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Assessment of Zinc and Sulphur Solubilizing Plant Growth Promoting Organisms for Potential Use for Lipid Hydrolysis and Cellulose Degradation in Compost for Enhanced Food Quality

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ABSTRACT: The use of zinc and Sulphur solubilizing bacteria to improve compost will help in the mineralization of these vital elements during its production process. Hence, the objective of this paper was to evaluate the assessment of the ability of some plant growth promoting bacteria to solubilize zinc and sulphur and hydrolyze starch and lipid and degrade cellulose in compost using appropriate standard techniques. Data obtained show out of 15 isolates screened for zinc and Sulphur solubilizing ability, only five were able to solubilize zinc and Sulphur as well as hydrolyze starch, lipid and degrade cellulose. The five isolates were selected and identified as *Enterobacter kobei, Thiobacillus sp., Aspergillus udagawae, Aspergillus terreus and Meyerozyma guillermondii*. *Meyerozyma guillermondii* showed the greatest ability to solubilize zinc and sulphur with solubilisation efficiency of 4.6 and 4.4, respectively. The five selected isolates showed great potential for possible use to fortify compost as a way of enriching the compost with zinc and sulphur for plant uptake.

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Compost is the product of breakdown of organic materials through the activities of microbes. The length of time used for composting is one of the critical way of measuring the success in compost making. The shorter the period of composting, the higher the success rate of the composting. Composting can occur naturally but it may require longer duration, the duration of composting greatly depends on the nature of composting materials. Microorganisms are considered as one of the major factor that can enhance or accelerate the period of composting and the utilization of agricultural beneficial organism such as plant growth promoting organisms can help not only to acceleration the composting time but will supplement some essential nutrients deficient in plants and as well lead to the restoration of degraded soil when applied (Sumiyati, *et al*. 2022). Soil degradation associated with rapid depletion of nutrients and soil organic carbon stocks is severely affecting soil productivity. Resource-constrained farmers often cannot afford external inputs to maintain soil optimum productivity under intensive cultivation. This problem

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is further exacerbated by erratic rainfall due to climate change. Over time, various interventions have been used to enhance agricultural productivity of which the use of inorganic inputs is one. A more sustainable approach is the use of organic amendments such as compost. Compost fortification can solve issues associated with low micronutrients such as zinc and sulphur which has led to empty harvest. Furthermore, the inclusion of plant growth promoting bacteria (PGPR) can help to address the problem of fertility decline by improving the solubilisation of complex minerals, enhance mineralization of micronutrients, produce phytohormones for plant growth enhancements as well as reduce compost maturity period((Bhatt and Maheshwari, 2020).Plant growth promoting organisms refers to those microorganisms which is capable of solubilizing insoluble nutrient in the soil or compost there by making them available to plants, they can as well promote essential phytohormones that can enhance plant growth and development (Akintokun *et al*.2019). The utilization of plant growth promoting organism especially zinc solubilizing and sulphur metabolizing bacteria to fortify compost will help to make these essential nutrient available for plant uptake and as well promote the production of sulphur and zinc rich food (Srithaworn *et al,* 2023). This will not only increase yield and improve crop quality but will also address issues associated with both food insecurity and hidden hunger. Initial decomposition can be improved with the addition of a soil microbe inoculant to increase the degree of humification and improve the composting process, also application of effective microorganisms in compost increases the soil macro and micronutrient content (Upadhayay *et al*. (2022). Hence, the objective of this paper was to evaluate the assessment of the ability of some plant growth promoting bacteria to solubilize zinc and sulphur and hydrolyze starch and lipid and degrade cellulose in compost

MATERIALS AND METHOD:

Isolation of PGPR: This group of bacteria was isolated from rhizosphere soils of cowpea and maize, swine dung and compost, these were screened in-vitro for their effectiveness in Zn solubilization and sulphur oxidization using standard procedures. The most efficient isolates were purified and identified using molecular method. These will be used for compost fortification. Zinc and Sulphur solubilisation: A loop full of bacterial culture of isolates were diluted in sterile distilled water using serial dilution method individually and spread on to petri plates containing liquid salt agar medium having insoluble sources of ZnO and $ZnCO_3$ separately. After incubation, the diameter of bacterial colony and halozone around colony were measured and the values were calculated using solubilizing index formula SI= (Colony 54 diameter + Halozone diameter / colony diameter) (Edi-Premono *et al.,* 1996). The ZSB isolates showed maximum value of solubilizing index named as RRT with serial number 1 to 38 and used for further study.

*Sulphur oxidizing bacteria***:** The isolates were further screened on the basis of production of sulphate ion (SO4 -2) The amount of sulphate ions (SO4 -2) produced during growth of sulphur-oxidizing bacteria on Thiosulphate broth medium was determined spectrophotometrically. A loopful of 48 hrs old culture of each isolate was inoculated into 10 ml of Thiosulphate broth. All the inoculated tubes were incubated at 30ºC for 7 days. After 7 days of incubation, the broths were centrifuged at 15000 rpm for 10 minutes to separate the supernatant from the cell growth. Sulphate production was measured by adding 1:1 barium chloride solution (10%, w/v) with bacterial culture supernatant followed by mixing the suspensions vigorously (Cha *et al*., 1999). A resulting white turbidity due to barium sulphate formation was measured at 450 nm. The values obtained were compared with the sulphate standard curve.

*Cellulose degrading ability***:** This was performed by streaking the test isolates on a cellulose congo red agar media consisting of 0.5g of KH_2PO_4, 0.25g MgSO4, 2g of cellulose, 15g of agar-agar, 0.2g of congo red, 2g of gelatin and 1L distilled water. The use of congo red as an indicator provides the basis for a rapid and sensitive screening test for cellulolytic bacteria. Colonies showing discolouration of congo red was taken as positive result for cellulose degrading bacteria and they were preserved for further studies. The hydrolysis capacity was estimated by calculating the ratio of the diameter of the clearing zone and the colony (Acharya *et al*, 2015).

Starch hydrolysis: One litre of Starch agar consisting of Beef extract(3g), soluble starch (10g), Agar-agar (12g) was prepared and sterilized at 121ºC for I5 minutes and it was then allowed to cool and then dispensed aseptically into the sterile petri dish and then allowed to solidify. After solidifying, a fresh pure culture of the test bacteria was spot inoculated on the surface of the agar medium and incubated for 24 to 48 hours at 35ºC. After incubation, the surface of the agar was flooded with iodine solution. Appearance of clear zone around the bacteria colony indicated that the starch has not been hydrolysed (negative result) while the non-appearance of the clear zone was taken as positive results.

*Lipid hydrolysis***:** Lipid agar consisting of Casein (10g), yeast extract (35g), Spirit blue (0.15g),

agar(12g) and all dissolved in 1 litre of distilled water. The mixture was heated to boiling so as to dissolve the medium completely, it was then sterilized at 121ºC for 15minutes, and 15ml of lipase substrate was added while agitating so as to obtain even distribution. The media was then dispensed into a sterile petridish and was allowed to solidify. The test isolates were streaked on the plates and incubated for 3 days. After incubation, the plates were examined for clear zones around the colonies. The presence of clear zone around the colony was taken as positive result while its absence was taken as negative result.

RESULTS AND DISCUSSION

A total of 15 zinc solubilising and sulphur metabolising microorganisms (bacteria and fungi) were isolated from rhizosphere soils of cowpea and maize, compost and swine dung. Five most efficient microbial isolates were selected based on their zinc solubilising and sulphur metabolising efficiency (table 1). The isolates were further screened for cellulose degrading ability, lipid and starch hydrolysis. Some of the results were able to show multiple abilities. The results of cellulose degrading ability, lipid and starch hydrolysis were presented in Table 2. Molecular identification revealed the identity of the five selected organisms as *Enterobacter kobei, Thiobacillus sp., Aspergillus udagawae, Aspergillus terreus and Meyerozyma guillermondii* (table 3 and plates 1a and 1b).

Utilization of Zinc and sulphur solubilizing bacteria to improve compost quality will help to produce compost rich in zinc and sulphur for enhance uptake by plants when applied. This will enhance the micronutrient level in the crops and human diet. Fifteen isolates were assessed for their ability to solubilize zinc and sulphur so as to know if they will be deployed in compost fortification. Some of these isolates were able to solubilize zinc and sulphur which is an indication that they will be useful in enhancing the mineralization of zinc and sulphur components of compost for easy plant uptake.

Table 1: Zinc and Sulphur metabolising abilities of the Isolates

S/N	Isolate code	Colony diameter (Zinc)	Colony diameter (Sulphur)	Halo zone (zinc)	Halo Zone (Sulphur)	Solubilization Efficiency (Zinc)	Solubilization Efficiency (Sulphur)
1	SA 3.2	0.5	-	1.4	-	3.8	-
2	CI 4	0.6	-	1.6	-	3.7	-
3	MI 3	0.6	-	1.5	-	3.5	-
4	Z2.3	0.6	0.9	1.4	2.8	3.3	4.11
5	M1.4	0.5	1.0	1.8	3.4	4.6	4.4
6	C1.4	0.5	1.2	1.0	3.2	3.0	3.7
7	Z2.1	0.5	1.2	2.2	2.5	3.0	3.1
8	CI 3	-	-	-	-	-	-
9	CI 2	0.6	1.2	1.6	3.5	3.66	-
10	SA1.3	0.7	1.4	1.3	2.2	2.9	2.6
11	M1.4	-	0.8	-	1.2	-	2.5
12	SA 13	0.6	1.5	1.0	3.5	2.6	3.3
13	C 3.6	0.5	0.6	1.2	2.0	3.4	4.3
14	M 1.2	-	-	-	-	-	-
15	SA 3.2	-	-	-	-	-	-

Table 2: Cellulose degrading, starch and lipid hydrolysis abilities of the Isolates

S/N	Isolate code	Cellulase degrading ability	Starch Hydrolysis	Lipid hydrolysis
1	SA 3.2	-	+	-
2	CI 4	-	-	-
3	MI 3	-	-	+
4	Z2.3	+	+	+
5	M1.4	-	-	-
6	C1.4	+	+	
7	Z2.1	-	+	-
8	CI 3	-	-	+
9	CI 2	-	-	-
10	SA1.3	-	-	-
11	M1.4	+	+	+
12	SA 1.3	-	-	-
13	C 3.6	+	+	+
14	M 1.2	+	+	+
15	SA 3.2	-	-	-

Table 3: Molecular identification of selected zinc solubilising and sulphur metabolising organisms

S/N	Isolate code	Source	Molecular identification
1.	C1.4	Cowpea rhizosphere	*Meyerozyma guillermondii*
2.	C3.6	Cowpea rhizosphere	*Enterobacter kobei*
3.	M1.2	Maize rhizosphere	*Thiobacillus sp.*
4.	M1.4	Maize rhizosphere	*Aspergillus udagawae*
5.	Z2.3	Swine dung	*Aspergillus terreus*

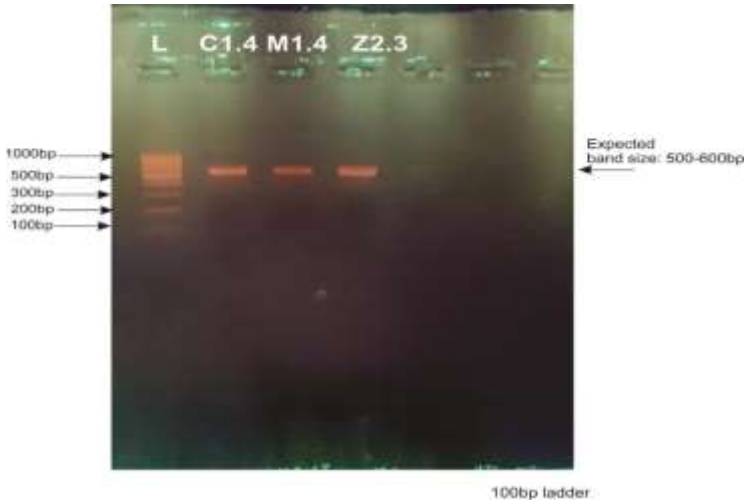

Plate 1a: Agarose gel electrophoresis of DNA fragments of isolates C1.4. M1.4, Z2.3 specifically amplified from genomic DNA with 16S rDNA primers

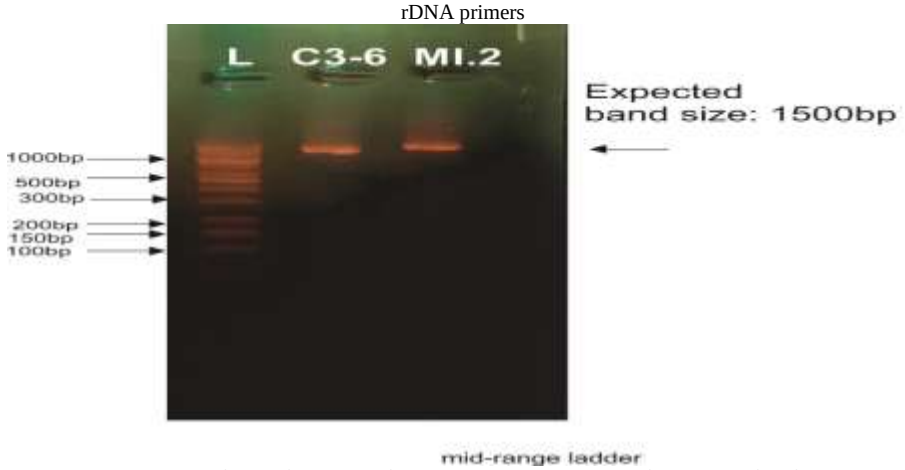

Plate 1b: Agarose gel electrophoresis of DNA fragments of isolates C3.6 and M1.2 specifically amplified from genomic DNA with 16S rDNA primers.

This agrees with Sumiyati *et al*. (2022) which reported that the utilization of local microorganisms can reduce composting time and enhance compost quality. Five isolates with multiple abilities identified as *Meyerozyma guillermondii*, *Enterobacter kobei*, *Thiobacillus sp*., *Aspergillus udagawae* and *Aspergillus terreus* were selected and kept for further studies following their efficiency in solubilizing zinc and sulphur. *Aspergillus udagawae* (M1.4) which is one of the microbial isolates showed the highest zinc and Sulphur solubilizing efficiency with zinc and Sulphur solubilizing efficiency of 4.6 and 4.4, respectively. Zn-solubilizing bacteria are very useful in enhancing zinc availability in the soil (Upadhayay *et al*. (2022), Saravanan, *et al*.(2011). Several bacteria such as *Bacillus* sp., *Pseudomonas* sp., Rhizobium, Azospirillum, *Burkholderia cenocepacia*, *Serratia liquefaciens* and *S. marcescens* etc have been reported to solubilize zinc.

Srithaworn *et al* (2023) reported that zinc-solubilizing rhizobacteria can convert insoluble zinc to soluble form and increase Zn bioavailability in soil, which help reduce Zn deficiency in crops. Several zinc-solubilizing bacteria, such as *Pseudomonas protegens*, *Bacillus megaterium* (Bhatt and Maheshwari, 2020) and *Bacillus altitudinis* (Kushwaha *et al.*, 2021), have been reported as plant growth-promoting bacteria due to the production of plant hormones and growth

factors and making zinc available to plants, which is beneficial for plant growth (Garcia and Kniffin, 2018).

The isolates were also assessed for their ability to solubilize lipid, cellulose and starch which are some of the essential components that needs to be degraded by the isolates for them to be considered for possible deployment in reducing composting time and improving compost quality. The five selected isolates were able to degrade cellulose as well as hydrolyse starch and lipid. This agrees with the findings of Ohkuma, (2003) which reported that Microorganisms such as bacteria and fungi are responsible for the cellulose degradation in the soil. Maki *et al*. (2011) reported that despite the large number of cellulose producer, there is still insufficient number of microorganisms that can produce reasonable amount of enzymes that can efficiently degrade cellulose to fermentable form.

Conclusion: The five identified isolates were able to solubilize zinc and sulphur as well as degrade cellulose and that's an indication that they have potentials that will be very useful in compost fortification for reduced composting time and enhanced Zn and Sulphur mineralization for uptake by plant so as to improve plant products. The isolates can enhance the uptake of essential micronutrients by plants and in turn enhance crop productivity and crop product quality.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the first author.

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