., 1988). Moreover, for *Geobacillus* sp. HTA426 in soil samples from a hot spring in Rucheng Reshui Town, China (Potprommanee *et al*., 2017). These results show the genus *Geobacillus* its ability to grow at high temperatures. In addition, the genus *Geobacillus* is a well-known, promising collection of organisms in biotechnological processes and a source of various thermostable enzymes, including α-amylase, cellulase, lipase, and xylanase (Novik *et al*., 2018).



a **b** **c**

Figure 1. **(a) The cellulase activity of G. *kaustophilus* strain SBKS0103 on CMC agar plates within the area delineated by a red rectangle. (b) 16S PCR amplification from *G. kaustophilus* strain SBKS0103 on 1.5% agarose gel electrophoresis (lane Marker: 100 bp DNA Marker; lane 1: negative control; lane 2: *G. kaustophilus* strain SBKS0103). (c) Phylogenetic tree analysis of strain SBKS0103 (ON909194) and closely related strains using MEGA 10.0 with the neighbor-joining method and 500 bootstrap replicates. Values over 50% were shown at the respective node. Bar = 0.01 nucleotide substitutions per site.**

Cellulase production in CMC medium

The activity of the crude cellulase produced by *G. kaustophilus* strain SBKS0103 was determined by measuring the reducing sugar released during the 5-day incubation period. The results showed that its activity was highest on day 2 at 0.02 ± 0.01 U/ml and remained stable until day 5 (Figure 2(a)). The protein profile of the crude cellulase showed differences in molecular weight by SDS-PAGE. Activity staining using a native zymogram with CMC pointed out the presence of endoglucanase at approximately 63 kDa (Figure 3). This result was consistent with a previous study that 66 kDa cellulase from hot spring-derived *Geobacillus* sp. KP43 (Khadka *et al*., 2022). Additionally, cellulases from other groups of microorganisms, such as *Cellulomonas uda* (66 kDa) (Nakamura and Kitamura, 1983) and *Bacillus* sp. AC-1 (67 kDa) (Li *et al*., 2005), have been reported.

a **b**

Figure 2. **(a) The condition of crude cellulose reaction from G. *kaustophilus* strain SBKS0103 using CMC as a substrate. (b) The effect of pH (ranging from 3-9) on the crude cellulase activity was determined with CMC as the substrate at 50°C for 30 min. The enzyme activity measures the amount of reducing sugar released by the DNS method. All data are the means ± SD of three experiments (n=3)**

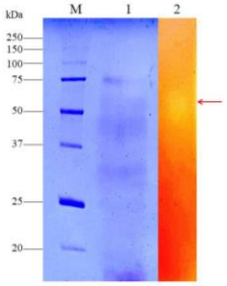


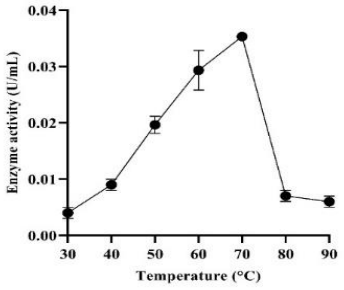
Figure 3. **SDS-PAGE and zymogram analysis of the crude cellulase from *G. kaustophilus* strain SBKS0103. Lane M: Precision Plus Protein™ All Blue Prestained Protein Standards; lane 1: crude cellulase; lane 2: zymogram of the cellulase activity of the crude enzyme.**

Effect of pH on crude cellulase activity

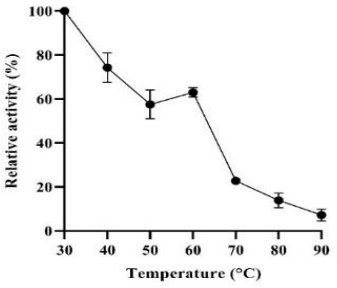
The effect of pH on the crude cellulase activity of *G. kaustophilus* strain SBKS0103 in CMC medium was determined at pH values ranging from 3 to 9, as shown in Figure 2(b). The optimum pH for enzyme activity was pH 6 in sodium citrate buffer. From the previous report, optimal pH of cellulase from *Geobacillus* was reported. The optimum pH for the highest activity of cellulase from *Geobacillus* sp. T1 is 6.5. (Assareh *et al*., 2012), while the maximum activities at pH 7 and 5 were found for *G. thermoleovorans* T4 and *Geobacillus* sp. WSUCF1, respectively (Tai *et al*., 2004; Rastogi *et al*., 2010). This information highlights the usefulness of broad pH range cellulase from *Geobacillus* spp.

Effect of temperature on crude cellulase activity and stability

The effect of temperature on the production of crude cellulase enzymes by *G. kaustophilus* strain SBKS0103 was determined at temperatures ranging from 30 to 90°C, as shown in Figure 4(a). The maximum cellulase activity was exhibited at 70°C, which decreased significantly at 80 and 90°C. Several previous reports have indicated that 70°C was the optimum temperature for cellulase activity from genus *Geobacillus*, such as that of *G. thermoleovorans* T4 (Tai *et al*., 2004) and *Geobacillus* sp. WSUCF1 (Rastogi *et al*., 2010). The thermal behavior of enzyme could be explained by protein folding or the enzyme structure, which provide protection against high temperatures. For instance, if denatured, the proteins refold to their original forms and restore their functionality (Haki and Rakshit, 2003). Additionally, the structure of cellulase contains large number of isoleucine, valine amino acids, which are hydrophobic elements. Hydrophobic interactions affect the thermal stability of the enzyme, so it is speculated that hydrophobic amino acids may enhance the high-temperature tolerance of cellulase (Li *et al*., 2008). The thermal stability of cellulase was investigated by heating crude enzymes for 2 h at temperatures between 30 and 90°C at optimum pH 6 before performing an activity assay. As shown in Figure 4(b), the relative enzyme activity at 40°C was retained at around 75%. At 50 and 60°C, the relative enzyme activity remained at 60%. At 70°C, the relative activity of cellulase decreased to 20%, and at the higher temperatures (80 and 90°C), more than 90% of the activity was lost. Tai and colleagues (2004) demonstrated that after 1 h of incubation at 70°C, the *Geobacillus* strain retained more than 90% of its cellulase activity, but at 80°C, the relative activity was reduced to 40%. The activity of cellulase from *Geobacillus* sp. WSUCF1 at 50 and 60°C was stable after 1 day, whereas at 70 and 80°C, the stability of cellulase activity decreased by 20% and 40%, respectively, and at 90°C, the enzyme activity completely diminished within 1 h (Rastogi *et al*., 2010).



a



b

Figure 4. (a) The effect of temperature (ranging from 30-90°C) on the crude cellulase enzyme activity on CMC medium at pH 6 for 30 min. The enzyme activity measures the amount of reduced sugar released by the DNS method. (b) The temperature to enzyme stability of the crude cellulase activity on CMC medium at temperatures of 30-90°C at pH 6 for 30 min. The enzyme activity measures the amount of reduced sugar released by the DNS method. The enzyme activity at 30°C is expressed as percentages of maximum activity (100%). All data are the means ± SD of three experiments (n=3)

Digestion of animal feed ingredients by crude cellulase

To evaluate the enzymatic digestion of animal feed ingredients, cassava pulp and sorghum seed were incubated with crude cellulase. Cassava and sorghum were selected in this study because of limited digestive experiments, especially on sorghum. Furthermore, there is a growing trend in countries that traditionally relied on sorghum as a food crop to now utilize it as a feed grain (Ronda *et al*., 2019). Each substrate was incubated with crude cellulase produced by the strain SBKS0103 at 37°C for 24 h, and sodium citrate buffer pH 3 and 6 were used as controls. These pH values were

selected to mimic the pH of the gastrointestinal tract; rumen pH typically ranges from 6.5 to 6.8, while the abomasum has an acidic pH of 3 due to the hydrochloric acid present in gastric juice (Valverde and Doherty, 2008). In addition, the small intestine has a pH of 4-8 (Bergmann, 2017). After 24 h, the dry weight reductions of cassava and sorghum at pH 3 were 7.10 ± 0.21% and 10.34 ± 0.17%, respectively, and at pH 6, the reductions were 11.63 ± 4.32% and 12.71 ± 1.44%, respectively (Figure 5(a)). To confirm the hydrolysis, the release of reducing sugar in the mixture was studied, and prolonged incubation times were observed from 2 h until 12 h in both pH 3 and pH 6 conditions. The result showed that at pH 6 the reducing sugar concentration of cassava kept increasing until 12 h (1.17 µg/ml), which is consistent with that of sorghum (0.85 µg/ml). In the case of pH 3, both substrates provided slightly increased and reached approximately 0.25 µg/ml (Figure 5(b)). This study has shown that crude cellulase from *G. kaustophilus* strain SBKS0103 is able to digest cassava pulp and sorghum seed by weight reduction and increasing concentration of reducing sugar. Several previous reports focused on alkaline-treated lignocellulolytic digestion for bioconversion to value-added products (e.g., ethanol and lactic acid) (Potprommanee *et al*., 2017; Mostafa *et al*., 2020; Rodhe *et al*., 2011). Alkaline pretreatments improve enzymatic lignocellulolytic saccharification by lignin removal at ether linkage (Rodhe *et al*., 2011; Kim *et al*., 2016). The pretreatment combination with lignocellulolytic enzymes improved the saccharification process and increased the release of the reducing sugar. In addition, the efficiency of the saccharification process depends on temperature, pH, enzyme, and substrate. (Cardoso *et al*., 2013; Ejaz *et al*., 2024; Rodhe *et al*., 2011). Rodhe and colleagues (2011) found that crude cellulase from *Trichoderma reesei* NCIM 992 to digest alkaline-treated sorghum straw displayed a hydrolytic efficiency of approximately 18% at 12 h of digestion. Our results from dry weight and reducing sugar concentration implied more efficiency of cellulase activity at pH 6 than at pH 3. Moreover, these results confirmed that these animal feed ingredients can utilize crude cellulase from *G. kaustophilus* strain SBKS0103.

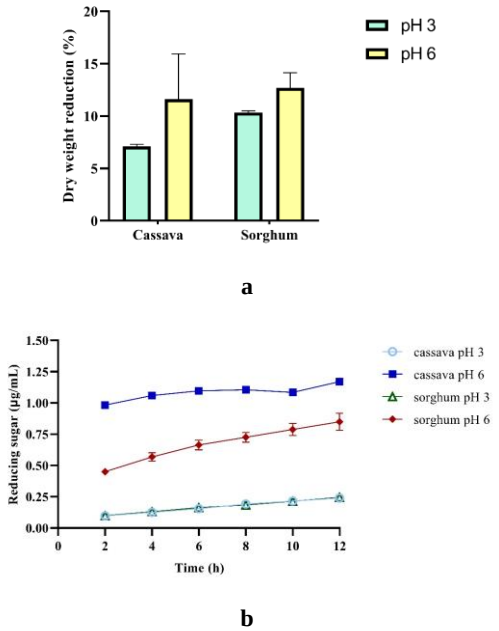


Figure 5. **(a) Percent weight reduction after 24 h enzymatic digestion normalized to the control (buffer only). (b) The concentration of reducing sugar released from animal feed ingredients mixed with crude cellulase enzymes from *G. kaustophilus* strain SBKS0103, incubated in sodium citrate buffer (pH 3 and 6) at 37 °C. All data are the means ± SD of three experiments (n=3)**

Conclusions

To summarize, the strain SBKS0103 was identified as *G. kaustophilus* SBKS0103 based on full-length 16S rRNA analysis and phylogenetic analysis. The optimum conditions for *G. kaustophilus* SBKS0103 to produce crude cellulase were 2 days on CMC medium at 55°C. The endoglucanase exhibited a molecular weight of around 63 kDa based on both SDS-PAGE and zymogram analysis. The enzyme activity was highest at pH 6 and 70°C. The enzyme remained stable at a wide pH range from pH 5-9 and retained 60% activity after preincubation at 40-60°C, demonstrating thermal stability. Crude enzymes could improve the degradation of cassava pulp and sorghum seed at both pH 3 and pH 6, with a 7.1-12.7% decrease in dry weight as well as an increase in releasing reducing sugar over time. Therefore, crude cellulase from *G. kaustophilus* SBKS0103 could be further developed as an animal feed additive to aid in lignocellulose digestion.

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