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Characterization of Bacillus species from fresh fruits and their biodegradation potential in the reduction of heavy metal contents in banana juices.

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ABSTRACT

Fresh fruits are important sources of bioactive compounds that are vulnerable to post-harvest spoilage. Spoilt fruits are reservoirs of both microorganisms and heavy metals. The biological method of decontamination of heavy metals has been reported to be safer for the environment and living organisms. This study was aimed to isolate and characterize *Bacillus* species and determining their biodegradation potential of heavy metals. Selected fresh fruits comprising of oranges, lime, apples, and pineapples were bought from a fruit market in the Ibadan metropolis for the enumeration of *Bacillus* species using nutrients and De Man Rogosa Sharpe media. Identification of *Bacillus* species was done using phenotypic and molecular tests. Amylolytic screening was done using the starch agar plate method. Biodegradation of lead, iron, zinc, cobalt and copper were monitored inside pasteurized banana juice inoculated with *Bacillus haynesii* and *Bacillus subtilis* and uninoculated banana juice for a period of five days using analytical methods. Statistical analysis was carried out by means of one-way analysis of variance. A total of thirty-five *Bacillus* species were obtained in this study, of which *Bacillus cereus*, *Bacillus subtilis* and *Bacillus haynesii* were amylolytic; isolates identified molecularly are *Bacillus subtilis* MG850841, *Bacillus* spp.MG850846 and *Bacillus haynesii* PX520826, only lead and copper were reduced in both control and inoculated juices, more than 50% reduction was recorded. While there was no significant difference in decreasing iron, zinc, and cobalt content in both inoculated and uninoculated banana juices. This study observed that *Bacillus* species are abundant in fresh fruits and they can be harnessed for decontamination of certain objectionable metals in the environment.

Keywords: Amylolytic, Bacillus species, Biodegradation, Heavy metal

Introduction

Heavy metals are usually regarded as elements with higher density that of water. They include metalloids, like arsenic that can be dangerous at low exposure levels. This group includes less common heavy metals such as manganese iron, cobalt and arsenic due to their physical and biochemical characteristics Khalef *et al*., 2022). Heavy metals are difficult to break down and are not biodegradable, hence, they are more persistent in the environment than organic pollutants. Continuous buildup of heavy metals in soils can harm the ecosystem and unintentionally disrupt a number of physicochemical characteristics of the soil, including pH, anion/cation exchange, thermal and electrical conductivity, microbial ecosystems, and the mobility of heavy metal ions in the soil (Kim *et al*., 2019)

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The genus *Bacillus* are rod-shaped, aerobic or facultative anaerobes, spore-forming, and Gram-positive bacteria are used in bioremediation. The species are a diverse collection of bacteria most frequently found in soil and in other sources like water, air, water, vegeTables, and food, as well as from the intestines of humans and animals. *Bacillus* spp. have numerous uses in the industries, agriculture, environment, and health (Pham *et al*., 2022; Miljakovic *et al*., 2020). Bacteria like *Bacillus subtilis* have been utilized in the food processing and agriculture as food preservatives and biopesticides. They also create a number of secondary metabolites, including antibiotics (Caulier *et al*., 2019). Additionally, industrial enzymes such as lipases, proteases, amylases, and phytases may be found in *Bacillus* species including *Bacillus subtilis* and *Bacillus amyloliquefaciens*.

Numerous micronutrients, including trace elements like arsenic, lead, tin, zinc, copper, and iron are abundant in fruit juices, they gain access into the juices from soil, processing ingredients and equipment. Fruit juices both natural and artificial contain these pollutants whose concentrations must be managed. These minerals, often known as trace metals exhibit direct and indirect health benefits (Onyeneto *et al*., 2015). Several authors have reported the role of *Bacillus* species in decontamination of heavy metals in the environment, though few reports have been recorded relating to food or fruit samples (Karmakar, *et al*., 2024 and Alotaibi *et al*. (2021). Amylolytic *Bacillus* species which include the *Bacillus subtilis*, *Bacillus cereus, B. thuringiensis*, *B. sterothermophilus*, *B. megaterium*, *B. pumilus*, *B. licheniformis*, and *B. jeotgali* have been reported to remove heavy metals most effectively (Pranay *et al*., 2019). This study is aimed at determining the biodegradation potential of amylolytic strains of *Bacillus species* isolated from fresh fruits in the reduction of heavy metal.

Materials and Methods

Isolation and Identification of *Bacillus* species

The bacteria were isolated from fruits by using pour plate method (Aneja, 2009). Each banana fruit was macerated in a pre-sterilized blender and sterile distilled water was added to form a solution which was serially diluted from 10^{-1} to 10^{-5} dilutions, and then inoculated into a sterilized nutrient agar and Man Rogosa Sharpe media. The inoculated plates were incubated at 37°C for 24 hours for bacterial growth. After incubation, the morphologically different colonies of bacteria were isolated and sub-cultured to obtain pure cultures.

Phenotypic Characterization of Isolates

Presumptive colonies were subjected to various morphological and biochemical tests which include Gram staining, endospore staining, indole, oxidase, catalase and starch hydrolysis following the methods of Dimri *et al*. (2020)

Molecular Identification of Bacteria

DNA Isolation, Polymerase Chain Reaction and 16S RNA Sequencing.

An overnight pure culture of *Bacillus* species was transferred into a tube, and the cells were pelleted by centrifugation at 7,500 rpm for 10 minutes. The bacterial cells were washed with Tris buffer comprising of 10 mM Tris/HCl, 1 mM Na2EDTA, 0.1 M NaCl, and 0.1% Sarkosyl and subsequently resuspended in TE buffer with 10 mM Tris/HCl, 1 mM Na2EDTA.The procedure was completed following the method of Lu *et al*. (2018)

Screening of isolated samples for amylase production

Exactly 25g of nutrient agar was weighed and suspended in 1000 ml of distilled water in a conical flask, the solution was gently shaken boiled to dissolve completely and also facilitate complete homogenization. The solution was sterilized in an autoclave at 121°C for 30 minutes, after sterilization, the agar was allowed to cool in a control aseptic environment before being poured into sterilized petri dishes. The bacterial culture was diluted and spread on the sterile Starch agar plates, the plates

Table 1: Morphological and Biochemical characteristics of the isolates

Isolate	Gram's reaction	Shape	Endospore	OXIDASE	Catalase	INDOLE	Starch Hydrolysis
B1	+	Rod	+	_	+	+	+
B2	+	Rod	+	_	+	+	+
B3	+	Rod	+	_	+	+	+
B4	+	Rod	+	_	+	+	+
B5	+	Rod	+	_	+	+	+
B6	+	Rod	+	_	+	+	+
B7	+	Rod	+	_	+	+	+

Key: + Positive, - Negative

were incubated at 37˚C for 24 hours. After flooding the plates with 1% iodine solution, amylase-positive strains were determined by the presence of a clear zone of starch hydrolysis around the colony on the starch plates, while presence of blue color around the growth indicated negative result.

Treatment of banana pulp with *Bacillus haynesii* and *Bacillus subtilis*

Overripe banana was blended using a pre-sterilized blender, the blended sample was pasteurized at 60°C for 30 minutes to eliminate microorganisms present, it was left to cool to room temperature and aseptically transferred into five (5) 100 ml transparent sterile bottles. An inoculum corresponding to 10^{-5} each of *Bacillus haynensii and Bacillus subtilis* was transferred into different pasteurized banana pulp except the control. The five bottles were stored at room temperature for 7 days. Heavy metal composition of the banana juice was determined using Atomic Absorption Spectroscopy as described by Qadir *et al.* (2022).

Results

Bacterial strains were isolated from the overripe banana fruit samples; Table 1 shows the result of the morphological and biochemical characteristics of the *Bacillus* isolates Fig 1. Shows the phylogenetic tree of the *Bacillus* spp. isolated from the banana fruits and its relationship with other strains of *Bacillus* strains isolated from other sources. The amplicon products of the *Bacillus* species obtained in this study revealed expected 1500 base pairs as depicted in Plate 1, the sequences of six of the *Bacillus* species have been deposited in Gen Bank and four have been assigned accession numbers which are *Bacillus subtilis* MG850841, *Bacillus* spp. MG850846 and *Bacillus haynesii* PX520826 and *Bacillus coagulans* PX520825 now taxonomically referred to as *Heyndrickxia*. Figure 1 shows the phylogenetic relatedness of the isolates in this study to other *Bacillus* species from other sources deposited in the Genbank.. Table 2 and 3 depicts the concentration in the presence of different metals in the blended banana. The heavy metals detected during Spectrophotometry analysis of the fruits sample and their corresponding concentration after inoculation with the two *Bacillus* strains after days of incubation were shown in Tables 2 and 3 Among the five heavy metals, Copper (CU) and Lead (Pb) were found to have reduced in concentration for both samples inoculated with *Bacillus subtilis* and *Bacillus haynesii*, for the banana juice inoculated with *Bacillus subtilis* (SBC), Copper (Cu) concentration was found to have reduced from 2.021 to 0.115 representing a 94.31% reduction, while that of Lead (Pb) was found to have reduced from 2.422 to 0.064, this indicated

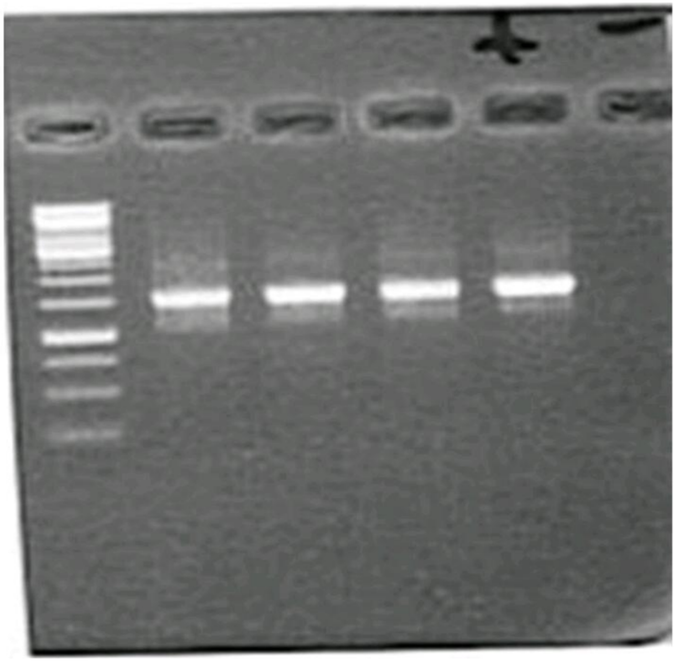

Plate 1: Gel Electrophoresis of the *Bacillus* species.

a 97.30% reduction. Also, for the sample inoculated with *Bacillus haynesii* (SBH), the concentration of Copper (Cu) decreases from 0.811 to 0.213 that is a 73.74% reduction in concentration, while that of Lead (Pb) was found to have reduced from 0.100 to 0.019 indicating 81.5% reduction.

In the present study, it is evident that both strains of *Bacillus* are effective bioremediator of Copper and Lead but unsuiTable bioremediator of both Zinc and Cobalt, it is however unclear why the *Bacillus* stains showed this activity (Table 4)

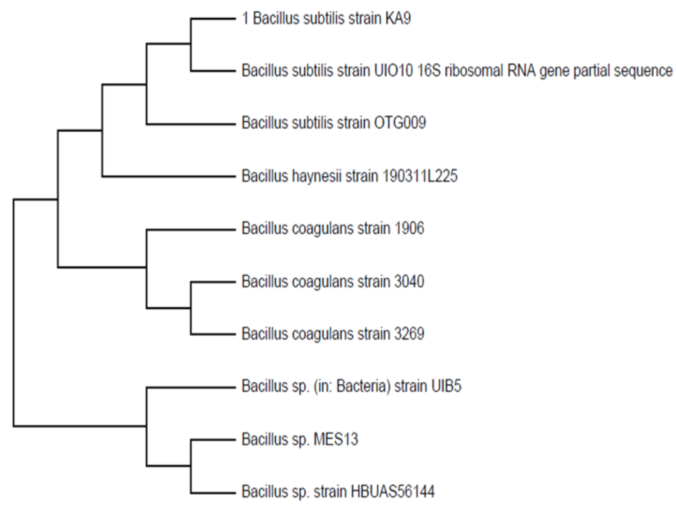

Figure 1: Phylogenetic tree of the *Bacillus* spp.

Discussions

In this study, isolates were characterized by genetic analysis and blast analysis of the sequences deposited at Genbank confirmed the isolates to be *Bacillus haynesii*, *Bacillus subtilis* and other *Bacillus* species. The occurrence of *Bacillus* species in fruits has been reported, though, only relatively few studies have reported isolation and characterization of *Bacillus* species from banana fruits. The sequences of *Bacillus* species isolated in this study have amplicons and blast

Table 2: Concentration of heavy metals in overripe banana (day zero)

Samples/Heavy metals (mg/kg)	SB	SBC	SBH
Iron	0.672	0.552	2.110
Copper	-0.099	2.021	0.811
Zinc	0.0034	-0.00022	-0.00986
Lead	0.289	2.422	0.100
Cobalt	0.044	0.104	0.078

Key
SB - Overripe banana
SBC - Overripe banana with *Bacillus subtilis*
SBH - Overripe banana with *Bacillus haynensii*

Table 3: Concentration of heavy metals in overripe banana (day five)

Samples/ Heavy metals(mg/Kg)	SB	SBC	SBH
Iron	1.018	2.021	1.330
Copper	0.142	0.115	0.213
Zinc	0.00167	0.00279	-0.01142
Lead	0.145	0.064	0.019
Cobalt	0.242	0.446	0.199

Key
SB - Overripe banana
SBC - Overripe banana with *Bacillus subtilis*
SBH - Overripe banana with *Bacillus haynensii*

Table 4: Analysis of % Reduction/Increase in Heavy Metal Concentration

METAL	Day 0			Day 5			% Reduction/Increment		
	SB	SBC	SBH	SB	SBC	SBH	SB	SBC	SBH
Iron	0.67	0.55	2.11	1.02	2.02	1.33	51.49	266.12	36.97
Copper	-0.10	2.02	0.81	0.14	0.12	0.21	43.43	94.31	73.74
Zinc	0.0034	-0.022	-0.099	0.0017	0.0028	-0.013	50.88	1168.18↑	15.82
Lead	0.29	2.42	0.10	0.15	0.06	0.02	49.83↓	97.34↓	81.5
Cobalt	0.04	0.10	0.08	0.24	0.45	0.19	450↑	328.85↑	155.13

analysis of more than 97% with isolates in Genbank similar to what has been reported by several authors (Huo *et al*.,2021; Koilybayeva *et al*., 2023)

Fruits are essential components of human diet; efforts and attention should be placed on its safety to avoid contamination. *Bacillus amyloliquefaciens* have been isolated from the surface of banana fruits and were found to possess in vitro antagonism against pathogens (Alvindia *et al.,* 2009. *Bacillus* species have equally been isolated from date palm in Saudi Arabia (Hamed *et al.,* 2024) Banana is a staple fruit in the world and its most desirable because of its taste and nutritional properties, it is a perishable food due to its peel and high sugar content, hence, microbes found a home in banana.

The reduction in heavy metal concentration was due to the ability of the two species to accumulate both Cu (II) and PB (II) ions (Sabae *et al*., 2016). However, in the un-inoculated sample that served as the control sample (SB), it was discovered that Lead (Pb) showed a 49.83% reduction in concentration while Copper showed an increase of 43.43% in concentration.

From the result, the concentration of Iron (Fe) increases from 0.552 to 2.021 in the sample inoculated with *Bacillus subtilis* (SBC), while the concentration of Iron (Fe) decreases from 2.110 to 1.330 for the sample inoculated with *Bacillus haynesii* (SBH). However, the concentration of Zinc (Zn) increases from -0.00022 to 0.00279 in the sample inoculated with *Bacillus subtilis*

(SBC), while the concentration of Zinc (Zn) increases from -0.00986 to -0.01142 for the sample inoculated with *Bacillus haynesii* (SBH). Also, the concentration of Cobalt (Co) increases from 0.104 to 0.446 in the sample inoculated with *Bacillus subtilis* (SBC), while the concentration of Cobalt (Co) increases from 0.078 to 0.199 for the sample inoculated with *Bacillus haynesii* (SBH). Similarly, in the control sample SB and on the fifth day of analysis Iron (Fe) increased in concentration by 51.49%, concentration of Zinc reduced by 50.88% while Cobalt reveals an astonishing 450% increase in concentration. The decontamination of heavy metals in banana pulp in this study is attributed to the ability of *Bacillus* species to serve as biocontrol agents and bioremediator which have been reported by several authors (Jamal and Ahmad, 2022 and Qadir *et al.,* 2022).

Syed and Chinthala (2015) reported that the use of *Bacillus licheniformis* NSPA3, *Bacillus cereus* NSPA8, *Bacillus subtilis* NSPA13 to detoxify lead through bioabsorption of copper and chromium was quiet low. Similarly, *Bacillus subtillis* 7611 was effective in the removal of lead followed by lower removal rates of cadmium and copper in water (Rocco *et al*., 2024)

Bacillus species detoxify the environment by absorbing heavy metals through distinctive processes: Biosorption, bioaccumulation and bioprecipitation or biomineralization. The process of bioaccumulation of heavy metals is applicable to detoxification of heavy metals in food. Heavy metals found their way into food through soil, industrial waste, mining, agricultural practices, food processing, equipments and additives. There is need to reduce the intake of heavy metals as it affects the vital organs of the body. (Jomova *et al.,* 2015).The application of *Bacillus* species for bioremediation promises to be a cost effective, environmentally sustainable and ecofriendly alternative.

Conclusions

The decontamination of heavy metals in banana pulp in this study is attributed to the ability of *Bacillus* species to serve as biocontrol agents and bioremediator. In conclusion, this study have shown that fresh fruits are rich in *Bacillus* species, and that these bacteria possesses potential for use in the decontamination of certain “objectionable metals” in the environment**.**

References

Alotaibi, B. S., Khan, M., and Shamim, S. (2021). Unraveling the Underlying Heavy Metal Detoxification Mechanisms of *Bacillus* Species. *Microorganisms*, *9*(8), 1628.

Alvindia, D., and Natsuaki, K. T. (2009). Bio-control activities of *Bacillus amyloliquefaciens* DGA14 isolated from banana fruit surface against banana crown rot causing pathogens. *Crop Protection*, *28*(3), 236-242.

Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., and Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Frontiers in microbiology*, *10*, 302

Dimri, A.G., Chaudhary, S., Singh gram-positive and gram, D., Chauhan, A. and Aggarwal, M., 2020. Morphological and biochemical characterization of food borne -negative bacteria. *Science Archives*, 1(1), 16-23.

Hu, Q., Fang, Y., Zhu, J., Xu, W. and Zhu, K., (2021). Characterization of *Bacillus* species from market foods in Beijing, China. *Processes*, *9*(5), p.866.

Kim, J., Kim, Y. S, and Kumar, V. (2019). Heavy metal toxicity: An update of chelating therapeutic strategies. *Journal of Trace elements in Medicine and Biology*, *54*, 226-231.

Koilybayeva, M., Shynykul, Z., Ustenova, G., Abzaliyeva, S., Alimzhanova, M., Amirkhanova, A., Turgumbayeva, A., Mustafina, K., Yeleken, G., Raganina, K. and Kapsalyamova, E. (2023). Molecular characterization of some Bacillus species from vegeTables and evaluation of their antimicrobial and antibiotic potency. *Molecules*, *28*(7), p.3210.

Jamal, Q. M. S, and Ahmad, V. (2022). Lysinibacilli: A biological factories intended for bio-insecticidal, bio-control, and bioremediation activities. *Journal of Fungi*, *8*(12), 1288.

Jomova, K., Alomar, S. Y., Nepovimova, E., Kuca, K., and

Valko, M. (2015). Heavy metals: toxicity and human health effects. *Archives of toxicology*, *99*(1), 153-209.

Karmakar, D., Magotra, S., Negi, R., Kumar, S., Rustagi, S., Singh, S., Rai, A.H., Kour, D. and Yadav, A.N., (2024) Bacillus species for sustainable management of heavy metals in soil: current research and future challenges.

Kim, J., Kim, Y. S, and Kumar, V. (2019). Heavy metal toxicity: An update of chelating therapeutic strategies. *Journal of Trace elements in Medicine and Biology*, *54*, 226-231.

Khalef, R. N., Hassan, A. I., & Saleh, H. M. (2022). Heavy metal's environmental impact. In *Environmental impact and remediation of heavy metals*. IntechOpen

.Lu, Z., Guo, W., & Liu, C. (2018). Isolation, identification and characterization of novel Bacillus subtilis. *Journal of Veterinary Medical Science*, *80*(3), 427-433.

Miljakovic Dragana, Jelena Marinković, and Svetlana Balešević-Tubić. (2020). "The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegeTable crops." *Microorganisms* 8(7), 1037.

Onyeneto, T., Nwachukwu, I., and Nwogwugwu, N. U. (2015). Trace metals and contaminants in commercial fruit juice sold in south eastern states, Nigeria. *Annals of Biological research*, *6*(10), 15-19.

Pham, V. H., Kim, J., Chang, S., and Chung, W. (2022). Bacterial biosorbent, an efficient heavy metals green cleanup strategy: prospects, challenges, and opportunities. *Microorganisms*, *10*(3), 610.

Pranay, K., Padmadeo, S.R., Jha, V. and Prasad, B., (2019). Screening and identification of amylase producing strains of Bacillus. *J Appl Biol Biotechnol*, *7*(4), 57-62.

Qadir, A., Hussain, M. M., Zafar, M. S. B., Hameed, M. A., and Farooqi, Z. U. R. (2022). Unveiling the potential of Bacillus sp. in bioremediation and biocontrol. In *Bacilli in Agrobiotechnology: Plant Stress Tolerance, Bioremediation, and Bioprospecting* (pp. 519-538). Cham: Springer International Publishing.

Rocco D. H., Freire, B. M., Oliveira, T. J., Alves, P. L. M., de Oliveira Júnior, J. M., Batista, B. L., and Jozala, A. F. (2024). Discover Applied Sciences.

Sabae S., Refat, B. M., and Tahoun, U. M. (2016). Biosorption of Copper, Lead and Cadmium using bacterial biomass of *Pseudomonas putida* isolated from El-Manzala Lake, Egypt. *African J. Biol. Sci*., 12 (1): 37-51

Syed, S., & Chinthala, P. (2015). Heavy metal detoxification by different *Bacillus* species isolated from solar salterns. *Scientifica*, *2015*(1), 319760.

Zhang, S., Han, S., Zhang, M., and Wang, Y. (2018). Noninvasive prenatal paternity testing using cell-free fetal DNA from maternal plasma: DNA isolation and genetic marker studies. *Legal Medicine*, *32*, 98-103.