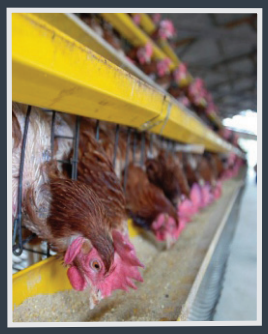
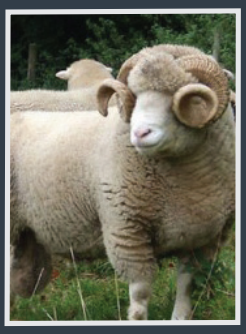
The 7th INTERNATIONAL SEMINAR ON TROPICAL ANIMAL PRODUCTION

"Contribution of Livestock Production on Food Sovereignty in Tropical Countries"

PROCEEDINGS





September 12 – 14, 2017
Yogyakarta,Indonesia

ISBN: 978-979-1215-29-9

Organized by :
Faculty of Animal Science, Universitas Gadjah Mada Yogyakarta
Indonesian Society for Sustainable Tropical Animal Production [ISSTAP]
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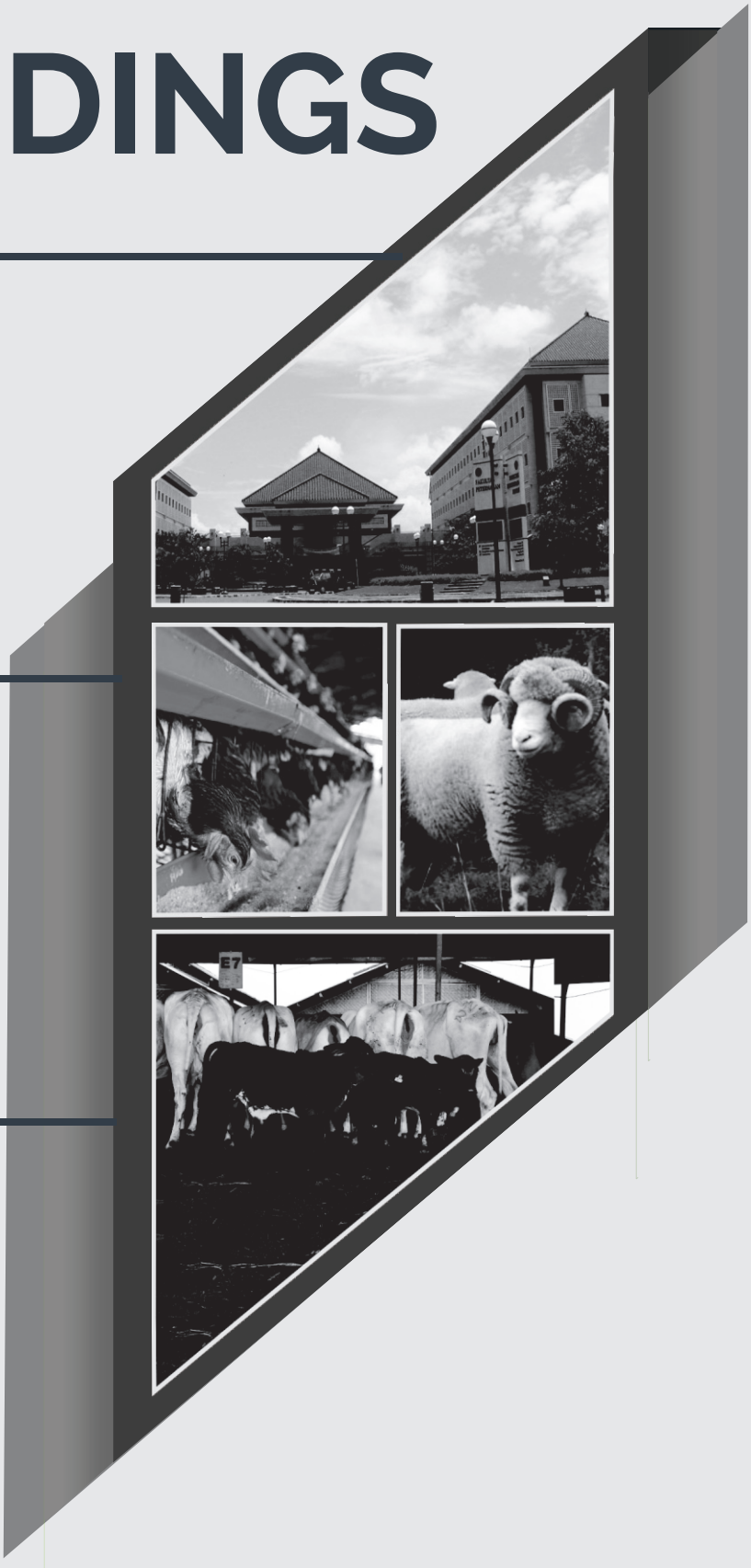

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PREFACE

On behalf of Faculty of Animal Science, Universitas Gadjah Mada, I am pleased to present you the 7th International Seminar on Tropical Animal Production (ISTAP) which is held on September 12-14, 2017 at Auditorium Drh. R. Soepardjo, Faculty of Animal Science UGM, Yogyakarta. Under the main theme "Contribution of Livestock Production on Food Sovereignty in Tropical Countries", we expect that information and ideas on animal production systems in the tropics and its related problems will be shared among participants, thus we can elaborate an integrated approach in developing sustainable tropical animal production. I believe, this can be achieved since more than 200 animal scientists, researchers, students, and producers from more than 10 countries join this seminar.

In this moment, I have to address my great thanks to all people who have contributed for the success of this seminar. First, to all participants, thank you for your contributions, time, and efforts in participating in all sessions in this seminar. We also would like to extend our gratitude to the reviewers and editors for dedicate their expertise and precious time in reviewing and editing the papers. I deeply appreciate the hard work of all members of the Steering Committee, Organizing Committee, and students of Faculty of Animal Science UGM for making this seminar achieved a great success!

I hope all of you enjoy the seminar and Jogja as well!

Dr. Cuk Tri Noviandi

Editor in Chief

REPORT FROM ORGANIZING COMMITTEE

Dear all scientists, delegates, participants, ladies and gentlemen,

Praise to The Almighty for His Merciful and Beneficent to gather us in this memorable moment of scientists and delegates from all over the world who are interested in Tropical Animal Production field can meet up together.

On behalf of the Board of Committee, it is my great pleasure and honor to welcome all participants to attend the 7th ISTAP in Yogyakarta, the city where nature, culture and people live in harmony.

As a chair in this seminar, let me report that, today, we have distinguished participants from all over the continents in the world to present their paper with the theme of "Contribution of Livestock Production on Food Sovereignty in Tropical Countries". There are around 250 scientists, delegates, and graduate students from 11 countries attending the seminar; and more than 170 research papers will be presented during these three days seminar. The great enthusiasm of all participants to share their research-based valuable information and knowledge on livestock production development in tropical areas as well as to contribute on developing human prosperity all over the world is expressed.

The 7th ISTAP programs are rich of scientific programs as well as social and cultural activities. The scientific programs offer six plenary sessions, eight parallel sessions (both oral and poster presentation) each day, and rural field trip. The social and cultural programs of the 7th ISTAP are also important as the scientific programs since the scientists' interaction, intercultural exchange, friendship and future scientific or research collaboration are also central to this seminar. In the evening, participants will attend a warm invitation from the Dean of Faculty of Animal Science UGM in a Welcome Dinner that will give you the most impressive moment to attend. Rural field trip activity offers a wonderful experience to the rural livelihood surrounded by the spectacular natural landmark, Ancient Volcano in Yogyakarta where many smallholder farmers live in harmony. We will also accompany all participants to experience the ancient civilization by enjoying the beautiful of Prambanan temple. We do hope that participants will take part of these wonderful opportunities.

During the seminar, the 7th ISTAP committee also creates a competitive atmosphere among all participants by granting awards for those who have outstanding paper and poster. Participants are encouraged to share their precious works in research and knowledge dissemination in an attractive way. The awards will be given to the outstanding participants immediately after the last session of parallel presentations where the closing ceremony will also be held on September 13th, 2017 afternoon. I wish all of the participants enjoying activities that we have organized.

Finally, on behalf of 7th ISTAP Committee, let me express the high appreciation and acknowledgement to the Rector of Universitas Gadjah Mada and Dean of Faculty of Animal Science UGM for the advice and suggestion in organizing this international seminar. Recognition should go to the Steering Committee, Scientific Committee, Reviewers and Editorial Boards and All Technical Committee members who have worked extremely hard for the details of important aspects of the seminar programs.

Terima kasih (Thank you).

Sincerely Yours,

R. Ahmad Romadhoni Surya Putra, Ph.D
Chairman
The Organizing Committee of the 7th ISTAP

WELCOME ADDRESS

Selamat pagi, Good morning, and *Assalamu'alaikum Wr. Wb.*

The honorable Rector Universitas Gadjah Mada, Invited Speakers, all of delegates, distinguished guests, participants, ladies and gentlemen.

First of all, it is our great pleasure and honor to extend a warm welcome to all of you at The 7th International Seminar on Tropical Animal Production (ISTAP), which be held on September 12 - 14, 2017 at Auditorium Drh. R. Soepardjo, Universitas Gadjah Mada, Yogyakarta, Indonesia. This seminar is proudly organized by Faculty of Animal Science Universitas Gadjah Mada, every 4 years since 1994. But, since last two years (2017) ISTAP has been conducting for every two years in collaboration with the Indonesian Society for Sustainable Tropical Animal Production (ISSTAP). We consider due to the rapid development of science and technology in animal production and also the need for exchange knowledge and experiences among the stakeholders, this scientific event is conducted for every two years.

The contribution of this seminar to the development of national food security is truly significant for introducing of new scientific knowledge and equipment that is much needed in Indonesia to maintain a safe and secure environment and to look at more effective ways to meet and anticipate the future challenges. We can see great enthusiasm of the entire participant to present their latest research finding as well as to share valuable information and knowledge for human prosperity all over the world.

In these 3 days of seminar, we have invited some important distinguished speakers for the plenary session and invited papers relevant to the animal production challenges for sharing their valuable information and knowledge. Other participants from over 11 different countries and from research institute and/or universities can deliver their precious research through oral and poster presentations at concurrent sessions.

At this opportunity, we would like to express our special thank you to the Steering Committee, Scientific Committee, Reviewers and Editorial Boards for their great contribution to make the seminar a great success. Also, we would like to congratulate and deliver high appreciation to the Organizing Committee as the organizer for their great contribution and generous efforts to make the seminar successfully organized. We are really indebt to your valuable time, effort and sacrifice to the success of this seminar.

To all of the participants, I do hope this seminar will enrich you with the new perspective of recent knowledge and of course with new friends for possible future partnership and collaboration in fostering the advancement of animal science. Also, I wish to all of the participants having a great achievement of success and fulfill the expectation as well as enjoying the interaction with all participants. Surely, with all of our hospitality, we have been trying our best to make your brief visit to our country become a wonderful and memorable moments. We are looking forward to meeting you in the future event.

Finally, we wish you all a very pleasant and most enjoyable stay in Yogyakarta, Indonesia, beside you scientific journeys.

Thank you very much for your attention, *Terima kasih, Wassalamu'alaikum Wr. Wb*.

Yogyakarta, 12 September 2017

Sincerely yours,

Prof. Dr. Ali Agus
Dean Faculty of Animal Science UGM

OPENING REMARKS

Dear Excellencies, Distinguished Delegates, Ladies and Gentlemen,

It gives me great pleasure to extend you all a very warm welcome on behalf of Universitas Gadjah Mada. We highly appreciate your participation in joining the 7th International Seminar on Tropical Animal Production hosted by the Faculty of Animal Science Universitas Gadjah Mada in Yogyakarta from 12-14 September 2017.

The theme of this conference is Contribution of Livestock Production on Food Sovereignty in Tropical Countries. We hope that this seminar will provide a perspective and insight into tropical livestock production systems and sustainable local resources management contribution in food sovereignty, also give a forum in order to exchange information and ideas on livestock production systems in the tropics and its related problems.

Food Sovereignty is a comprehensive concept which involves not only guaranteed access to food, but also to define their own food compatible with local resource potentials which may ensure food appropriateness and sufficiency. In the Livestock Production, Indonesia and other tropical countries have a variety number of livestock genetic resources and animal biodiversity. Those can be potential assets and capital to gain advantages in domestic and global market. However, achieving food sovereignty need a synergy to work together among government, people, farmer, researcher, and academia. These three days seminar denote those synergy among stakeholders in food sovereignty. We believe that challenges to realize the food sovereignty in tropical countries will be discussed; and technical solution as well as recommendation will be provided to solve the existing problems in tropical animal production.

Finally, on behalf of Universitas Gadjah Mada, we would like to congratulate and appreciate to the Faculty of Animal Science, UGM as the organizer for their great efforts to make the seminar successfully organized. To all of participants, I wish all of you have a very fruitful, dynamic and constructive seminar also great discussion and interaction with other scientists participating in the seminar as well as enjoying your time in Yogyakarta.

Thank you

Rector of Universitas Gadjah Mada
Prof. Ir. Panut Mulyono, M.Eng.,D.Eng

Isolation of Bacteria Producing Enzyme Collagenase From Waste of Pufferfish (*Arothon reticularis*) Skin

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ABSTRACT

Puffer fish is one of the waste fisheries catch. It is not only can be a waste of fisheries that is difficult to degrade, but also can be used as a source of collagenolytic bacteria.The objective of this research was to obtain bacterial isolates from the waste of puffer fish skin (*Arothon reticularis*) as one of the sources of collagenases. Sample from puffer fish skin waste was inoculated in enrichment media and colonies producing clear zone in skim milk agar were selected and identified as *B. cereus* BRAW_KM. Medium optimization to grow the selected collagenase producing bacterial strain was checked with various parameters such as temperature (at 31 and 33ºC), pH (8 to 9), substrate concentration (15 g/L), osmotic pressure (4%), inoculum concentration (8%), and agitation speed (100 to 120 rpm). The bacteria produced extracellularly collagenase enzymes in enrichment media and its collagenase activity was 1,029 U/mg.

Keywords: Bacteria, Collagenase, Isolation, Puffer fish skin, Waste

INTRODUCTION

It is estimated that the potential of Indonesia's marine fisheries to be sustainable reaches 6.4 million tons per year that spread over Indonesian territorial waters and the Exclusive Economic Zone with an allowable catch of up to 5.12 million tons per year or about 80% of the sustainability potential. This potential comprises one of the opportunities to increase fish production, both for capture fisheries and culture fisheries. According to Data from the Directorate of Product Processing (2006), it has been estimated that there are 28,400 species of fish in the world, and in Indonesian founded more than 25,000 species. Nevertheless, only a few of them fit for consumption, i.e. by 1-5% and are used as ornamental fishes, i.e. by less than 1%, while the rest is predicted to play a role in the food chain system in aquatic ecosystems. Puffer fish are one of the fisheries waste from the catch that can still be used because they are rich in proteases, which among others serve as a source of enzyme collagenase.

Collagenases are a hydrolytic enzyme which can be used for multiple purposes and applications for industrial needs, medicine, and research. This enzyme in fisheries processing is used in fish tanning, membrane removal, and protein hydrolysates (Bjarnason 2001). Some researchers have isolated collagenases from fishes, such as from pyloricaeca of yellowtail fish (Shahidi and Botta 1994), mackerel, the pancreas of catfish *Parasilarus asotus* (Kim et al., 2002) and digestive organs found in 19 fish species (Shahidi and Botta 1994).

Among the components of fisheries waste is the skin. In general, the skin contains many proteins in the form of collagen fibers that have substantial grace power. Collagen is a connective tissue consisting of collagen fibers and elastin containing polysaccharides as well as various organic and inorganic components. Collagen has a unique amino acid composition. About one-third of the amino acids contained is glycine, 6-10% of them is hydroxyproline, and 10-12% of them is proline (de Man, 1997). Its main characteristics are its triple-helical conformation and its amino acid content, in which the amount of hydroxyproline residues present is much higher than the amount found in other proteins existing in nature due to their rigid structure, and only a limited number of proteases may cleave collagen, such as collagenolytic proteases or collagenases (Harrington, 1996). Collagenases are a hydrolytic enzyme which can be used for multiple purposes and applications for industrial needs, medicine, and research. This enzyme in fisheries processing is used in fish tanning, membrane removal, and protein hydrolysates (Bjarnason, 2001). Some researchers have isolated collagenases from fishes, such as from pyloricaeca of yellowtail fish (Shahidi and Botta 1994), mackerel, the pancreas of catfish *Parasilarus asotus* (Kim et al., 2002) and digestive organs found in 19 fish species (Shahidi and Botta 1994).

Considering the importance of all of those things describes, collagen hydrolisate production from fish waste source enzymatically as an effort to handle enviromental problem and to increase economicalvalue is important to perform. Therefore, this study was aims to obtain bacterial isolates from the waste of pufferfish (*Arothon reticularis*) skin as one of the sources of enzyme collagenase.

MATERIALS AND METHODS

Bacteria isolation. The materials used in the present study were 30 sheets of pufferfish skin that underwent 30 days of decay. The growth medium for the isolation process was based on Macedo et al. (2005) with several modifications added, namely pufferfish skin flour as a single carbon and nitrogen source by 10 g; minimum minerals that consisted of NaCl by 0.5 g; K_2HPO_4 by 0.3 g; and KH_2PO_4 by 0.4 g. The medium for stock solution consisted of 1 g of Yeast extracts, 1 g of biological Peptone, 0.5 g of NaCl, and 100 ml of Aquadest.

Collagenase activities. Based on the modified method proposed by Pilai and Archana, (2008), collagenase activities were examined using 0.2 ml of coarse enzymes dissolved in the pure collagen solution by 0.4 mg (Sigma-Aldrich, St. Louis, USA) in 0.4 ml of the 50 mM Tris-Cl buffer solution at pH 8. The reaction was incubated at 30°C for 30 minutes. The enzyme reaction was discontinued using 0.6 ml of 15% TCA and afterwards stored in ice for 15 minutes. The solution was centrifuged at 12,000 g for 10 minutes. The absorbance of supernatants was measured at a wavelength of 520 nm. A unit of enzyme collagenase is defined as the quantity of enzymes that can increase absorbance by 0.01 according to the test conditions.

RESULTS AND DISCUSSION

Collagenase Activities in Bacteria

Collagenases are a type of enzymes with the ability to degrade collagen. Generally, they are defined as an enzyme capable of degrading polypeptide bonds. This enzyme is classified into two different types based on its physiological function. Serine collagenases take part in hormonal production and pharmacological activities. These functions include protein digestion, blood clotting, fibrinolysis, complex activation, and fertilization (Neurath 1984; Park et al. 2002). Based some founded isolate from the research, isolates with the highest collagenase activities was BRAW_KM by 1,029 U/mg.

Morphological Identification

Observation made on the morphology of bacterial colonies included their shape, edges, internal structure, elevation, and color. The obtained isolate showed the following properties: nonmotile, oxidase-positive, and catalase-positive. There were numerous Gram-positive bacteria *Bacillus subtilis* SLC (Cedrola et al., 2011); *B. subtilis* 1271, *B. licheniformis* 1269, and *B. cereus* 1268 (Mazoto et al., 2011), and *Streptomyces* sp. Strain AB1 (Jouadi et al., 2010). Based on the observation results, it was revealed that the shape, edges, internal structure, and elevation of the colonies of bacterial isolates were: they had a circular shape, efuse elevation, an entire edge shape, and a translucent internal structure. The cell morphology showed that all bacterial isolates had a gram-positive stem cell shape and were acid negative in acid staining. For the carbohydrate test, the result was positive for glucose, fructose, sucrose, and lactose tests and had spores. The catalase and oxidase tests generated positive results. According to Cappucino and Sherman (1987), bacterial colonies may have a round and irregular shape with a convex, concave or flat surface and flat or wavy edges.

Then, BRAW_KM isolates were observed in terms of their optimum growth based on the number of substrates, growth temperature condition, pH, inoculum concentration, agitation difference, and osmotic pressure. To determine the optimal bacterial growth, the growth was measured using various substrate concentrations. The substrates used were Pufferfish Flour at the concentrations of 5, 10, 15, 20, and 25 g/L. The obtained results suggested that the optimal use f substrates was achieved at the concentration of 15 g/L. The pH treatment in this study was given at pH levels 6, 7, 8, 9, 10, 11 and 12. Based on the obtaine result, it was revealed that the optimal pH ranged from pH 8 to 9. Different organisms showed maximum enzyme production at different pH levels. For example, *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus pumilus* produced enzymes maximally at pH f 7.0, 5.9, and 5.6 (Sivakumar et al. (2012), respectively. According to Rochimaa et al. (2015), the optimum pH

of enzyme collagenase from *Basilus subtilis* ranges from 7 to 9 (from 1,298 units per mL to 1,321 units per mL).

The temperature treatment to examine bacterial growth was given at 25, 27, 29, 31 and 33ºC. The result of the study showed that the most optimum bacterial growth occurred at a temperature between 31 to 33ºC. The inoculum concentrations were given at the following percentages: 1, 2, 3, 4, and 5% in growth media. The result suggested that the higher concentration of inoculum given, the higher level of growth. This was because in the growth medium there were more bacteria, thus causing growth competition. The different agitation (60, 80, 100, 120, 140 and 160 rpm) affected to collagenolytic bacterial growth. The optimum growth of it was speeds at 100-120 rpm. The treatment of osmotic pressure difference was the difference of NaCl concentration, such as 1, 2, 3, 4, and 5%. It was revealed that the isolates were able to grow to a concentration of 4%. This was based on the modified method proposed by Rao and Narasu (2007) stating that bacterial growth requires the NaCl concentration by 1, 2, 3, 4, 5, 6 and 7. The maximum enzyme activity was found at 215 U/ml.

Analysis of the Base Sequence of 16S rRNA Genes

According to Clarridge III (2004), the sequence of 16S rRNA genes has been determined for many strains. GenBank as the largest nucleotide sequence data bank has more than 20 million nucleotide sequences and nearly 90,000 of them are 16S rRNA genes. This indicates that many of the previously stored nucotide sequences are used to compare the sequence of a new strain discovered. Besides, 16S rRNA genes have universal properties in bacteria so that they can be used to analyze the family relationship between bacteria from the genus level of various phyla to the strain level, namely species and subspecies. The base sequence is presented in Figure 1.

The nitrogenous base sequence of isolates and that of strains used as a reference or as a comparison were used to be analyzed to determine the family relationship in the form of a phylogenetic tree. The phylogenetic tree obtained from the analysis results is presented in Figure 2.

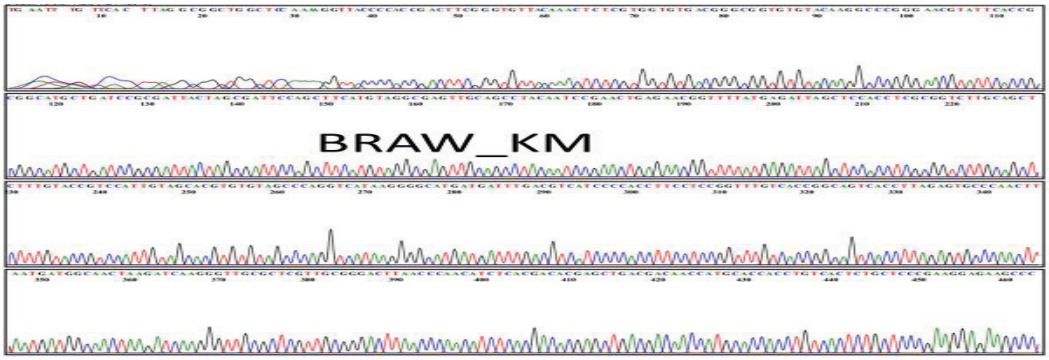

Figure 1. The Base Sequence of the isolates of BRAW_KM

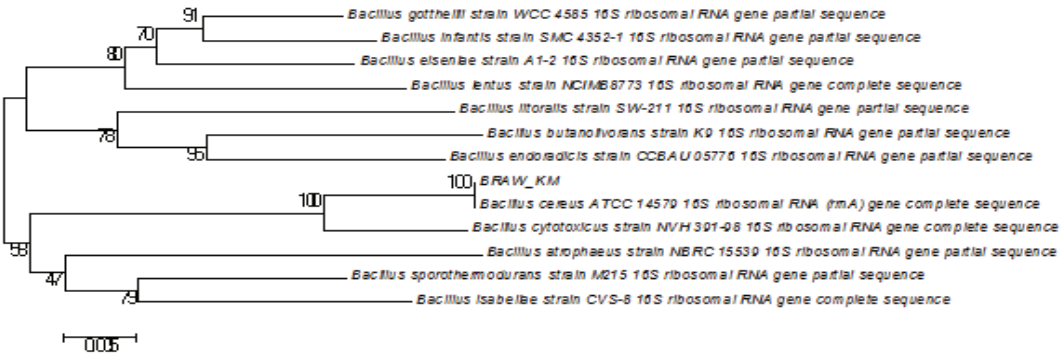

Figure 2. The Phylogenetic Tree of Bacteria BRAW_KM

Based on the obtained phylogenetic tree, it is revealed that BRAW_KM has a very close family relationship with the family *Bacillaceae,* i.e. with *Bacillus cereus*, as indicated by the value of similarities by 100%.

CONCLUSIONS

1. BRAW_KM isolates will have their optimum performance at a temperature between 31 and 33ºC; the optimum pH ranges from 8 to 9; the optimum quantity of substrates is equal to 15g/l; the osmotic pressure is equal to 4%; the optimal speed ranges from 100 to 120 rpm; and the inoculum concentration is equal to 8%.
2. The collagenase activity of bacteria BRAW_KM reaches 1,029 U/mg.
3. They have a very close family relationship with the family *Bacillaceae*, i.e. with *Bacillus cereus*, as indicated by the value of similarities by 100%.

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