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Fruit-peel-degrading Potential of Some Legumes-associated Bacteria

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Abstract

This research aimed at determining the digestive ability of nine legumes-associated bacteria on the peels of some fruits (orange, watermelon, plantain, banana, pineapple and pawpaw). The bacteria were cultivated separately on each peel for 18 hours at 28°C; the amount of glucose released was quantified using Dinitrosalicylic acid reagent method. The results obtained showed that the bacteria degraded all the peels with the highest (0.297 mg/mL) and lowest (0.087 mg/mL) glucose concentrations produced by *Rhizobium leguminosarum* FUBO001 and *Bonitrorhizobium winogradskyi* FUBO004 in banana and pawpaw peels, respectively. The *Bo. winogradskyi* FUBO004 synthesized 0.101 mg/mL as minimum sugar amount in the former peel while *Bradyrhizobium nigeriasis* FUBO005 produced the highest glucose quantity (0.167 mg/mL) in the latter peel. The lowest amounts of glucose in orange, watermelon, pineapple and plantain peels produced by *Bradyrhizobium nigeriasis* FUBO003, *Rhizobium nigeriasis*, *R. nigeriasis* and *Br. nigeriasis* FUBO003 were 0.095, 0.132, 0.09 and 0.248 mg/mL respectively. In these peels, the highest amount of the reducing sugar made was 0.131 mg/mL by *Br. nigeriasis* FUBO005, 0.211 mg/mL by *Br. nigeriasis* FUBO005, 0.156 mg/mL by *Bo. winogradskyi* FUBO004 and 0.291 mg/mL by *R. nigeriasis*. These results suggest that the bacteria catabolized the fruit peels, reflecting their high potential in the conversion of the fruit peels to useful products.

Keywords: Fruit peel, Degradation, Legumes-associated bacteria, Glucose concentration.

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Introduction

Fruits and vegetables are covered by a protective layer called peel (Ajayi and Boboye, 2012; Pranav et al., 2017). Based on thickness and tastes of fruit peels, the peels could be eaten as part of the fruits; but in some instances, fruit peels are discarded as wastes particularly when they have unpleasant tastes or constitute inedible portions as seen in banana (Pranav et al., 2017). After the consumption of the inner succulent part of a fruit or during its use in fruit juice production, fruit peels are considered as wastes in order to prevent contamination (Olukunle et al., 2007; Oladiji et al., 2010). Fruits and vegetables are responsible for about 22% of food losses and wastes along the supply chain (Santos et al., 2022). Solid wastes such as peel, core, unripe and over-ripe fruits, as high as 50% of raw materials, are generated by fruit processing industries (Lekhuleni et al., 2021).

Fruit peels consist of pectin and related substances occurring in the cell walls and middle lamellae of all higher plants (Dutta, 1981; Singh et al., 2003). These substances hold cells together in all plants (Okafor, 1987; Pretel et al., 2018). The cell walls of plants generally contain different components. However, the primary cell wall is majorly made up of eight polymers which are pectin existing in three forms containing D galacturonic acid, cellulose, three glycans having neutral sugars and structural proteins (Pretel et al., 2018). Wongkaew et al. (2021) reported that

mango peel is a potential source of dietary fibre and depending on fruit varieties and methods of extraction, it contains 5 – 11% pectin.

Peels are manually, mechanically or enzymatically separated from other parts of plants (Boboye and Ajayi, 2012; Pranav et al., 2017). The separation of plant's individual cells occurs by the degradation of pectin and similar polymers and hence loss of tissue coherence (Shigetaka, 1977; Kumar, 2015). The removal of fruit peels by enzymes is based on the principle of digestion of pectic substances present in the cell wall of the plants (Bruemmer et al., 1978; Berry et al., 1988; Ajayi and Boboye, 2012). Maceration is important industrially for the removal of the segment of fruit's membrane and this can positively affect the integrity of the fruit juice (Ben-Shalom et al., 1986; Boboye and Ajayi, 2012). Previous research efforts have shown that extraction of fruit juices using enzymes to peel fruits leads to higher yields and improvement of juice appearance (Kumar, 2015).

Many researchers including Zerva et al. (2019), suggested and shown that maceration of plant tissues is carried out by many microorganisms. Pathogenic activity of many of these microorganisms limit their agricultural and industrial applications. It is important to search for non-pathogenic microbes which can be used for fruit-peel removal. Rhizobia are non-pathogenic soil bacteria that form nodules on the roots of legumes using the mechanism of intracellular infection. Based on this, the organisms are useful for the removal of fruit peels, particularly *Rizhobium* spp. CWP G34B (Ajayi and Boboye, 2012). This research was proposed to screen some tropical rhizobia for their ability to catabolize peels of some Nigerian fruits.

Materials and Methods

Samples and Their Preparation

The fruits (orange, pineapple, watermelon, plantain, banana and pawpaw) from which peels were prepared for this study were obtained from different markets namely: "Sasha", "Oba", "Isinkan", "Agagu Road", "Mojere" and "Iloro" in Akure, Southwestern, Nigeria. The fruits were washed thoroughly in sterile water to remove any dirt and peeled using a sterilized knife, sun dried (between 30°C and 40°C) for 5 hours daily for 7 days. The samples were then ground using Marlex Electroline Blender and kept in the refrigerator maintained at 4°C until needed.

Culture Media and Their Preparation

The culture media used in this study were nutrient agar, nutrient broth (Lab M, Topley House, England), basal medium and fruit-peel medium (FPM) (Composed in this Work). The first two media were prepared according to the manufacturer's specification. Basal medium containing 0.1% (w/v) NH_4NO_3, 0.5% (w/v) KH_2PO_4, 0.05% (w/v) $MgSO_4.7H_2O$ and 0.01% (w/v) $CaCl_2.2H_2O$ was prepared in 100 mL of distilled water at pH 5.6. The FPM was prepared with 10 mL of the basal medium and 1% (w/v) fruit peel. All media were sterilized by autoclaving at 121°C for 15 minutes.

Source and Preparation of Bacterial Inocula

The bacteria (*Rhizobium leguminosarum* FUBO001, *R. leguminosarum* FUBO002, *Bradyrhizobium nigeriasis* FUBO003, *Bonitrorhizobium winogradskyi* FUTABO004 *Bradyrhizobium nigeriasis* FUBO005, *Borhizobium nigeriasis* FUBO006, *Borhizobium nigeriasis* FUBO007, *R. leguminosarum* FUBO008 and *Rhizobium nigeriasis*) were provided by the Department of Microbiology, Federal University of Technology, Akure, Nigeria. Inoculum of each bacterium was prepared by inoculating them separately into nutrient broth which was incubated at 28°C for 24 hours.

Determination of the Fruit-Peel-Degrading Potential of the Bacteria

Cultivation of bacteria

The sterilized fruit-peel medium (FPM) was inoculated with 0.1 mL of the 24 hours old nutrient broth inoculum (prepared above) and incubated at 28°C for 24 hours. The test was carried out in triplicates for each bacterial isolate. Uninoculated FPM was used as control. Each culture and the control were centrifuged at 3600 rpm for 15 minutes and the supernatants were used to assay for the quantities of glucose formed by the bacteria.

Measurement of Glucose Released from Fruit Peel

Standard glucose curve was prepared according to the Dinitrosalicylic acid (DNSA) reagent method (Bernfeld, 1955) as described by Boboye and Alao (2008). Amount of glucose released into the medium by each microbe was measured by subjecting the culture supernatant of individual

bacterium to the DNSA method and the optical density (OD) measured at 540 nm was referred to the standard curve to obtain the concentration of glucose in the culture supernatant of each microorganism. Glucose standard curve value of the control (FPM) was subtracted from the glucose standard curve reading of each of the supernatants of the bacterial grown in FPM to obtain the actual glucose concentration.

Statistical Analysis of Data

The data collected were analysed using the analysis of variance (ANOVA) technique and expressed as a mean of value. Duncan Multiple Range Test was carried out to determine differences in the means using SPSS Software Package (Duncan, 1955) as applied by Ajayi and Boboye (2012).

Results

All the legumes-associated bacteria showed considerable (at 95% confidence limit) degradation ability on all the fruit peels used in this study. The amount of glucose released by the individual bacterium into the growth medium of each fruit-peel is shown in Figures 1 – 6. The highest and lowest glucose concentrations of 0.297 mg/mL and 0.087 mg/mL were produced by *Rhizobium leguminosarum* FUBO001 and *Bonitrorhizobium winogradskyi* FUTABO004 in banana and pawpaw peels respectively (Figures 1 and 2). In the banana peel, *Bo. winogradskyi* formed 0.101 mg/mL as the lowest glucose concentration (Figure 1) and *Bradyrhizobium nigeriasis* FUBO005 produced 0.167 mg/mL as the highest quantity of the sugar in the pawpaw peel (Figure 2).

In orange peel, *Br. nigeriasis* FUBO003 released the lowest (0.095 mg/mL) and *Br. nigeriasis* FUBO005 the highest (0.131 mg/mL) amounts of the sugar (Figure 3). Similarly, the concentrations of glucose made in watermelon by *Rhizobium nigeriasis* and *Br. nigeriasis* FUBO005 ranged from 0.132 mg/mL to 0.211 mg/mL (Figure 4). *Rhizobium nigeriasis* produced 0.09 mg/mL and *Bonitrorhizobium winogradskyi* FUBO004 made 0.156 mg/mL as the lowest and highest glucose concentrations in pineapple peel (Figure 5). *Bradyrhizobium nigeriasis* FUBO003 and *R. nigeriasis* catabolised plantain peel to release 0.248 mg/mL and 0.291 mg/mL of glucose as the lowest and highest concentrations respectively (Figure 6).

It was observed that none of the concentrations of glucose released from plantain peel was below 0.2 mg/mL in contrast to other peels. Generally, *Br. nigeriasis* FUBO005 produced the highest amounts of glucose in the peels of three fruits (pawpaw, orange and watermelon) (Figures 2, 3 and 4). Comparatively, the bacterium made considerable glucose amounts of 0.253 mg/mL, 0.146 mg/mL and 0.290 mg/mL in banana, pineapple and plantain peels respectively (Figures 1, 5 and 6).

Discussion

The results of this study showed that all the bacteria produced glucose from the peels of the fruits (orange, watermelon, plantain, banana, pineapple and pawpaw) tested, meaning that the bacteria degraded the substrates. Generally, peels and plant outer coverings contain pectin and its derivatives which are made up of polymers of glucose, hence the glucose formed from the peels by these microbes. The difference in the amounts of glucose released, is an indication that the ability of the bacteria to degrade the peels differs. Those bacteria that made the lowest and highest amounts of the sugar have lesser and better peel degrading potentials respectively on the same peel in contrast to their counterpart microbes. This result is supported by the findings of some researchers like Kong et al. (2022).

The pattern of the data obtained in this study also suggests that the bacteria prefer one peel to the other; besides *Rhizobium leguminosarum* FUBO001 that performed best overall and in banana peel, along with *Rhizobium nigeriasis* in plantain peel and *Bradyrhizobium nigeriasis* FUBO005 in watermelon peel, other test organisms individually established catabolism preference for different fruit peels. Other biodegraded peels did not contain up to 0.2 mg/mL glucose compared with plantain peel (Fig. 6) except banana and except banana and

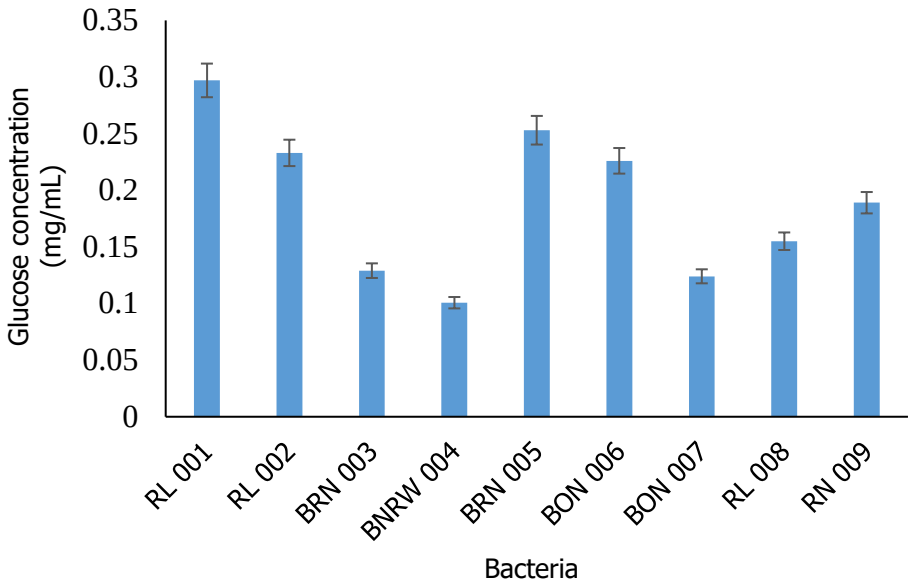

Figure 1: Banana peel degradation potential of some legumes-associated bacteria
Legend:
RL 001: *Rhizobium leguminosarum* FUBO001
RL 002: *Rhizobium leguminosarum* FUBO002
BRN 003: *Bradyrhizobium nigeriasis* FUBO003
BNRW 004: *Bonitrorhizobium winogradskyi* FUTABO004
BRN 005: *Bradyrhizobium nigeriasis* FUBO005
BON 006: *Borhizobium nigeriasis* FUBO006
BON 007: *Borhizobium nigeriasis* FUBO007
RL 008: *Rhizobium leguminosarum* FUBO008
RN 009: *Rhizobium nigeriasis*

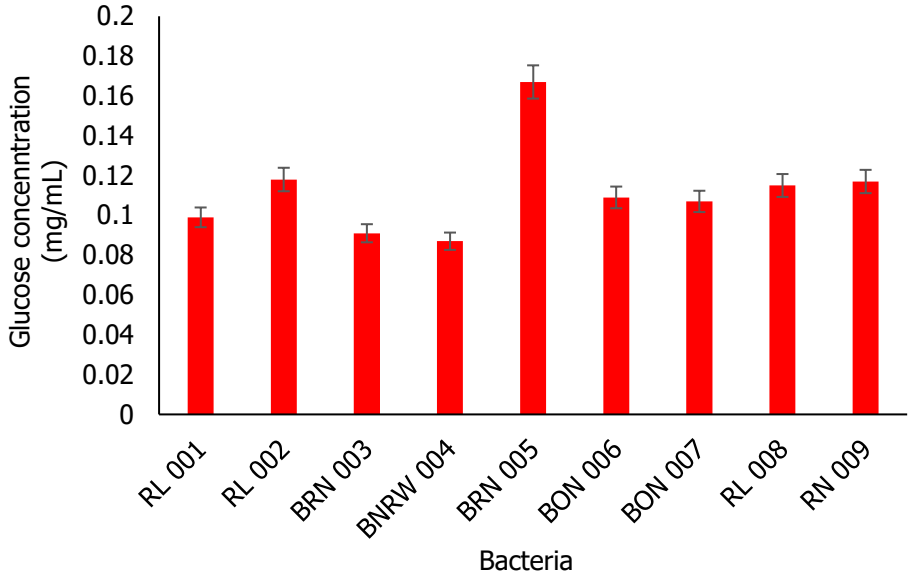

Figure 2: Pawpaw peel degradation potential of some legumes-associated bacteria
Legend:
RL 001: *Rhizobium leguminosarum* FUBO001
RL 002: *Rhizobium leguminosarum* FUBO002
BRN 003: *Bradyrhizobium nigeriasis* FUBO003
BNRW 004: *Bonitrorhizobium winogradskyi* FUBO004
BRN 005: *Bradyrhizobium nigeriasis* FUBO005
BON 006: *Borhizobium nigeriasis* FUBO006
BON 007: *Borhizobium nigeriasis* FUBO007
RL 008: *Rhizobium leguminosarum* FUBO008
RN 009: *Rhizobium nigeriasis*

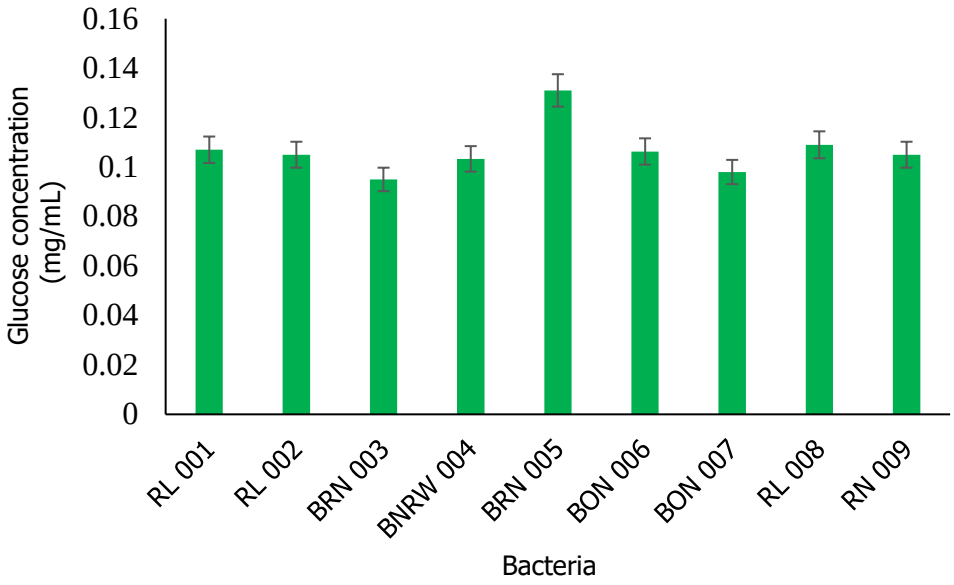

Figure 3: Orange peel degradation potential of some legumes-associated bacteria
Legend:
RL 001: *Rhizobium leguminosarum* FUBO001
RL 002: *Rhizobium leguminosarum* FUBO002
BRN 003: *Bradyrhizobium nigeriasis* FUBO003
BNRW 004: *Bonitrorhizobium winogradskyi* FUBO004
BRN 005: *Bradyrhizobium nigeriasis* FUBO005
BON 006: *Borhizobium nigeriasis* FUBO006
BON 007: *Borhizobium nigeriasis* FUBO007
RL 008: *Rhizobium leguminosarum* FUBO008
RN 009: *Rhizobium nigeriasis*

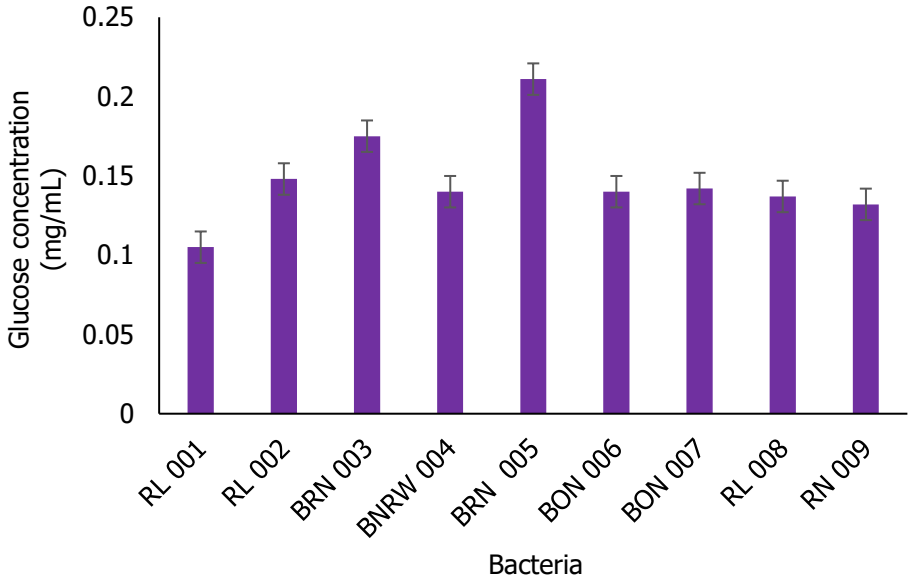

Figure 4: Watermelon peel degradation potential of some legumes-associated bacteria
Legend:
RL 001: *Rhizobium leguminosarum* FUBO001
RL 002: *Rhizobium leguminosarum* FUBO002
BRN 003: *Bradyrhizobium nigeriasis* FUBO003
BNRW 004: *Bonitrorhizobium winogradskyi* FUBO004
BRN 005: *Bradyrhizobium nigeriasis* FUBO005
BON 006: *Borhizobium nigeriasis* FUBO006
BON 007: *Borhizobium nigeriasis* FUBO007
RL 008: *Rhizobium leguminosarum* FUBO008
RN 009: *Rhizobium nigeriasis*

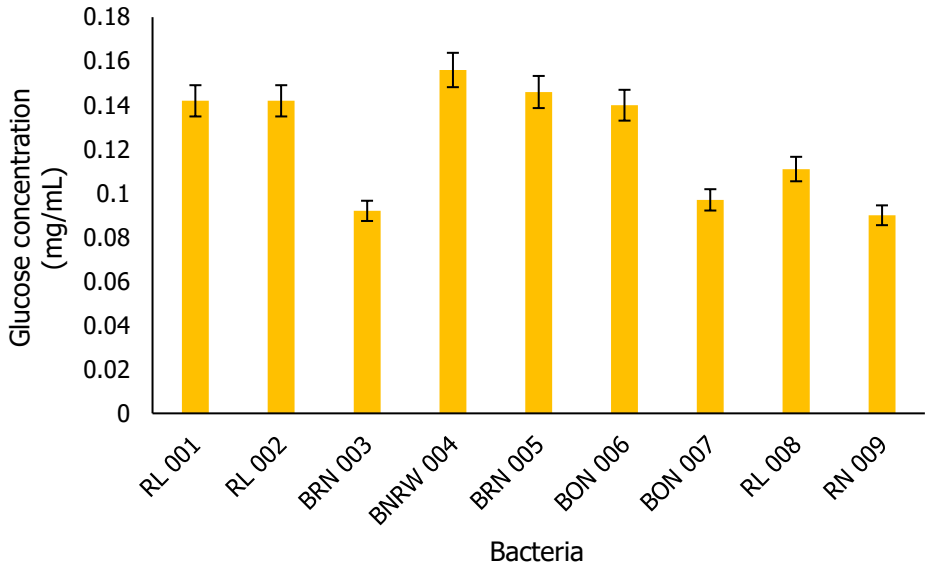

Figure 5: Pineapple peel degradation potential of some legumes-associated bacteria
Legend:
RL 001: *Rhizobium leguminosarum* FUBO001
RL 002: *Rhizobium leguminosarum* FUBO002
BRN 003: *Bradyrhizobium nigeriasis* FUBO003
BNRW 004: *Bonitrorhizobium winogradskyi* FUBO004
BRN 005: *Bradyrhizobium nigeriasis* FUBO005
BON 006: *Borhizobium nigeriasis* FUBO006
BON 007: *Borhizobium nigeriasis* FUBO007
RL 008: *Rhizobium leguminosarum* FUBO008
RN 009: *Rhizobium nigeriasis*

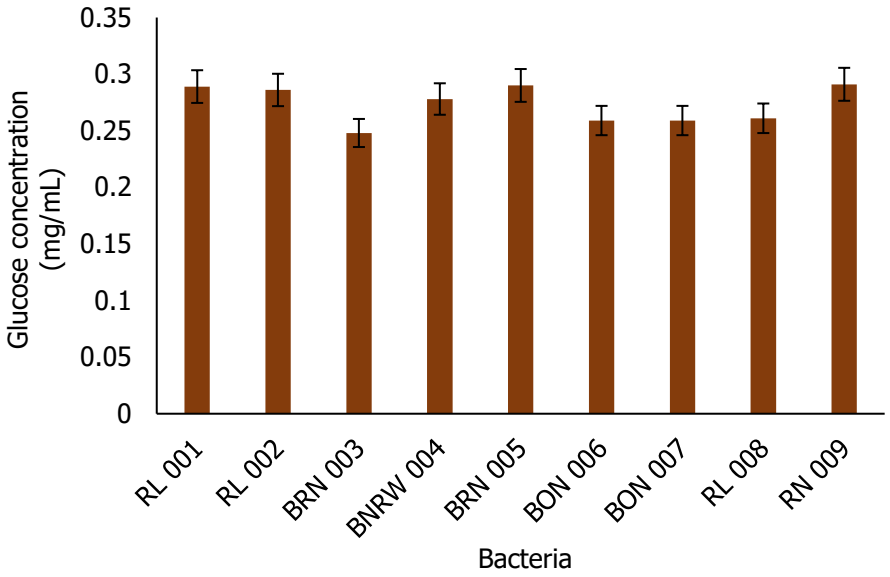

Figure 6: Plantain peel degradation potential of some legumes-associated bacteria
Legend:
RL 001: *Rhizobium leguminosarum* FUBO001
RL 002: *Rhizobium leguminosarum* FUBO002
BRN 003: *Bradyrhizobium nigeriasis* FUBO003
BNRW 004: *Bonitrorhizobium winogradskyi* FUBO004
BRN 005: *Bradyrhizobium nigeriasis* FUBO005
BON 006: *Borhizobium nigeriasis* FUBO006
BON 007: *Borhizobium nigeriasis* FUBO007
RL 008: *Rhizobium leguminosarum* FUBO008
RN 009: *Rhizobium nigeriasis*

watermelon peels (Fig. 1 and 4) in which 44.44% and 11.11% of the bacteria produced >0.2 mg/mL glucose respectively and hence, the banana peel is rated as the second best degraded after plantain peel. The higher glucose concentrations obtained in the plantain peel than in other peels (apart from banana) infers that any of the bacteria can be used to degrade plantain peel with the best being *Rhizobium nigeriasis* followed by *Br. Nigeriasis* FUBO005 and *R. leguminosarum* FUBO001.

The better catabolism of the plantain peel than other peels may be associated with their nutrient's composition. Relatively, all the peels are high in fibre content since they contain >5g/100g; fruit peels are commonly rich in fibres as proven by many researchers including Dias et al. (2020). Fibre is a structural carbohydrate and it aids digestion; the fibre concentrations in banana, pawpaw, orange, watermelon and pineapple peels have been reported to be 11.81 ± 0.06, 12.16 ± 0.06, 14.19 ± 0.01, 26.31 and 14.80 ± 0.01 g/100g dry weight respectively (Feumba et al., 2016). Similarly, Morais et al. (2017) reported the fibre concentrations of banana, pawpaw, watermelon and pineapple peels to be 20.1 ± 0.27 – 23.5 ± 3.8, 16.7 ± 0.5 – 18.7 ± 1.8, 32.3 ± 4.6 – 37.4 ± 7.1, and 13.9 ± 1.1 – 15.9 ± 2.4 g/100g dry weight respectively. For plantain peel, the total dietary fibre (TDF) is 64.33 g/100g (Arun et al., 2015) while from the studies of Emaga et al. (2007) on peels of five different varieties of plantain, the TDF varied from 32.9 to 49.9 g/100g. The higher fibre content of the plantain peel could have made it easier for the bacteria to penetrate this peel than other peels and thus breakdown the carbohydrate to the reducing sugar.

Conclusion

The fruit peels used in this study were catabolized by the legume-associated bacteria. This implies that the bacteria have potential for use in the peeling of fruits and for the production of enriched animal feeds. Based on the highest glucose quantities formed by the bacteria, *Rhizobium leguminosarum* FUBO001, *Bonitrorhizobium winogradskyi* FUBO004 and *R. nigeriasis* may be particularly useful in the removal of banana, pineapple and plantain peels while *Bradyrhizobium nigeriasis* FUBO005 is suitable for the removal of pawpaw, orange and watermelon peels.

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References

Ajayi, G. O. and Boboye, B. (2012). Intracellular and extracellular fruit-peel-degrading enzymes synthesized by *Rhizobium* species CWP G34B. J. Pure and App. Microb. 6(1): 23-28.

Arun, K. B., Persia, F., Aswathy, P. S., Chandran, J., Sajeev, M. S., Jayamurthy, P. and Nisha, P. (2015). Plantain peel - a potential source of antioxidant dietary fibre for developing functional cookies. J. Food Sc. and Tech. 52(10): 6355–6364.

Ben-Shalom, N., Levi, A. and Pinto, R. (1986). Pectolytic enzyme studies for peeling of grape fruit segment membrane. J. Food Sc. 51(2): 421-423.

Bernfeld, P. (1955). Amylases α and β. Methods in Enzymology, 1: 149-158.

Berry, R. E., Baker, R. A. and Bruemmer, J. H. (1988). Enzyme separated sections: A new lightly processed citrus product. In: Proceedings of the 6th International Citrus Congress, Tel-Aviv, Israel, March 6 – 11, 1988. Edited by Gorel, R. and Hendel, K. Balaban Publishers, Philadelphia. pp. 1711 – 1716.

Boboye, B. E. and Ajayi, G. O. (2012). Biodegradation of selected Nigerian fruit peels by the use of a non-pathogenic *Rhizobium* species CWP G34B. Open Microb. J. 6: 88–93.

Boboye, B. E. and Alao, A. (2008). Effect of mutations affecting the synthesis of pectate lyse in *Xanthomonas campestris*. W. J. Microbiol. and Biotech. 9: 240 – 242.

Bruemmer, J. H., Grifflin, A. W. and Onayomi, O.

(1978). Sectioning grapefruit by enzyme digestion. Proceedings of Florida State Horticultural Society, 91: 112-114.

Dias, P. G. I., Sajiwanie, J. W. A. and Rathnayaka, R. M. U. S. K. (2020). Chemical composition, physicochemical and technological properties of selected fruit peels as a potential food source. Inter. J. Fruit Sc. 20(4): S240-S251.

Duncan, D. M. (1955). Multiple Range and Multiple F-Test. In: Biometric, 11: 1 – 42.

Dutta, A. C. (1981). Botany for Degree Students. Oxford University Press, Delhi. 5th Edition. pp. 106-161.

Emaga, T. H., Andrianaivo, R. H., Wathelet, B., Tchango, J. T. and Paquot, M. (2007). Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. Food Chem. 103: 590–600.

Feumba, D. R., Ashwini, R. P. and Ragu, S. M. (2016). Chemical composition of some selected fruit peels. Europ. J. Food Sc. and Tech. 4(4): 12-21.

Kong, X., Dong, R., King, T., Chen, F. and Li, H. (2022). Biodegradation potential of *Bacillus* sp. PAH-2 on PAHs for oil-contaminated seawater. Open Acc. 124: 83-89.

Kumar, S. (2015). Role of enzymes in fruit juice processing and its quality enhancement. Adv. in App. Sc. Res. 6(6): 114-124.

Lekhuleni, I. L. G., Kgatla, T. E., Mashau, M. E. and Jideani, A. I. O. (2021). Physicochemical properties of South African prickly pear fruit and peel: Extraction and characterisation of pectin from the peel. Open Agric. 6: 178–191.

Okafor, N. (1987). Biocatalysts: Immobilized Enzymes and Immobilized Cell. In: Industrial Microbiology. 1st Edition, University Press Limited, Nigeria. pp. 305-312.

Oladiji, A. T., Yakubu, M. T., Idoko, A. S., Adeyemi, O. and Salawu, M. O. (2010). Studies on the physicochemical properties and fatty acid composition of oil from ripe plantain (*Musa parasidiaca*) peel. Afr. Sc. 11: 73-78.

Olukunle, J. O., Oguntunde, P. G. and Olukunle, O. F. (2007). Development of a system for fresh fruit juice extraction and dispensary. Conference on International Agricultural Research for Development. Tropentag 2007. University of Kasssel-Witzenhausen and University of Gottingen, Germany. October 9-11, 2007. pp. 1-4.

Pranav, D. P., Sachin, A. M. and Bhaskar, D. K. (2017). Fruit peel waste: characterization and its potential uses. Curr. Sc. 113(3): 444-445.

Pretel, M. T., Sanchez-Bel, P., Egea, I. and Romojaro. F. (2018). Enzymatic peeling of citrus fruits: factors affecting degradation of Albedo. Tree and Forestry Sc. and Biotech. 2: 52–59.

Morais, D. R., Rotta, M., Sargi, S. C., Bonafe, E. G., Suzuki, R. M., Souza, N. E., Matsushita, M. and Visentainer, J. V. (2017). Proximate composition, mineral contents and fatty acid composition of the different parts and dried peels of tropical fruits cultivated in Brazil. J. Braz. Chem. Soc. 28(2): 308-318.

Santos, D., Lopes da Silva, J. A. and Pintado, M. (2022). Fruit and vegetable by-products' flours as ingredients: A review on production process, health benefits and technological functionalities. LWT - Food Science and Technology 154 (2022) 112707: 1-11.

Shigetaka, I. (1977). Purification and characterization of a factor that stimulates tissue maceration by pectolytic enzyme. Phyto. 67(8): 994-1000.

Singh, A. P., Kim, Y. S., Wi, S. G. and Lee, K. H. (2003). Evidence of the degradation of middle lamella in a waterlogged archaeological wood. Holzfor. 57: 115-119.

Wongkaew, M., Kittiwachana, S., Phuangsaijai, N., Tinpovong, B., Tiyayon, C., Pusadee, T., Chuttong, B., Sringarm, K., Bhat, F. M., Sommano, S. R. and Cheewangkoon, R. (2021). Fruit characteristics, peel nutritional compositions

and their relationship with mango peel pectin quality. Plants. 10(6): 1148 (Online pp. 1-20).

Zerva, I., Remmas, N. and Ntougias, S. (2019). Biocatalyst potential of cellulose-degrading microorganisms isolated from orange juice processing waste. Bev. 5(1): 21-31.