AIP Conference Proceedings

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Cite as: AIP Conference Proceedings **2021**, 030004 (2018); https://doi.org/10.1063/1.5062728
Published Online: 17 October 2018

Fitria N. Aini, Erika B. Laconi, and Maggy T. Suhartono

The Potential of Agricultural By-products as a Carbon Source in the Growth of *Paenibacillus* sp.

Fitria N. Aini[1,a)], Erika B. Laconi[1], and Maggy T. Suhartono[2,b)]

[1]*Department of Nutrition and Feed Technology, Bogor Agricultural University, Bogor, West Java, Indonesia*
[2]*Departmemt of Food Science Technology, Bogor Agricultural University, Bogor, West Java, Indonesia*

[a)]Corresponding author: nurolaini12@gmail.com
[b)]mthenawidjaja@yahoo.com

Abstract. *Paenibacillus* sp. is a bacterium that contains the expected lignocellulolytic enzyme. Agricultural by-products like rice straw, corn straw, soybean straw, and sugarcane bagasse are lignocellulose biomass that have potential to be used as carbon source. This study aimed to measure the growth of *Paenibacillus* sp. inoculated on different carbon media. The treatments consisted of nutrient broth (NB) and five formulations which including carboxy methyl cellulose (CMC) and four agricultural by-products as the varied carbon sources. The media was composed of peptone (0.5%), yeast extract (0.5%), $K_2HPO_4.3H_2O$ (0.1%), $MgSO_4.7H_2O$ (0.02%), and a carbon source (0.5%). A loop-full of *Paenibacillus* sp. was used to inoculate the variable media and was then incubated at 30°C. Total numbers of bacteria in stationary phase was calculated using the total plate count method and it was found that sugarcane bagasse provided the highest number of bacteria. Overall the total number of bacteria in stationary phase was determined to be significantly different ($p < 0.05$). Corn straw obtained the least number of bacteria (9.5×10^5 CFU ml^{-1}), whereas the sugarcane bagasse medium had the highest yields with 1.4×10^7 CFU ml^{-1}. The other media NB, soybean straw, rice bean straw, and CMC yielded 2.5×10^6 CFU ml^{-1}, 5.2×10^6 CFU ml^{-1}, 9.9×10^6 CFU ml^{-1}, and 1.13×10^7 CFU ml^{-1}, respectively. This study concludes that agricultural by-products, especially sugarcane bagasse can be used as the carbon source for growing *Paenibacillus* sp.

Keywords: bacteria, carbon source, *Paenibacillus* sp., sugarcane.

INTRODUCTION

The use of alternatives to natural resources are important to be developed for both human and environmental purposes. Agricultural by-products as alternate natural resources has come to be an important source of lignocellulosic biomass. In Indonesia alone, the availability of agricultural by-products such as rice straw, corn straw, soybean straw and sugarcane bagasse has been reported at approximately 17 million, 15 million, 278 032, and 10 million tons/year, respectively.[1,2,3] Together these could be used as the carbon source for many industries including pulp, paper, textile, or to produce biofuels, enzymes and proteins. However, to date it has not been possible to use them in the growth of bacteria.

Currently, microbiology use bacteria in advanced biotechnology protocols as they are fast growing, easily genetically modified, and a specific yield can be produced reproducibly. *Paenibacillus* sp. is a genus isolated from the gastrointestinal tract of termites and is predicted to play an important role in the lignocellulose degradation process, as it was found to grow well on media containing lignin.[4,5] This genus was first isolated from soil and appeared as a novel cellulolytic bacterium.[6] Additionally, it has been isolated from the gut of a soil-feeding termite and was suggested to produce xylanase.[7] This study aimed to measure the growth of *Paenibacillus* sp. On media containing agricultural by-products.

MATERIALS AND METHODS

This research was conducted using samples prepared from agricultural by-products (paddy straw, corn straw, soybean straw, and sugarcane bagasse). Paddy straw and corn straw were collected from Muara Field, Ciapus, Bogor, soybean straw was collected from Cikarawang Field, Dramaga, Bogor, and sugarcane bagasse was

The 8th Annual Basic Science International Conference
AIP Conf. Proc. 2021, 030004-1–030004-5; https://doi.org/10.1063/1.5062728
Published by AIP Publishing. 978-0-7354-1739-7/$30.00

collected from Kediri Field, East Java. The chemical content and fiber fraction were analysed for each by-product, and they were used as the carbon source in the media for growing the bacteria. *Paenibacillus* sp. was obtained from the Indonesian Institute of Science, Cibinong, Bogor (InaCC B63). *Paenibacillus* sp. were grown on six different media consisting of nutrient broth (NB) only as the control, in addition to four agricultural by-products and carboxymethylcellulose (CMC) as carbon sources (peptone 0.5%, yeast extract 0.5%, $K_2HPO_4.3H_2O$ 0.1%, $MgSO_4.7H_2O$ 0.02% and carbon source 0.5%).[8] Turbidity and total number of colonies were observed as growth estimates.

Preparation of Samples and Bacteria

Agricultural by-products were sun-dried for 2 days and ground as to pass through either a 0.6 mm particle width sieve (Retsch 5657 Haan w. Germany Mesh No.30) in preparation for chemical content and fiber fractionation analysis, or a 0.150 mm particle width sieve (No.100) for the media growth comparison. Inoculum of *Paenibacillus* sp. was regenerated on nutrient agar (NA) medium for 2-3 days at room temperature. A stock of bacterium was kept at 4°C.

Chemical Content Analysis

Proximate analysis was conducted according to AOAC 2005.[9] Fiber fractionation was performed as previously described.[10] Both analyses were conducted in duplicate.

Bacterial Growth

One loop of *Paenibacillus* sp. inoculum was used to inoculate medium (4 mL) and was then incubated in a shaking water bath at 30°C for 3 days. Samples of media containing bacteria were collected every 8 h for turbidity analysis using a Spectrophotometer UV-200 at 600 nm, with the medium at 0 hours used as the blank. Total number of colonies were determined by a total plate count (TPC) analysis as previously described.[11] The samples used for the TPC analysis were collected at stationary phase. The turbidity analysis was performed in triplicate and TPC in duplicate.

Data Analysis

Data were analyzed based on a completely randomized design using analysis of variance (ANOVA). The data were compared using the Duncan's multiple range test, and if the result presented $p < 0.05$, it was considered significant. SAS software was used for all statistical analyses.

RESULTS AND DISCUSSION

All agricultural by-products in this study commonly contained low level of ash, i.e below 10% DM; however, rice straw was found to contain high levels (19% DM) of ash. Rice straw and corn straw generally contained higher levels of CP (above 10% DM) when compared to soybean straw and sugarcane bagasse (below 10% DM). In addition, the EE contents of the by-products were commonly below 1% DM. Furthermore, soybean straw and sugarcane bagasse contained higher amounts of CF (above 30%) compared to that found in rice straw and corn straw (below 30%). The NFE content was nearly the same across the by-products between 42-53% (Table 1).

Generally, the sugarcane bagasse was found to contain high levels of cellulose (above 40% DM), while the other by-products contained 31-37% DM of cellulose. In addition, rice straw commonly contained higher level of hemicellulose (above 35%), followed by corn straw, sugarcane bagasse, and soybean straw, respectively. Furthermore, rice straw and soybean straw generally contained higher amounts of lignin (above 10% DM) compared to corn straw and sugarcane bagasse (below 10% DM). Generally, the silica content was highest in the rice straw at 9.05%, while the other by-products contained between 1-5.5% of silica (Table 2).

TABLE 1. Proximate composition of agricultural by products (%DM).

Agricultural by products	Ash (%)	CP (%)	EE (%)	CF (%)	NFE (%)
Rice straw	19.00	10.21	0.92	27.73	42.14
Corn straw	4.02	15.00	0.21	28.35	52.40
Soybean straw	6.26	9.53	0.56	40.10	43.55
Sugarcane bagasse	2.39	6.43	0.14	38.71	52.32

Note : DM = dry matter; CP = crude protein; EE = ether extract; CF= crude fiber; NFE=nitrogen free extract

TABLE 2. Fiber fractination of agricultural by products (%DM).

Agricultural by products	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Silica (%)
Rice straw	31.35	35.01	15.52	9.05
Corn straw	33.20	30.34	7.99	5.37
Soybean straw	36.56	12.22	13.19	1.08
Sugarcane bagasse	42.27	24.28	7.27	2.86

Note : NDF = neutral detergent fiber; ADF = acid detergent fiber

TABLE 3. Colony forming unit of *Paenibacillus* sp.

Type of media or carbon source	Total colonies ($\times 10^5$ CFU ml^{-1})
Nutrient broth (NB)	25±0.00cd
Rice straw (RS)	99±14.1b
Corn straw (CS)	9.5±0.71d
Soybean straw (SS)	52.5±6.36c
Sugarcane bagasse (SB)	140±21.21a
Carboxy methyl cellulose (CMC)	113±14.14ab

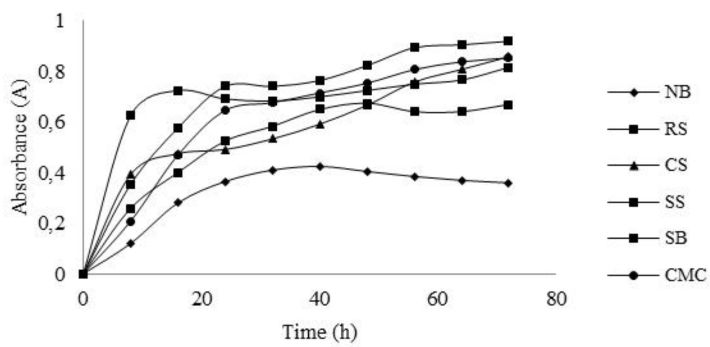

FIGURE 1. Growth of *Paenibacillus* sp.

The TPC analysis showed that there was a difference among the varying media. Sugarcane bagasse produced the highest number of colonies, while corn straw produced the lowest one (Table 3). Furthermore, the turbidity analysis revealed that NB showed the lowest level of turbidity, indicting minimal growth. CMC, corn straw, and sugarcane bagasse curves presented nearly the same level of growth, however the sugarcane bagasse produced the fastest growth up until 16 h (Fig. 1). Overall the fastest growth was seen when soybean straw was used as the carbon source.

Chemical Content of Agricultural By-products

The main constituent of all the by-products was carbohydrate, consisting of CF and NFE. Previously studies in which agricultural by-products have been used, they have concentrated on the CF content.[7,12] The CF content of the four agricultural by-products used in this study have already been described in other studies. Some studies reported that the CF of rice straw ranged between 25.26-37.68%,[1,13] while corn straw, soybeans straw and sugarcane bagasse have been found to contain similar levels of CF to what was determined in this study.[14,15] The difference in the CF content may be caused by a varying proportion of the cell wall, which is influenced by the species and different growth phases.[16] CF is a structural carbohydrate that is usually used as a poor-quality indicator of feedstuffs in farm animal nutrition; however in many cases, i.e. tropical grasses and straws, it is not a true representation of the digestible fibrous part of the plant.[16]

The Van Soest analysis separated the CF fractions depending on nutritional availability, and is an acceptable indicator of quality.[17] The cellulose content of rice straw was relatively similar to what has been established in previous studies,[18,19] while the cellulose content of corn straw and soybean straw was lower.[19] On the other hand, the sugarcane bagasse contained a higher level of cellulose than what has previously been described.[20,21] The high cellulose content of sugarcane bagasse suggests that it is potentially the source of the carbon in this by-product. The proportion of cellulose can have an impact on the varying uses of the by-product materials, and therefore the nutrition value of by-products may be estimated by the cellulose amount.[22]

The hemicellulose content of rice straw, corn straw, and sugarcane bagasse obtained in this study was relatively similar to previously established values.[19,24] However, the hemicellulose content of CS in this study was found to be higher.[20] Hemicellulose is commonly known as the “filler”, together with cellulose and lignin, and is composed mainly of glucose, xylose, galactose, arabinose, and mannose.[23] It is an alkali-soluble cell wall polysaccharide that is closely related to cellulose.[16]

The lignin content of rice straw has previously been reported to be lower than what was obtained in this study.[19,24] The lignin content of corn straw and soybean straw in this study was relatively similar to previous studies.[20,25,26,27] However, the lignin content of sugarcane bagasse determined in this study was three times lower than what has been previously described.[21,22] Lignin is of interest in animal nutrition, as it provides protection to chemical degradation. It acts as a physical layer of plant fibers that makes the materials inaccessible to enzymes which would digest them.[16] The low lignin content of sugarcane bagasse supports its use as a carbon source for bacterial growth.

There has been several studies investigating the silica content of rice straw, but there are no studies reporting the silica content of the other agricultural by-products used in this study. Rice straw is known to have a high content of silica, ranging from 13 - 15%;[18,28] however, the result obtained in this study was lower. The function of silica in plants remains unknown, however it has been suggested that it may play a role in the defense against a predator by rising the abrasiveness, and in turn, decreasing the digestibility of the material.[29]

All microbial life need nutritional requirements for their normal growth and function.[30] Suitable materials need to be tested to investigate which one will provide optimum growth, in addition these studies can help understand the use of varying constituents of the materials by the bacteria grown on it. In this study, sugarcane bagasse medium provided the highest number of colonies of *Paenibacillus* sp. and this may be due to the good nutritional value of sugarcane bagasse, including high levels of cellulose and low levels of ash, lignin, and silica. Apart from the high content of cellulose that make it a potential good source of carbon, the low content of ash also offers numerous benefits for application in bioconversion using bacterial cultures.[31] Furthermore, the low content of lignin and silica increasingly support the use of sugarcane bagasse to feed bacteria. Lignin is a long chain of phenyl propane units, and functions by filling the room between the cellulose and hemicellulose. It binds the cell walls together and make it inaccessible to enzymes, and in turn cannot be digested by bacteria.[32] This result was also proven in the sugarcane bagasse growth curve of *Paenibacillus* sp. which showed the fastest growth until 16 h which corresponded to the exponential growth phase. On the other hand, corn straw showed the least number of colonies, possibly due to the slow rates of digestion of corn straw as was explained in previous studies.[25,33] In conclusion, the sugarcane bagasse is the best carbon source for growing *Paenibacillus* sp. Further studies are needed to analyze its enzyme activity for future use as feed for ruminants.

ACKNOWLEDGEMENT

The authors would like to thank Lembaga Pengelola Dana Pendidikan (LPDP) Indonesia for funding Master Degree Programme in Feed and Nutrition Science Study Programme (IPB Post Graduate) that has been conducted this research and technician in Faculty of Animal Science Laboratory for helping some analysis.

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