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Isolation and identification of bacteria with capability of starch, cellulose and protein decomposition from Frass of Black Soldier Fly (*Hermetia Illucens*) Larvae

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Abstract

This research isolated and identified bacteria with capability of starch, cellulose and protein decomposition from frass of black soldier fly (*Hermetia Illucens*) larvae (BFL). Dilute suspension of BFL frass was spread on petri dishes with solid Nutrient Agar for bacteria isolation. Selection and evaluation of bacteria with capability of starch, cellulose and protein decomposition was based on their ability of creating halo zone on starch agar, CMC and skim milk media. Identification of selected bacterial isolates was based on 16S rDNA sequences. There were 44 bacterial strains isolated from BSFL frass. Bacterial strains with the best capability of starch, cellulose and protein decomposition were CCRL21 and CCRL16 with halo zone radii of 1.5 cm and 0.8 cm; CCRL16, CCRL20 with halo zone radii with the similar size of 0.83 cm; and CCRL44 has the best ability of protein decomposition with an average halo zone radius about 2.47 cm, respectively. Six bacterial strains with good ability of degrading cellulose, starch and protein were identified as *Bacillus paramycoides* CCRL1; *Bacillus altitudinis* CCRL16, *Bacillus spizizenii* CCRL20, *Bacillus altitudinis* CCRL21, *Lysinibacillus pakistanensis* CCRL32 and *Paenibacillus silvae* CCRL43.

Keyword: Bacterial flora; black soldier fly; BSFL frass; halo zone; starch, cellulose and protein decomposition

1 Introduction

The black soldier fly (BSF), *Hermetia Illucens* had many uses and applications in practice. Black soldier fly larvae (BSFL) were commonly used to convert a variety of organic wastes such as food waste, animal carcasses and manure and agricultural waste to produce animal feed and fuel [1]. Black soldier fly powder was used to replace fish meal in aquaculture. Fresh larvae of the black soldier fly were used directly as food for fish, chickens, ducks and eels. Black soldier fly larval manure was used as an organic fertilizer in agriculture [2].

According to Klammsteiner et al., (2020) [1], gut microorganism of an organism was not only responsible for most of the metabolic processes associated with food ingest and digestion but also can strongly affect health and behavior. A stable gut microflora provided general metabolic competences for substrate decomposition and was against external disturbances such as diet changes or pathogens. Microbes in the gut could also substantially support nutrient uptake processes and affect the host immune system [3].

Therefore, several researches conducted to investigate the microorganism system in and on BSFL with purposes on better understanding of the BSFL microbial community composition and function. The dissected insect larvae gut or whole larvae was often used for microbiota analysis. A specific sample type selection depended on the purposes of the study. However, the microbiota composition of sample types could be different [4]. Researches of black soldier fly larvae gut microflora included antibacterial peptide active substances extraction [5], intestinal specific microbiota (Xie ei al., 2010) [6], conserved microbiota analysis [7], active enzymes analysis and identification [8], enhanced protein

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degradation by BSF and its gut microbes [9]. Most of researches of the BSFL gut microflora involved metagenomics, culture-independent molecular methods while studies by using culture-dependent methods provided direct evidences of the characteristics and roles of on BSF microflora were limited.

In order to understand more insight into characteristics and functions of the bacterial flora, especially, bacteria with good properties of organic matter degradation in black soldier fly, this research isolated and identified bacteria capable of degrading starch, cellulose and protein from faeces of Black soldier fly (*Hermetia Illucens*) larvae.

2 Material and methods

2.1 Materials

Fresh BSFL faeces samples were taken from the Black Soldier Flies farm located in Cu Chi dictrist, Ho Chi Min City, Vietnam, placed in a sterile container and sent to the laboratory for further studies.

2.2 BSFL faeces bacteria isolation

One gram of the fresh BSFL frass sample was suspended in 99 mL of sterile distilled water and shaken for 2 to 5 minutes [10]. The solution was thoroughly mixed and diluted with levels of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. 30 µL of samples at each dilution was then spread on petri dishes with solid Nutrient Agar (6 g peptone, 01 g beef extract, 2 g yeast extract, 5 g sodium chloride, 14 g agar, pH 7.3) [11]. These dishes were incubated aerobically at 30 ^{o}C for 72 hours. Discrete colonies were selectted and re-streaked nutrient agar plates for purification. Pure isolates were stored temporarily in agar slant tubes at 4 °C for further studies.

2.3 Morphological characterization of bacterial isolates

Morphology of colonies including form, elevation, margin, surface and size were investigated after 48 hours of cultures on solid LB media. Size and shape of bacteria cellular were observed by light microscopy. Gram of isolates were determined by the method as described by Nguyen et al., (2003) [12].

2.4 Investigation of the decomposition of organic matter

2.4.1 Preparation of bacteria suspension

A loop with the biomass of each pure strain was transfered to a test tube containing 5 mL of liquid LB medium, and incubate on a thermostatic shaker at 30 ^{o}C, 120 rpm. After 48 hours, the suspension of each isolate was collected and measured the cell density with a spectrophotometer at 600 nm and adjusted to the McFarland standard 0.5 corresponding to 1.5 x 10^{8} CFU/mL [13]. This standard cell density was applied to all following experiments.

2.4.2 Investigation of starch, cellulose and protein degradation ability

Media used to investigate starch, cellulose and protein degrading ability were starch agar, skim milk and *carboxyl methyl cellulose (CMC)*. Chemical composition of these media and reference sources were described as in Table 1. pH of the culture media was adjusted to reach the requirement. The media were solidified with 15 g agar per L.

Table 1 Chemical composition of culture media and experimental purposes

Media	Experimental purposes	Chemical composition/L	References
Starch agar	Decomposition of starch	3 g of meat broth, 5 g of peptone, 2 g of soluble starch, 15 g agar, pH 7.0	[14]
Skim milk	Decomposition of protein	5 g peptone; 3 g meat extract; 1 g yeast extract; 300 mL skim milk; 15 g agar, pH 6.5	[15]
CMC	Decomposition of cellulose	1 g (NH4)2SO4; 1g K2HPO4; 0.5 g MgSO4; 9 mg NaCl; 10 g CMC; 10 mL Congo red; 15 g agar, pH 6.8	[16]

Agar well diffusion method was used to evaluate bacteria's capability of degrading organic compounds [17]. Wells with a diameter of 6 mm were made on the agar plate. Each well was filled with 50 mL of the suspension of each bacterial

isolate (cell density 1.5×10^8 CFU/mL). Then, the agar plates were incubated at 28 ± 2 ℃ for 48 hours. The formation of halo rings was used to evaluate the results. Based the sizes of the halo zones, the ability of organic compound decomposition of bacterial strains was evaluated. The halo zone size was calculated by subtracting bacterial colony diameter from the halo ring and bacterial colony diameter [18]. The larger the halo zone diameter/radii, the greater bacteria's capability of degrading starch, cellulose and protein.

In case of evaluation of cellulose decomposition of bacteria isolates, at the end of incubation period, the agar plates were stained with Congo red dye 0.1% for 15 min, then washed with NaCl 1 M to clarify halo rings. For the starch hydrolysis assay, after incubation period, the plates were flooded with Lugol's iodine solution (5 g iodine and 10 g potassium iodide added into water up to 100 mL), kept for a minute and then poured off. Iodine reacted with starch to form a blue colored compound. The color less zone (halo zone) appeared surrounding bacteria colonies [19].

2.5 Identification of selected bacteria isolates

Bacterial strains expressed good results in the above tests were identified through 16S rRNA gene. 16S rRNA DNA of the selected bacteria was isolated and amplified by using primer pairs 27F and 1492R as described by Hoang and Cao [20]. PCR product was separated and visualized in 1% agarose gel using standard electrophoresis procedure. The satisfied PCR products were sequenced by 1st BASE Pte Ltd, Singapore (bi-directional sequencing). The sequencing results were processed by BioEdit software version 7.2 and compared with the reference sequences of the 16S rRNA genes in the GenBank of National Center for Biotechnology Information (NCBI) using Nucleotide BLAST tool. Query and reference sequences were applied to build up the phylogenetic tree by Neighbor-Joining method with bootstrap 1,000 using MEGA 11 software [21].

2.6 Experiment design and data analysis

The experiments were arranged in a completely randomized design with three replicates. Data for quantitative experiments was analyzed by using One-way ANOVA and comparisons of means were carried out based on Duncan's test at 5% level of confidence with the support of IBM SPSS Statistics software version 20.0.

3 Results and discussion

3.1 Morphology of bacterial strains isolated from BSFL frass

There were 44 bacterial strains isolated from BSFL frass, named as CCRL1 to CCRL44 and described morphological characteristics (Table 2). Colonies with circular shapes accounted for 86.6% and with irregular one accounted for 13.6%. Most of colonies were opaque white (43.2%), ivory white (20.5%) and brown (11.4%). 65.9% and 25% of colonies had entire and undulated margins, respectively. Percentages of colonies with raised and flat elevation were similar of 50%. Colonies' diameters were from 0.69 mm to 9.39 mm. In isolated bacterial strains, bacterial cells with short rod-shaped occupied 54.55%, with long rods accounted for 43.18%, and with sphere accounted for 2.27%. Percentages of Gram-negative and Gram-positive bacteria were similar, 54.55% and 45.45%, respectively.

Table 2 Morphology of bacteria colonies and cells

Isolates	Colonies					Cells		
	Color	Shape	Margin	Elevation	Size (mm)	Shape	Gram (-)	Gram (+)
CCRL1	Ivory white	Circular	Entire	Raised	2.48	Short rod		✓
CCRL2	Ivory white	Circular	Entire	Flat	4.05	Long rod	✓	
CCRL3	Ivory white	Circular	Entire	Flat	4.85	Short rod	✓	
CCRL4	Brown	Irregular	Rose	Flat	7.42	Short rod	✓	
CCRL5	Ivory white	Circular	Undulate	Raised	8.01	Short rod	✓	
CCRL6	Ivory white	Irregular	rose	Flat	7.71	Short rod	✓	
CCRL7	Transparent	Irregular	Undulate	Raised	2.08	Long rod	✓	
CCRL8	Opaque white	Irregular	Undulate	Raised	4.15	Long rod		✓

CCRL9	Opaque white	Circular	Entire	Flat	3.07	Long rod	✓	
CCRL10	Opaque white	Circular	Entire	Flat	4.25	Long rod		✓
CCRL11	Opaque white	Circular	Undulate	Raised	4.45	Long rod		✓
CCRL12	Brown	Circular	Entire	Raised	0.89	Short rod	✓	
CCRL13	Brown	Circular	Entire	Raised	1.19	Short rod	✓	
CCRL14	Opaque white	Circular	Entire	Flat	4.95	Short rod		✓
CCRL15	Ivory white	Circular	Entire	Flat	9.40	Long rod		✓
CCRL16	Opaque white	Circular	Undulate	Flat	0.89	Short rod		✓
CCRL17	Opaque white	Circular	Entire	Raised	0.69	Long rod	✓	
CCRL18	Ivory white	Circular	Undulate	Raised	3.26	Short rod		✓
CCRL19	Opaque white	Circular	Entire	Flat	4.66	Long rod		✓
CCRL20	Transparent	Irregular	Undulate	Flat	7.71	Short rod		✓
CCRL21	Opaque white	Circular	Entire	Flat	1.78	Short rod		✓
CCRL22	White	Circular	Entire	Raised	3.36	Long rod		✓
CCRL23	White	Circular	Entire	Raised	3.76	Long rod		✓
CCRL24	white	Circular	Entire	Flat	7.42	Long rod		✓
CCRL25	Opaque white	Circular	Undulate	Flat	2.87	Long rod		✓
CCRL26	Brown	Circular	Entire	Raised	4.05	Short rod	✓	
CCRL27	Brown	Circular	Entire	Raised	1.88	Short rod	✓	
CCRL28	Brown white	Circular	Entire	Raised	3.56	Short rod	✓	
CCRL29	Opaque white	Circular	Entire	Raised	0.99	Short rod	✓	
CCRL30	White	Circular	Entire	Flat	1.98	Short rod	✓	
CCRL31	Opaque white	Circular	Entire	Flat	7.12	Long rod		✓
CCRL32	Opaque white	Circular	Undulate	Raised	1.19	Short rod	✓	
CCRL33	Grey	Circular	Entire	Raised	2.57	Short rod		✓
CCRL34	Opaque white	Circular	Entire	Raised	2.37	Short rod	✓	
CCRL35	Transparent	Circular	Entire	Raised	0.99	Short rod	✓	
CCRL36	Ivory white	Circular	Undulate	Flat	2.47	Short rod	✓	
CCRL37	Opaque white	Circular	Entire	Flat	3.46	Sphere		✓
CCRL38	Ivory white	Circular	Entire	Raised	0.69	Short rod	✓	
CCRL 9	Opaque white	Circular	Entire	Flat	1.98	Long rod	✓	
CCRL40	Transparent	Circular	Undulate	Flat	1.48	Long rod		✓
CCRL41	Opaque white	Circular	Entire	Raised	2.27	Long rod	✓	
CCRL42	Opaque white	Irregular	Undulate	Raised	1.38	Short rod	✓	
CCRL43	Yellow white	Circular	Entire	Flat	2.97	Long rod		✓

CCRL44	Opaque white	Circular	Undulate	Flat	3.96	Long rod	✓	

3.2 Starch degradation ability

This section represented results of surveying the starch-degrading ability of 44 bacterial strains isolated from BSFL feces. 30 out of 44 bacteria strains accounting for 65.15% secreted starch-degrading enzyme in to the media to decompose starch in living processes. Thus, halo rings were created around colonies on starch agar medium (Figure 1).

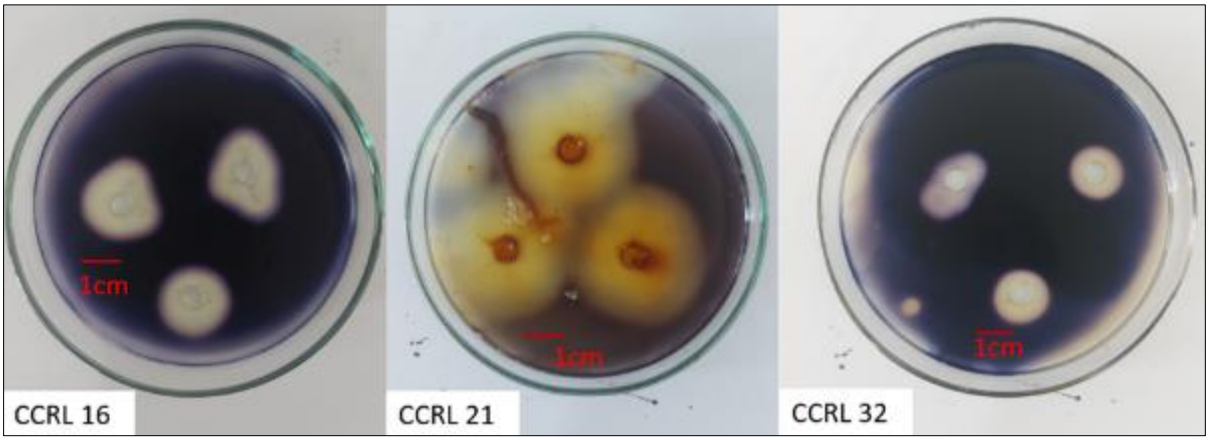

Figure 1 Halo rings of some bacterial strains in starch decomposition experiments

The starch-degrading ability of the bacteria through halo zone size was presented in Table 3. The average halo ring radii ranged from 0.07-1.5 cm. Among 30 bacterial strains with starch degradating ability, there were 5 strains produced the largest halo zones (radii ranged from 0.73-1.5 cm): CCRL3, CCRL4, CCRL22, CCRL16, CCRL21. Two isolates CCRL16 and CCRL22 showed the best ability to hydrolyze starch with the largest halo zone radius of 1.5 cm and 0.8 cm. The ability to break down starch by bacteria was also reported in some researches. Research by Mai et al. (2019) [22] isolated 28 bacterial strains from the guts of *Holotrichia parallela and Lubricus terrestris*. There were 27 strains capable of degrading starch. Sizes of clear zone were about 0.57 - 2.34 cm in diameter when stained with Lugol's solution. Anand et al. (2010) [23] isolated three species of cultivatable starch degrading bacteria *B. Circulans, S. liquefaciens* and *K. peumoniae* from gut of larva of *Bombyx mori* L. Amylase activity of *B. circulans* were higher than that of the other strains. This strain was present in greatest numbers in the gastrointestinal tract of fifth-year-old *B. mori larvas.*

Table 3 Halo zone size on the starch agar media produced by 44 bacterial strains

Isolates	Halo zone radius (cm)	Isolates	Halo zone radius (cm)
CCRL1	0.47 ± 0.153^{de}	CCRL23	0.07 ± 0.06^{ab}
CCRL2	0.53 ± 0.12^{e}	CCRL24	0 ± 0^{a}
CCRL3	0.73 ± 0.06^{gh}	CCRL25	0.05 ± 0.05^{ab}
CCRL4	0.73 ± 0.06^{gh}	CCRL26	0.07 ± 0.12^{ab}
CCRL5	0.4 ± 0.1^{d}	CCRL27	0.05 ± 0.05^{ab}
CCRL6	0.17 ± 0.06^{bc}	CCRL28	0.07 ± 0.03^{ab}
CCRL7	0 ± 0^{a}	CCRL29	0 ± 0^{a}
CCRL8	0.2 ± 0^{c}	CCRL30	0.07 ± 0.03^{ab}
CCRL9	0 ± 0^{a}	CCRL31	0.03 ± 0.06^{a}
CCRL10	0 ± 0^{a}	CCRL32	0.57 ± 0.21^{ef}
CCRL11	0.67 ± 0.06^{fg}	CCRL33	0.01 ± 0.01^{a}
CCRL12	0 ± 0^{a}	CCRL34	0.11 ± 0.08^{abc}

CCRL13	0.2±0.1^{c}	CCRL35	0±0^{a}
CCRL14	0±0^{a}	CCRL36	0.05±0.05^{ab}
CCRL15	0±0^{a}	CCRL37	0.04±0.01^{ab}
CCRL16	0.8±0^{h}	CCRL38	0±0^{a}
CCRL17	0.37±0.06^{d}	CCRL39	0±0^{a}
CCRL18	0.07±0.12ab	CCRL40	0±0 ^{a}
CCRL19	0.57±0.06ef	CCRL41	0±0 ^{a}
CCRL20	0.47±0.06de	CCRL42	0.02±0.03^{a}
CCRL21	1.5±0.1^{i}	CCRL43	0.13±0.06^{bc}
CCRL22	0.77±0.06^{gh}	CCRL44	0±0^{a}

Values in the same vertical column followed by one or more of the same letters are not significantly different at the 0.05 significance level according to Duncan's test.

3.3 Cellulose degradation ability

There were 36 out of 44 isolated bacteria strains (accounting 81.8%) showed their ability to degrade cellulose on CMC media. These isolates produced halo rings on the culture media after Congo red staining (Figure 2). Halo zone radii arranged from 0.033 to 0.833 cm (Table 4). Five strains with better capability of decomposing cellulose were CCRL16, CCRL20, CCRL43 and CCRL21. CCRL16 and CCRL20 produced the halo zones with the similar size of 0.833 cm, showing best ability of cellulose degradation in the above strains. The results were similar to findings of the previous studies. In the research on isolation of soil bacteria with cellulose decomposing ability, Mathmood et al., (2014) [24] reported that 57% of 42 bacterial isolated strains had capability of creating halo zone, where nine isolates produed halo zone radii from 0.7-0.8 cm. In addition, when surveyed cellulose decomposing capability of bacteria and fungi strains isolated from frass of termite collected in Binh Tan district, Vinh Long province, Vietnam, the researchers found out 28 bacterial strains being able to degrade CMC, while three strains with high CMC decomposition capability (calculated by percentage ratio of diameter of the bacterial colony to clear zone) were 3BTT6 (65.6%), 1BAL6 (61.7%) and 3BTT4 (59.7%) [25].

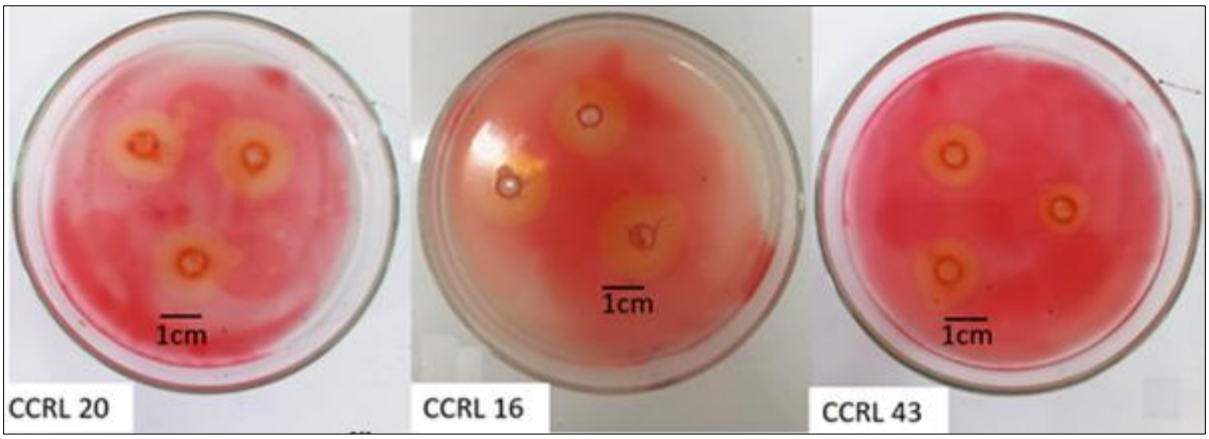

Figure 2 Halo rings of some bacterial strains in cellulose decomposition experiments

Table 4 Halo size on the CMC media produced by 44 bacterial strains

Isolates	Halo zone radius (cm)	Isolates	Halo zone radius (cm)
CCRL1	0.33±0.058^{ghi}	CCRL23	0.17±0.06^{bcdef}
CCRL2	0±0^{a}	CCRL24	0.03±0.06 ^{ab}
CCRL3	0.07±0.12^{abc}	CCRL25	0±0 ^{a}

CCRL4	0.27±0.12^{efghi}	CCRL26	0.17±0.06^{bcdef}
CCRL5	0.3±0.1^{fghi}	CCRL27	0.03±0.06 ^{ab}
CCRL6	0.03±0.06^{ab}	CCRL28	0.17±0.06^{bcdef}
CCRL7	0.37±0.0 ^{hij}	CCRL29	0.07±0.12^{abc}
CCRL8	0.23±0.06^{defgh}	CCRL30	0±0 ^{a}
CCRL9	0.08±0.03^{abcd}	CCRL31	0.17±0.06^{bcdef}
CCRL10	0.17±0.06^{bcdef}	CCRL32	0.33±0.12^{ghi}
CCRL11	0.17±0.06^{bcdef}	CCRL33	0.07±0.06^{abc}
CCRL12	0.4±0.2^{ij}	CCRL34	0.07±0.12^{abc}
CCRL13	0.07±0.06^{abc}	CCRL35	0.2±0.1^{cdefg}
CCRL14	0.13±0.06^{abcde}	CCRL36	0±0 ^{a}
CCRL15	0±0 ^{a}	CCRL37	0.05±0.0^{abc}
CCRL16	0.83±0.15^{k}	CCRL38	0.03±0.06^{ab}
CCRL17	0±0^{a}	CCRL39	0.17±0.06^{cdefg}
CCRL18	0±0^{a}	CCRL40	0.1±0^{bcd}
CCRL19	0.2±0.1^{cdefg}	CCRL41	0.13±0.06^{bcde}
CCRL20	0.83±0.15^{k}	CCRL42	0.13±0.06^{bcde}
CCRL21	0.4±0.1^{ij}	CCRL43	0.5±0.1^{j}
CCRL22	0.1±0.1^{abcd}	CCRL44	0±0^{a}

Values in the same vertical column followed by one or more of the same letters are not significantly different at the 0.05 significance level according to Duncan's test.

3.4 Protein degradation ability

After period of incubation, 42 out of 44 bacterial strains produced clear rings on the skim milk media, showing their capability of degrading proteins (Figure 3). Degree of proteolytic ability of 42 bacteria measured by radius size of halo zone arranged from 0.17 cm - 2.47 cm (Table 5). Six strains with good capability were CCRL44, CCRL41, CCRL5, CCRL22, CCRL15 and CCRL16; where CCRL44 has the best ability with an average halo zone radius about 2.47 cm. The five remained isolates produced halo zone with radii of 1.97, 1.97, 1.8, 1.57 and 1.57 cm, respectively.

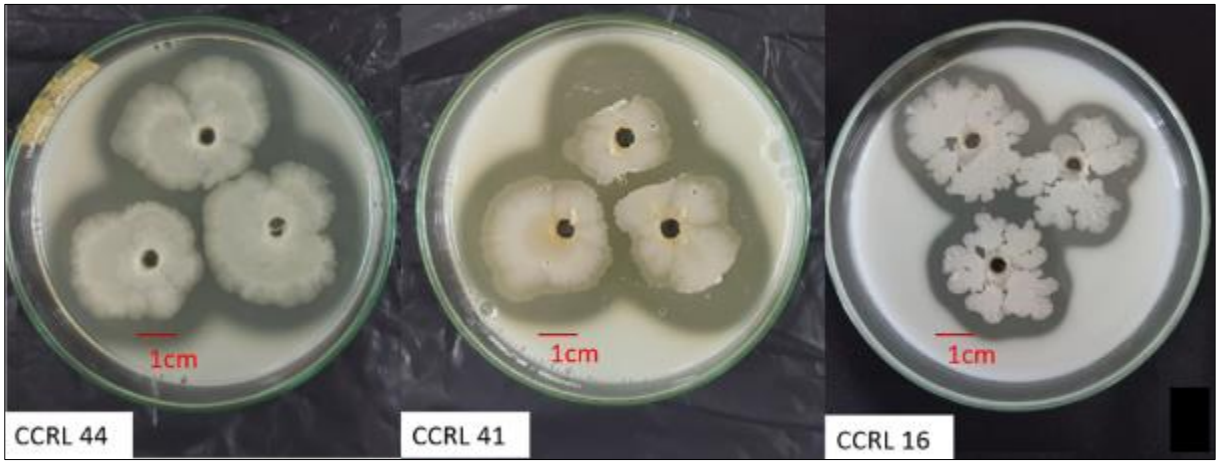

Figure 3 Halo rings of some bacterial strains in protein decomposition experiments

Table 5 Halo size on the skim milk produced by 44 bacterial strains

Isolates	Halo zone radius (cm)	Isolates	Halo zone radius (cm)
CCRL1	1.3±0.35^{hi}	CCRL23	0.33±0.06^{abcd}
CCRL2	1.13±0.25^{gh}	CCRL24	0.73±0.15^{defg}
CCRL3	0.57±0.46^{bcde}	CCRL25	1.23±0.21^{hi}
CCRL4	0±0^{a}	CCRL26	0.23±0.06^{ab}
CCRL5	1.97±0.15^{k}	CCRL27	0.17±0.06^{ab}
CCRL6	0.23±0.058^{ab}	CCRL28	0.43±0.06^{bcd}
CCRL7	0.9±0.44^{efgh}	CCRL29	0.45±0.06^{bcd}
CCRL8	1.07±0.15^{fgh}	CCRL30	0.33±0.06^{abcd}
CCRL9	0.73±0.06^{defg}	CCRL31	1.2±0.1^{hi}
CCRL10	1.03±0.15f^{gh}	CCRL32	1.03±0.46^{fgh}
CCRL11	0.73±0.12^{defg}	CCRL33	0.33±0.15^{abcd}
CCRL12	0.7±0.1^{cdef}	CCRL34	0.37±0.06^{abcd}
CCRL13	0.17±0.06 ^{ab}	CCRL35	0.37±0.06^{abcd}
CCRL14	1±0.1^{fgh}	CCRL36	0.3±0^{abc}
CCRL15	1.57±0.42^{ij}	CCRL37	0.27±0.06^{ab}
CCRL16	1.57±0.21^{ij}	CCRL38	0.5±0^{bcd}
CCRL17	0±0 ^{a}	CCRL39	1.2±0.26 ^{hi}
CCRL18	1±0.06f^{gh}	CCRL40	1±0.35^{fgh}
CCRL19	0.97±0.16^{fgh}	CCRL41	1.97±0.21^{k}
CCRL20	0.47±0.06^{bcd}	CCRL42	0.27±0.12^{ab}
CCRL21	1±0.1^{fgh}	CCRL43	0.93±0.55^{efgh}
CCRL22	1.8±0.26^{jk}	CCRL44	2.47±0.12^{l}

Values in the same vertical column followed by one or more of the same letters are not significantly different at the 0.05 significance level according to Duncan's test.

The bacterial strains that produced a lysis ring around the agar well demonstrate that they were capable of secreting protease enzymes to degrade the protein source in the medium. Proteolytic results in bacteria were also reported some researches. In order to utilize effective bacteria for degrading complex protein into simpler amino acids under optimum conditions, Gill et al. (2016) [26] isolated protein degrading bacteria from kitchen waste in India. Two out of six isolated bacterial strains (B1 and B2), showed effective protein degrading capability. Radii of halo rings on skimmed milk medium made by B1 and B were about 2.4 and 2.8 cm, respectively. Among 22 bacterial strains isolated from pepper plant roots in Binh Phuoc province, Vietnam, there were 20 strains with proteolytic ability with halo zone radii reaching from 0.4 to 1.94 cm [19]. In 46 bacterial strains capable of secreting protease isolated from organic waste collected from markets, restaurants and households in Can Tho City, ten isolates created halo rings with highest protein decomposing index calculated by ratio of combined colony and halo zone diameter to colony diameter from 3.0-3.29 [27].

3.5 Identification results of selected bacteria

Based on the good ability of cellulose, starch and protein decomposition, six bacterial strains CCRL1, CCRL16, CCRL20, CCRL21, CCRL32, CCRL43 were selected for identifying through 16S rRNA gene. The results of comparing the selected bacteria's sequences with the reference sequences of the 16S rRNA genes in the GenBank of National Center for Biotechnology Information (NCBI) using Nucleotide BLAST tool were summarized in Table 6. Apart from CCRL 16 and CCRL43 with Query Coverage about 56% and 83%, respectively, four remained trains all had Query Coverage from 92 to 100%, respectively. The 16S rDNA sequences of all selected bacterial isolates showed matching with the closest

BLAST sequence greater than 88%. BLAST results were confirmed by the following phylogenetic tree (Figure 4). Based on that six bacterial strains belonged to three genera: Bacillus, Lysinibacillus and Paenibacillus; and were identified as *Bacillus paramycoides* CCRL1; *Bacillus altitudinis* CCRL16, *Bacillus spizizenii* CCRL20, *Bacillus altitudinis* CCRL21, *Lysinibacillus pakistanensis* CCRL32 and *Paenibacillus silvae* CCRL43.

Table 6 Identification results of six selected strains by Blastn tool in the NCBI database

Strains (query sequence)	**Query Cover**	**Per. Ident**	**Accession**	**Reference strain names**
CCRL1 (4697590)	92%	97.76%	NR_157736.1	*Bacillus tropicus* strain MCCC 1A01406 16S ribosomal RNA, partial sequence
	92%	97.76%	NR_157734.1	*Bacillus paramycoides* strain MCCC 1A04098 16S ribosomal RNA, partial sequence
CCRL16 (4697592)	56%	88.56%	NR_118439.1	*Bacillus aerius* strain 24K 16S ribosomal RNA, partial sequence
	56%	88.56%	NR_042337.1	*Bacillus altitudinis* 41KF2b 16S ribosomal RNA, partial sequence
CCRL20 (4697593)	99%	99.09%	NR_112686.1	*Bacillus spizizenii* strain NBRC 101239 16S ribosomal RNA, partial sequence
	99%	99.01%	NR_115282.1	*Bacillus halotolerans* strain CR-95 16S ribosomal RNA, partial sequence
CCRL21 (4697594)	100%	95.17%	NR_118441.1	*Bacillus stratosphericus* strain 41KF2a 16S ribosomal RNA, partial sequence
	100%	94.81%	NR_042337.1	*Bacillus altitudinis* 41KF2b 16S ribosomal RNA, partial sequence
CCRL32 (4697596)	99%	96.97%	NR_113166.1	*Lysinibacillus pakistanensis* strain NCCP-54 16S ribosomal RNA, partial sequence
	99%	97.62%	NR_042073.1	*Lysinibacillus sphaericus* strain DSM 28 16S ribosomal RNA, partial sequence
CCRL43 (4697597)	83%	93.01%	NR_156979.1	*Paenibacillus intestini* strain LAH16 16S ribosomal RNA, partial sequence
	83%	94.74%	NR_156836.1	*Paenibacillus silvae* strain DB13031 16S ribosomal RNA, partial sequence

Bacteria flora of BSF were diverse but dependent on diets. Gorrens et al., (2021) [28] in research of isolation and identification of dominant bacteria from black soldier fly larvae envisaging practical applications isolated 172 bacteria from the highest serial dilutions of BSFL extract. They were belonged to either the Proteobacteria (66.3%), the Firmicutes (30.2%), the Bacteroidetes (2.9%) or the Actinobacteria (0.6%). In which, twelve genera collected with the most abundantly present ones were Enterococcus (29.1%), Escherichia (22.1%), Klebsiella (19.8%), Providencia (11.6%), Enterobacter (7.6%) and Morganella (4.1%). In a molecular survey of bacterial species in the guts of black soldier fly larvae reared on two urban organic waste (chicken manure (CM) and kitchen waste (KW)) in Kenya, based on both culture-dependent sequence-based and 16S rDNA amplification analysis, it was found that *Providencia* sp. was the most dominant bacterial species detected from the guts of BSFL fed on CM and KW. *Morganella* sp., *Brevibacterium* sp. were found from the exact of BSFL reared on CM and while *Staphylococcus* sp. and *Bordetella* sp. were detected in KW [29]. When screened and isolated bacteria from the gut of Black Soldier larvae treated rice straw feed, Supriyatna, and Ukit (2016) [30] found that six out of nine cellulose degrading bacteria belonged to *Bacillus*, where *Bacillus* sp. had highest ability of cellulose degradation. In another study, it found that protein decomposing bacteria of intestinal bacteria of Gnotobiotic BSFL created by incubating germ free BSFL with BSFL gut fed on a high-protein artificial diets were diverse including *Pseudomonas* spp., *Orbus* spp., *Campylobacter* spp., *Dysgonomonas* spp., *Issatchenkia* spp., *Campylobacter* spp., *Pediococcus* spp., *Lactobacillus* spp., *Lactobacillus* spp., and *Bacillus* spp., and *Issatchenkia* spp. [9].

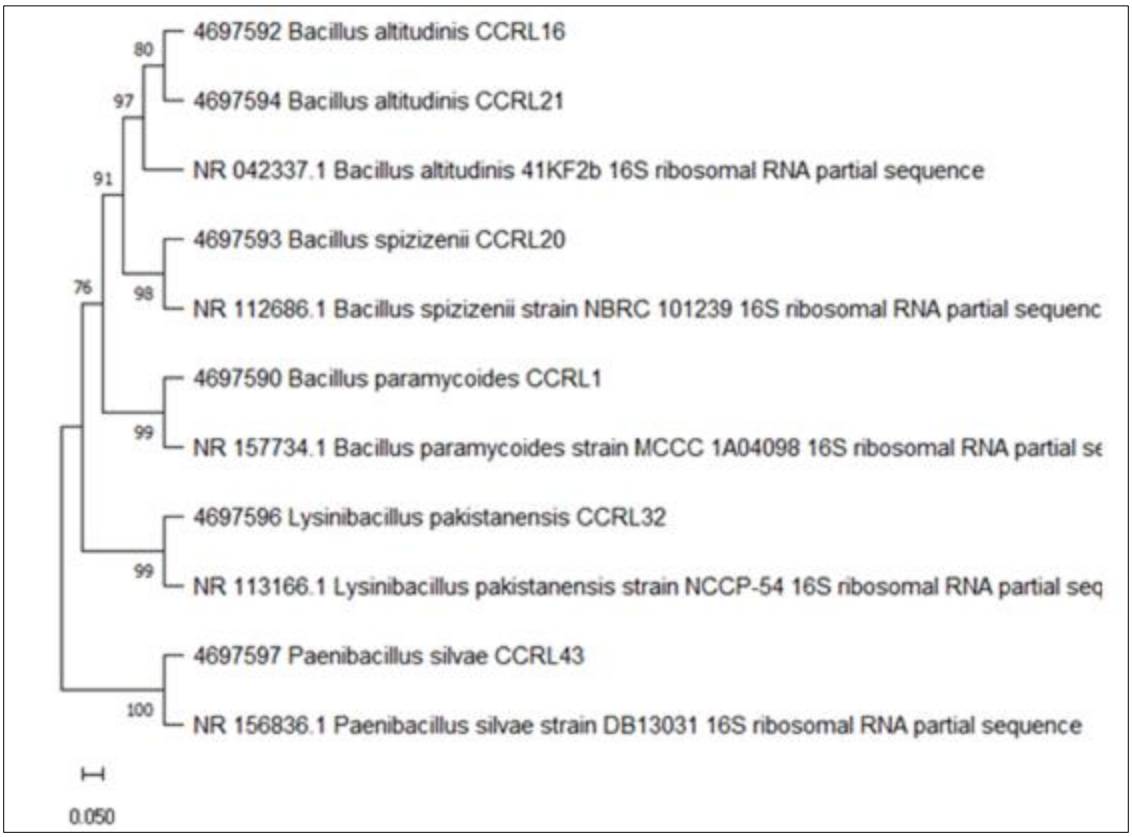

Figure 4 The phylogenetic tree showed the relative positions of six selected bacterial strains with reference strains in the GenBank, with bootstrap of 1,000

4 Conclusion

There were 44 bacterial strains isolated from BSFL frass. 30 out of 44 bacteria strains were able to decompose starch, where two isolates CCRL21 and CCRL16 showed the best results with the largest halo zone radius of 1.5 cm and 0.8 cm, respectively. 36 out of 44 isolates were of ability to degrade cellulose, in which, CCRL16 and CCRL20 produced the largest halo zone radii with the similar size of 0.833 cm. 42 out of 44 bacterial strains showed their capability of degrading protein. Six strains with good capability were CCRL44, CCRL41, CCRL5, CCRL22, CCRL15 and CCRL16; where CCRL44 has the best ability with an average halo zone radius about 2.47 cm. Six bacterial strains with good ability of degrading cellulose, starch and protein were identified as *Bacillus paramycoides* CCRL1; *Bacillus altitudinis* CCRL16, *Bacillus spizizenii* CCRL20, *Bacillus altitudinis* CCRL21, *Lysinibacillus pakistanensis* CCRL32 and *Paenibacillus silvae* CCRL43.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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