Plant Archives Vol. 18 No. 2, 2018 pp. 1455-1462 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PRODUCTION OF ECOFRIENDLY BIOFERTILIZERS PRODUCED FROM CRUDE AND IMMOBILIZED ENZYMES FROM *BACILLUS SUBTILIS* CH008 AND THEIR EFFECT ON THE GROWTH OF *SOLANUM LYCOPERSICUM*

Adetunji Charles Oluwaseun[1,2]*, Paomipem Phazang[2] and Neera Bhalla Sarin[2]

[1]Landmark University, Department of Biological Sciences, Applied Microbiology, Biotechnology and Nanotechnology Laboratory, P.M.B. 1001, Omu Aran, Kwara State.

[2]Jawaharlal Nehru University, School of Life Sciences, Laboratory of Genetic Manipulation for Stress Alleviation and Value Addition in Plants, New Delhi - 110 067, India.

Abstract

This work was performed in order to determine the effect of crude keratinase enzyme and the immobilized keratinase enzyme on the biodegradation of chicken feather wastes and to evaluate their effect on the growth of tomato plant. Moreover, the effect of variably formulated biofertilizer on soil enzymatic activity and soil carbon and soil respiration when compared to chemical fertilizer using different assay techniques. The order of bio-fertility activity is BCKE > BIKE >positive control. Additionally, the biofertilizer produced from the immobilized enzymes showed a significant difference on all the tested growth parameters like shoot length, root length, number of leaves, area of leaves and wet and dry biomass and enhanced the chlorophyll content of *Solanum lycopersicum* when compared to the control. After the 60th day period of experiment, the biofertilizer formulation from BCKE showed the highest value of 374 mg CO_2 kg^{-1} soil hr^{-1} followed by BIKE with a value of 298 mg CO_2 kg^{-1} soil hr^{-1} as against the positive control (NPK fertilizer) that had a value of 84 mg CO_2 kg^{-1} soil hr^{-1} . The level of organic carbon content also showed that the biofertilizer treated soil exhibited increased soil carbon content and improved soil enzymatic activity when compared to the NKP treated soil. Therefore, this work has established that biofertilizer derived from microbial biodegradation of feather wastes could be utilized as a permanent replacement to the high cost and environmental unfriendly fertilizer. This will also go a long way in the total reduction of environmental pollution and a sustainable green technology for solid wastes management.

Key words : Environmental unfriendly fertilizer, waste management, environmental pollution, *Bacillus subtilis*.

Introduction

Keratinase enzyme is a unique type of protease normally utilized in the breaking-down of hard and insoluble keratin substrates. The keratinase enzyme of microbial origin has been documented as one of the most utilized enzymes with a lot of biotechnological potential in various sectors like environment, industry, agriculture (Fang *et al*., 2017). Their uniqueness is based on the fact that they have the capability to highly hydrophobic keratinous substances as well as their stability and their capability to break the disulfide bonds cross-linked proteins (Liu *et al*., 2014). The microbial secreted keratinase can be produced from the utilization of cheap fermentation substrates like a chicken feather and the remaining biodegraded substrates can be converted into useful and value-added products like cheap and nutritious value-added animal feeds as well as biofertilizer (Brandelli *et al*., 2010). Currently, the keratinolytic proteases from microorganism are more prefer to chemically synthesized protease because of their economic feasibility, excellent thermostability, and their higher rate of catalytic activity. This form the major reason behind their greater demand in commercial market and the industry (Fang *et al*., 2016).

The utilization of chemical for a subset of agrochemicals has added tremendously to the high rate and increase in food production globally (Adetunji *et al*., 2018). However, a lot of detrimental effects has been

***Author for correspondence :** E-mail: adetunjicharles@gmail.com

reported whenever agricultural input like chemically synthesized fertilizers is applied. Some of these has created a lot of health hazards, and environmental pollution in the soil, water, and air (Adetunji *et al*., 2017). Therefore, there is a need to search for an alternatives green, clean, cheap and eco-friendly sustainable organic fertilizers most especially from microbial origin. This might be linked to the fact that application of microorganism is abundant in nature, they have a high capacity to biodegrade agricultural wastes and helps interactions within the agro-ecosystem that is important for both agricultural production and environment protection (Mondal *et al*., 2017).

A lot of agricultural and industrial waste product is discharged into the environment after utilizing the major product of significant. Chicken waste form one of the major wastes that are discharged into the environment due to the high consumption of chicken meat and their high industrial demand globally. The yearly generated chicken wastes have been estimated around 8.5 million tons (£aba *et al*., 2018). The disposal of these chicken wastes posse a lot of ecologies and economic challenges most especially in the developing countries. Moreover, most of these chicken wastes possess a very hard keratin which poses a look of difficulties during their disposal through the process of incineration or sent to landfill (Kopec *et al*., 2014). Therefore, there is a need to look for a sustainable waste management through biotechnology for effective bioconversion of these chicken wastes into biofertilizer (Adetunji *et al*., 2012).

The world population has been stipulated to increase drastically to 9 billion in 2030. Therefore, there is a need to increase the production of healthy, safe, nutritious food in order to meet the demand of this ever-increasing population (FAO, 2017). There is a need to revisit the constant dependent on chemical fertilizer normally used in the soil management due to the issue of food safety, pesticide residue, and food security. In view of the aforementioned, there is a need implement a policy that will support the application of beneficial microorganisms in the production of biofertilizers and their end-products derived from biodegradation of agricultural wastes (Bhardwaj *et al*., 2014). Biofertilizers has a lot of advantages because they increase nutrient and water uptake, resistance to abiotic and biotic stress, improved soil fertility, increase in yield and growth of the plant and play a key role in the sustainability of soil health (Rubin *et al*., 2017). Thereby protecting the ecosystem and give maximum benefit to the farmers due to the cost-effectiveness of biofertilizers when compared to the chemical fertilizers (Schütz *et al*., 2018).

The eating of fruit and vegetable-based diets has been documented to minimize the consequence of diet-related chronic diseases which has led to the high rate in the number of death recorded worldwide (Ahmed *et al*., 2018). Fruits and vegetables contain necessary micronutrients required for healthier diets (Schreinemachers *et al*., 2018). The World Health Organization has recommended a minimum intake of 400 g per day to avoid chronic diseases and provide necessary micronutrients (WHO, 2015). Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops that is consumed every day for the most part of the world. Moreover, Most tomatoes farmers normally record a high level of losses due to instability experience in the climate also cumulates to the high spoilage rate documented whenever pest and diseases attack tomato during production as well as the high level of soil infertility (Etissa *et al*., 2013).

Therefore, the aims of the present study were to compare the effectiveness of the keratinase enzyme and the immobilized keratinase enzyme in the biodegradation of chicken feather wastes and to evaluate their effect on the growth of tomato plant. Moreover, we determined the impact of the biofertilizer produced from chicken waste applied on the soil enzymatic activity and soil carbon and respiration when compared to chemical fertilizer.

Materials and Methods

Microorganisms and growth conditions

The *Bacillus subtilis* (CH008) utilized in this study was isolated from the feather-dumping site of Landmark University Farm. The *Bacillus subtilis* (CH008) was cultured on nutrient agar and incubated at 37°C for 48 h. The culture was stored at 4°C for further experiment.

Inoculum development

The inoculum seed was prepared a liquid cultural, which has 1% sterilized feather meal in addition to 0.2% yeast extract and was later incubated at 37°C using a rotatory incubator at 100 r/min for a period of one day.

Production of keratinase enzyme

The fermentation medium used for the production keratinase enzyme contained 6 g/L of $NaNO_3$; 6 g/L of NaCl; 0.3 g/L of $CaCO_3$; 2 g/L of KH_2PO_4; 0.15 g/L of $MgSO_4$, 0.03 g/L of $FeSO_4.7H_2O$, 0.03; 20 g/L of chicken feather in 100 mL flask and was sterilized at 121°C for 15 min. Liquid fermentation was carried out for a period of 120 hours at 37°C on a rotatory shaker at 150 rpm. The crude keratinase was separated from the fermentation broth by centrifugation at 5000 rpm at 10°C for 20 min. The supernatant collected was then membrane

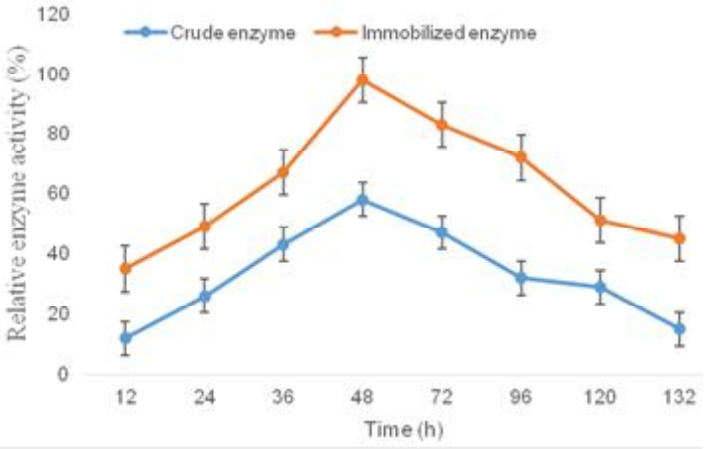


Fig. 1 : Effect of time on keratin biodegradation by *Bacillus subtilis* (CH008). The mean values and standard error of three replicates are presented.

filtered and stored at 4 °C.

Immobilization of *Bacillus subtilis* (CH008) crude enzyme by Entrapment in Ca-alginate

The immobilization of the crude enzyme from *Bacillus subtilis* (CH008) was carried out using a protocol developed by El-Borai *et al*. (2013). Autoclaved 3% sodium alginate prepared in distilled water was used for entrapping the keratinase enzyme. The crude enzyme was then added to the sodium alginate solution making the final concentration as 3%. This resultant mixture was dropped gently onto a 250 mL flat bottom conical flask containing the cross-linked solution made of 3% calcium chloride. The calcium alginate gel entrapping crude keratinase enzyme was confirmed by the formation of spherical beads with an average size of 2 to 3 mm. The mixture was placed on a rotatory shaker for a period of 2 hours at 100 rpm and was allowed to stand still for a period of 2 hours for proper solidification. The filtrate present was later decanted and the beads were was sieved to remove the presence of unbound enzymes and it was later stored at 4°C.

Preparation microbial hydrolysis of the chicken feather to biofertilizer

This was carried out following the protocol developed by Adetunji *et al*. (2012) with little modification. It was carried out in a bioreactor connected to an air pump for effective distribution and supply of aeration into the bioreactor. The bioreactor consists of three different treatments, with immobilized enzyme, crude enzyme, and only basal media, respectively. The chicken feather was used as a substrate in addition to the basal media containing; 6 g/L of $NaNO_3$; 6 g/L of NaCl; 0.3 g/L of $CaCO_3$; 2 g/L of KH_2PO_4; 0.15 g/L of $MgSO_4$, 0.03 g/L of $FeSO_4.7H_2O$, 0.03; 20 g/L of chicken feather. The basal media was autoclave at 121°C for 15 minutes. The submerged fermentation was carried out for a period of 72 hours after which the fermented broth was discarded and oven dried for two days at 45°C. The fermented feather from the different treatments was later ground to smaller size ranging between 1 to 2 mm and they were later coded accordingly; 1) Biofertilizer produced from chicken feather degraded by the crude keratinase enzyme (BCKE); 2) Biofertilizer produced from chicken feather degraded by immobilized keratinase enzyme (BIKE).

Evaluation of the keratinolytic activity of *Bacillus subtilis* (CH008)

This was performed by adding 2 mL of an immobilized and crude enzyme, 3 mg of biodegraded feather powder, 5 mL of phosphate buffer pH (7.0) while the combination of phosphate buffer and the biodegraded feather without any enzyme constitute the control. The reaction was carried out on a rotatory shaker at 150r/min at 45°C for 4 hours. The reaction was put to an end by adding 3 mL of 10% trichloroacetic acid and the precipitated protein was obtained by centrifugation at 5000 r/min for 15 min. The increase in absorbance at 280 nm from the cultural filtrate of the control to the test samples was determined as the release of protein and it was changed into keratinase units.

The effect of incubation on keratinase production

The influence of incubation period on the activity of keratinase production was studied by determining the residual enzyme activity after a reaction time of 12h, 24 h, 36, 48 h, 72 h, 96 h, 120 h and 132h respectively.

Soil sampling and soil enzyme activities

The soil was collected from 20 cm depth randomly from agriculture farmland where pepper and tomatoes were planted at three different plots. The freshly collected soil was mixed and sieved with using 2-mm mesh for the removal of obvious plant materials. The sieved soil was utilized for the assessment of various soil enzymes. The soil was later treated with biofertilizers (BCKE and BIKE) at the rate of 1.0 g fermented chicken substrate/pot while NPKF was used as positive control and unfermented chicken substrate without any enzyme degradation served as the negative control. Further, 2 g of the treated soil was weighed into a glass test tube containing different buffer solutions for different assays. The different supernatant obtained was then incubated at different temperatures for different enzymatic assays (Phosphatase activity; catalase activity; cellulase activity; urease activity) determined by colorimetric methods using

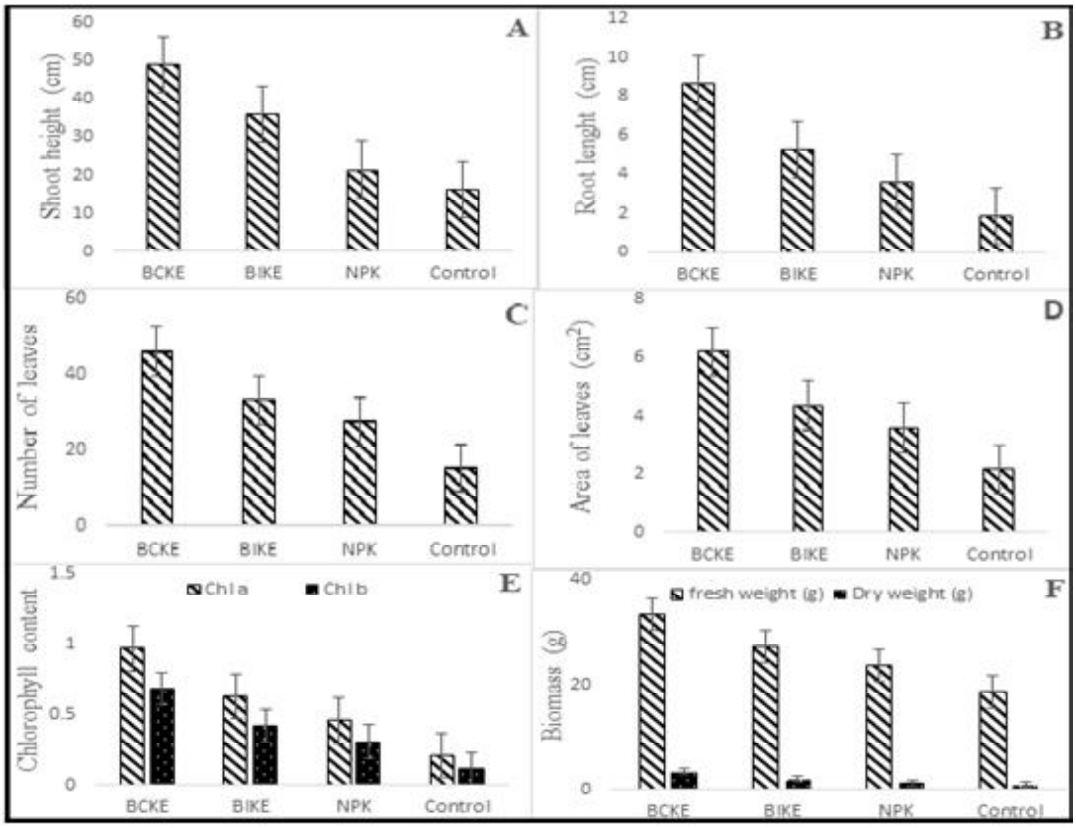


Fig. 2 (A-F) : Effect of biofertilizer prepared from immobilized and crude enzyme of *Bacillus subtilis* (CH008) on the growth of *Solanum lycopersicum*. The mean values and standard error of three replicates are presented. (A) Shoot length (cm) (B) Root length (cm) (C) Number of leaves (D) .Area of leaves (E). Chlorophyll content. (F) Wet and dry Biomass (g). BCKE = Biofertilizer produced from chicken feather degraded by crude keratinase enzyme; BIKE = Biofertilizer produced from chicken feather degraded by immobilized keratinase enzyme. Positive control = NPK fertilizer, Negative control = unfermented chicken substrate without any enzyme degradation.

a UV/Vis spectrophotometer as described by Chong Cao *et al*. (2017). The impact of the formulated biofertilizer was determined on the first day and 60 days after the soil was treated with various treatments.

Soil Organic Carbon and Microbial Biomass-C

The effect of biofertilizer on the soil organic carbon and microbial biomass-C was carried out using the protocol of Adetunji *et al.* (2018). The total amount of microbial biomass-C was determined by using fumigation-extraction method through back titration against 0.04N $(NH_4)_2Fe(SO_{4)2}.6H_2O$ using ferroin as indicator. The total amount of soil organic carbon was evaluated using a modified protocol developed by Walkley and Black, which involved partial oxidation through titration against 1N $(NH_4)_2Fe(SO_{4)2}.6H_2O$ using diphenylamine as indicator.

Greenhouse experiments

The seeds of *Solanum lycopersicum* were placed under running water for 3 hours and were later immersed in 200 mL 3% sodium hypochlorite solution for 30 min. The seeds were rinsed with sterilized water for seven times before they were sowed in 25 cm height × 15 cm width pots filled with soil. The biofertilizers (BCKE and BIKE) were later applied at the rate of 1.0 g fermented chicken substrate/pot while NPKF was used as a positive control and unfermented chicken substrate without any enzyme degradation as the negative control. The following growth parameters were assessed after 60 days of growth; Shoot length, Root length, Number of leaves, Area of leaves, Wet and dry Biomass, Chlorophyll content.

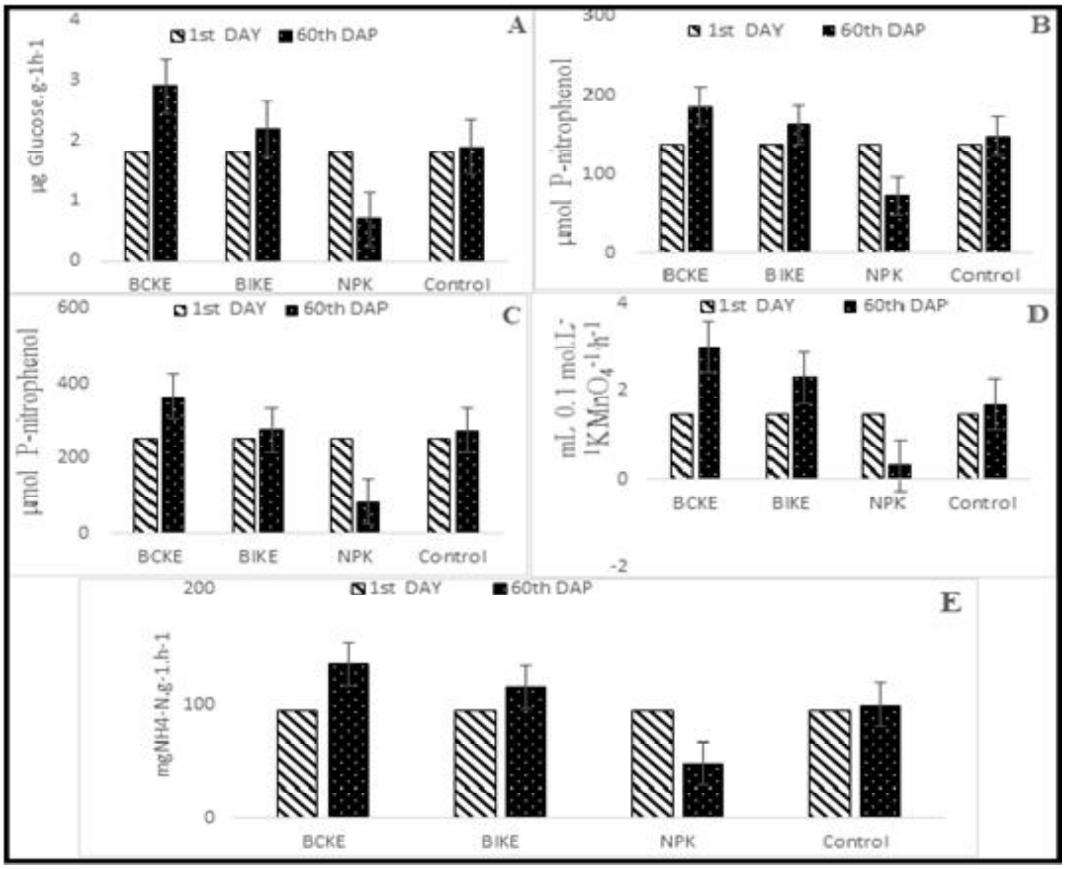
Statistical analysis

The mean values were subjected to an analysis of variance using SPSS (Version 21). Significant means were analyzed using Duncan's multiple range tests at $\alpha = 0.05$.

Results and Discussion

Effect of incubation on crude and immobilized keratinase production

The immobilized enzymes demonstrated the highest relative activity of 98% while the crude enzyme



DAP= Day after application. BCKE = Biofertilizer produced from chicken feather degraded by crude keratinase enzyme; BIKE = Biofertilizer produced from chicken feather degraded by immobilized keratinase enzyme. Positive control = NPK fertilizer, Negative control = unfermented chicken substrate without any enzyme degradation.

Fig. 3 : Effect of biofertilizer prepared from immobilized and crude enzyme of *Bacillus subtilis* (CH008) on soil enzyme activities. The mean values and standard error of three replicates are presented. (A) cellulase (B) alkaline phosphatase (C) acidic phosphatase (D) catalase (E) urease.

demonstrated the relative activity of 58% at 48 hours after incubation. It was observed that immobilized enzymes showed a relative activity of 35% while the crude enzyme demonstrated the relative activity of 12 % at 12 hours after incubation (fig. 1). The application of environment-friendly biotechnological-based formulations could be looked upon as an alternative for chemical fertilizers. In this study, we exploited a strain of *Bacillus subtilis* (CH008) for the production of a cheap, eco-friendly and growth improvement biofertilizer for plants. Several authors have reported the application of *Bacilli* species in the production of biofertilizers. They have shown plant growth promoting attributes, which manifested in terms of increased crop productivity (Ramirez and Kloepper, 2009; Patel *et al*., 2016; Ge *et al*., 2016). The enhanced enzyme activity shown by the immobilized enzymes indicate that the biocatalyst produced from the metabolites exhibit a very high rate of stability and specificity in reaction during the process of entrapment on alginates. This, therefore, substantiates for utilization of the immobilized enzymes for the production of biofertilizer (Franssen *et al*., 2017).

Effect of biofertilizer prepared from the immobilized and crude enzyme of *Bacillus subtilis* (CH008) on the growth of *Solanum lycopersicum*

The parameters like shoot length, root length, number of leaves, area of leaves and wet and dry biomass were determined to study the effect of the biofertilizer produced from the immobilized and crude enzyme of *Bacillus subtilis* (CH008) on the growth and development of *Solanum lycopersicum*. A significant difference was observed in all the parameters when compared to the control. Additionally, the biofertilizer produced from the immobilized enzymes enhanced the chlorophyll content of *Solanum lycopersicum* with the highest value (Chl a 0.92; Chl b 0.68) when compared to the negative control that had Chl a 0.21; Chl b 0.17) (fig. 2A-F). The tomato plants treated with the biofertilizers showed that the immobilization enhanced the availability of nutrient of the

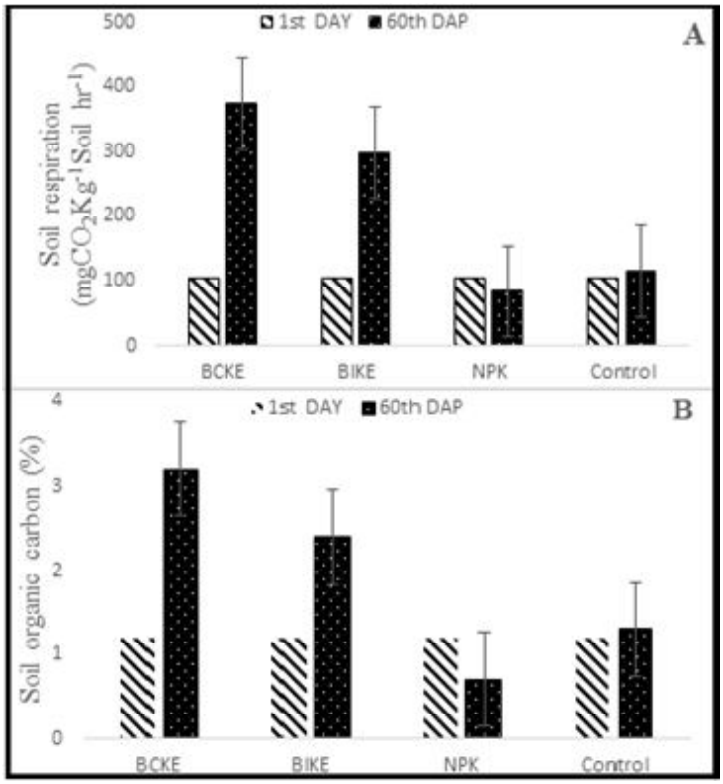


Fig. 4 : Effect of biofertilizer prepared from immobilized and crude enzyme of *Bacillus subtilis* (CH008) on soil respiration ($mgCO_2$ kg^{-1} Soil hr^{-1}) and soil organic carbon (%). The mean values and standard error of three replicates are presented. BCKE = Biofertilizer produced from chicken feather degraded by crude keratinase enzyme; BIKE = Biofertilizer produced from chicken feather degraded by immobilized keratinase enzyme. Positive control = NPK fertilizer, Negative control = unfermented chicken substrate without any enzyme degradation.

degraded feather to the plant and also increased the assimilation rate of the plant. This manifested in enhanced photosynthesis rate and growth rate when compared to the control plants. The enhancement may also be linked to the increased macronutrient content in plant tissues (Zlatev *et al*., 2013; Marquez-Hernandez *et al*., 2013; Mehdizadeh *et al*., 2013; Mesallam *et al*., 2017). Several authors have also established that bio fertilizers especially from a microorganism or their biodegraded products enhanced the growth attributes of tomato plants more than the chemical fertilizers (Kochakinezhad, *et al.,* 2012; Reeve and Drost, 2012). This efficacy of biofertilizer has also been established on other plants like pepper (Khan *et al*., 2012; Dawa *et al*., 2012) and cowpea (Adetunji *et al*., 2012).

Effect of biofertilizer prepared from the immobilized and crude enzyme of *Bacillus subtilis* (CH008) on soil enzyme activities

The result obtained revealed that the immobilized enzymes showed better soil activities when compared to the NPK treated soil and soil treated with unfermented chicken substrate without any enzyme degradation. It was observed that the negative treated soil containing NPK fertilizer showed the least soil activity (fig. 3). The increase in enzymatic activity observed in the soil treated with biofertilizer might be linked to the fact that the more the availability of nutrient the higher the rate of metabolically active microorganisms as well as higher biological activity (de Araújo *et al*., 2013). The increase in microbial activity thus substantiated the enhancement in the soil fertility (Caravaca *et al*., 2004). Further, the higher phosphatase activity showed by the biofertilizer treated soil revealed that only required amount of phosphorus was released for the plants and micro-organisms by the bio-formulated fertilizer (Singh *et al*., 2016). Rao and Tarafdar (1992) had also established that increase in phosphatase activity showed that there is an increase in the amount and the quality of phosphoryl substrates available in the soil.

Effect of biofertilizer prepared from the immobilized and crude enzyme of *Bacillus subtilis* (CH008) on soil respiration and soil organic carbon

The biofertilizer prepared from the enzymes of *Bacillus subtilis* showed an increased level of soil respiration when compared to the control and NPK treatment. The soil respiration before treatment was 105. After treatment, the BCKE showed the highest value of 374 mg CO_2 kg^{-1} soil hr^{-1} followed by BIKE with a value of 298 mg CO_2 kg^{-1} soil hr^{-1} then the positive control (NPK fertilizer) that had a value of 84 mg CO_2 kg^{-1} soil hr^{-1} and lastly the negative control that showed a value of 115 mg CO_2 kg^{-1} soil hr^{-1}after 60 days of application. The level of organic carbon content also showed that the biofertilizer treated soil exhibited increased soil carbon content when compared to the NKP treated soil. BCKE had the highest value with 3.2%, BIKE (2.4%), NPK (0.7%) while the negative control had 1.3% after 60 days of application (fig. 4). The improved rate as well as increase in the level of soil respiration and soil carbon content could be linked to the fact that the application of biofertilizer to the soil enhanced the rapid release of carbon as he biodegraded feather that was used as a substrate during the submerged fermentation served as a carbon source (Santos *et al*., 2012). This is also in line with Schweinsberg-Mickan & Müller (2009), who discovered that soil treated with

biofertilizer showed an increase in the amount of CO_2 release from the soil. These observations thus indicate that the functionality of microorganism in the soil. They are able to readily utilize the available carbon source from the biodegraded feather and releases CO_2 in exchange after metabolizing the biofertilizer (Alef, 1995).

Conclusion

This study has therefore established that *Bacillus subtilis* (CH008) could be utilized in the production of an environmental friendly biofertilizer as a permanent replacement to chemical fertilizer. The result obtained from the effect of the biofertilizer on tomatoes and soil activities shows that the newly formulated biofertilizer has no adverse effect on soil beneficial activities. This shows that it could be a sustainable and innovative technology that could contribute significantly towards the achievement of food and nutrition security, production of safe and healthy food and end hunger as well as helps in the promotion of sustainable agriculture and maintenance of green and clean ecosystem.

Acknowledgements

The authors are grateful to the Department of Biotechnology (DBT), New Delhi, India, the World Academy of Science (TWAS) for TWAS-DBT post-doctoral given Dr. Adetunji. FR number: 3240293141 and UGC and Ministry of Tribal affairs, Government of India for UGC-RGNF fellowship to Paomipem Phazang.

References

Adetunji, C. O., J. K. Oloke and O. O. Osemwegie (2018). Environmental fate and effects of granular pesta formulation from strains of *Pseudomonas aeruginosa* C1501 and *Lasiodiplodia pseudotheobromae* C1136 on soil activity and weeds. *Chemosphere*, **195** : 195:98-107. https://doi.org/10.1016/j.chemosphere.2017.12.056.

Adetunji, C., J. Oloke, A. Kumar, S. Swaranjit and B. Akpor (2017). Synergetic effect of rhamnolipid from *Pseudomonas aeruginosa* C1501 and phytotoxic metabolite from *Lasiodiplodia pseudotheobromae* C1136 on *Amaranthus hybridus* L. and *Echinochloa crus-galli* weeds. *Environ Sci Pollut Res*., **24** : 13700–13709 DOI 10.1007/s11356-017-8983-8.

Ahmed, S., C. B. Shanks and T. Smith (2018). Fruit and vegetable desirability is lower in more rural built food environments of Montana, USA using the Produce Desirability (ProDes). *Tool. Food Sec.*, **10** : 169. https://doi.org/10.1007/s12571-017-0748-1.

Alef, K. (1995). Estimation of soil respiration. In: Alef, K.; Nannipieri, P. Methods in soil microbiology and biochemistry. New York: Academic Press, 1995. p.464-470.

Bhardwaj, D., M. W. Ansari, R. K. Sahoo and N. Tuteja (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb. Cell Fact.*, **13** : 66. doi: 10.1186/1475-2859-13-66.

Brandelli, A., D. J. Daroit and A. Riffel (2010). Biochemical features of microbial keratinases and their production and applications. *Appl. Microbiol. Biotechnol.*, **85(6)** : 1735–1750.

Caravaca, F., J. M. Barea, J. Palenzuela, D. Figueroa, M. N. Alguail and A. Roldán (2003). Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. *Appl Soil Ecol.*, **2(2)** :103-111 <http://dx.doi.org/10.1016/S0929-1393(02)00136-1>.

Chong, C., H. Juan, C. Wen-Shu, Y. Chun-Ni, L. Jia-Liang and J. Yao-Dong (2017). Effects of Silver Nanoparticles on Soil Enzyme Activity of Different Wetland Plant Soil Systems. *Soil Sediment Contam.,* **26** : 5, 558-567. DOI: 10.1080/15320383.2017.1363158

Dawa, K. K., H. M. E. Abd El – Nabi and W. E. Swelam (2012). Response of sweet pepper plants (vegetative growth and leaf chemical constituents) to organic, bio fertilizers and some foliar application treatments. *J. Plant Production*, *Mansoura, Univ*., **3 (9)** : 2465 –2478.

de Araújo, Ademir S. F., R. Oliveira, José, M. Araújo, Raul and L. F. Gomes Regina (2014). Biofertilizers on soil microbial biomass and activity. *Revista Brasileira de Ciências Agrárias*, **9(4)** : 545-549.

Etissa, E., N. Dechassa, T. Alamirew, Y. Alemayehu and L. Desalegne (2013). Household Fertilizers Use and Soil Fertility Management Practices in Vegetable Crops Production: The Case of Central Rift Valley of Ethiopia. *Science, Technology and Arts Research Journal*, **2(4)** : 47.DOI.10.4314/star.v2i4.9

El-Borai, A. M., A. S. Youssef, A. E. Abd El-Salam and S. A. El-Assar (2013). Optimization of Keratinase Production by *Aspergillus terreus* Thom by Statistical and Immobilization Methods. *Life Sci J*., **10(3)** : 1833-1839.

Fang, Z., J. Zhang, G. Du and J. Chen (2017). Rational protein engineering approaches to further improve the keratinolytic activity and thermostability of engineered keratinase KerSMD. *Biochemical Engineering Journal*, **127** : 147–153.

Fang, Z., J. Zhang, B. Liu, G. Du and J. Chen (2016). Enhancement of the catalytic efficiencyand thermostability of *Stenotrophomonas sp*. keratinase KerSMD by domainexchange with KerSMF. *Microb. Biotechnol.,* **9(1)** : 35–46.

Franssen, Maurice C.R., Elinor, Peter Steunenberg, L. Scott, Zuilhofac, Han, Sanders, P. M. Johan (2013). Immobilised enzymes in biorenewables production. *Chem. Soc. Rev.*, **42** : 6491-6533

Ge, B., B. Liu, T. T. Nwet, W. Zhao, L. Shi and K. Zhang (2016). *Bacillus methylotrophicus* Strain NKG-1, Isolated from Changbai Mountain, China, Has Potential Applications as a Biofertilizer or Biocontrol Agent. *PLoS ONE,*. **11(11)** : e0166079. https://doi.org/10.1371/journal.pone.0166079

Khan, Z., S. A. Tiyagi, I. Mohamood and R. Rizvi (2012). Effect of N-fertilization, organic matter and biofertilizers on growth and yield of chilli in relation to management of plantparasticnematods. *Turk. J. Bot.*, **36** : 73-81.

Kopeæ, M., K. Gondek, K. Orowska and Z. Kulpa (2014). The use of poultry slaughterhouse waste to produce compost. *Ecol Eng.*, **37** : 143–150.

£aba, W., B. Arowska, D. Chor¹yk, A. Pud³o, M. Piegza, A. Kancelista and W. Kopeæ (2018). New keratinolytic bacteria in valorization of chicken feather waste. *AMB Express*, **8** : 9. 1-14. doi: 10.1186/s13568-018-0538-

Liu, B., J. Zhang, Z. Fang, G. Du, J. Chen and X. Liao (2014). Functional analysis of the C-terminal propeptide of keratinase from *Bacillus licheniformis* BBE11-1 and its effect on the production of keratinase in *Bacillus subtilis*. *Process Biochem.,* **49(9)** : 1538–1542.

Marquez-Hernandez, C., P. Cano-Rios, U. Figueroa-Viramontes, J. A. Avila-Diaz, N. Rodriguez-Dimas and J. L. Garcia-Hernandez (2013). Yield and quality of tomato with organic sources of fertilization under greenhouse conditions. *Phyton (Buenos Aires)*, **82** : 55-61.

Mehdizadeh, M., E. I. Darbandi, H. Naseri-Rad and A. Tobeh (2013). Growth and yield of tomato (*Lycopersicon esculentum* Mill.) as influenced by different organic fertilizers. *International Journal of Agronomy and Plant Production*, **4(4)** : 734-738.

Mesallam, M. G., S. Shafshak Nadia, F. A. Abo-Sedera and M. M. Abou Elmagd (2017). Effect of bio fertilizers, organic and mineral nitrogen fertilizer on growth and yield of tomato plants grown under sandy soil conditions. *Annals of Agric. Sci., Moshtohor.,* **55(2)** : 343 –354.

Mondal, T., J. K. Datta and N. K. Mondal (2017). Chemical fertilizer in conjunction with biofertilizer and vermicompost induced changes in morpho-physiological and bio-chemical traits of mustard crop. *Journal of the Saudi Society of Agricultural Sciences*, **16** : 135–144.

Patel, G., S. Singh, S. K. Saxena and K. J. Kaur (2016). Isolation, Biochemical Characterization and Production of Biofertilizer from *Bacillus megaterium*. *Int. J. Life. Sci. Scienti. Res.*, **2(6)** : 749-752. DOI:10.21276/ijlssr.2016.2.6.16

Ramirez, C. A. and J. W. Kloepper (2009). Plant growth promotion by *Bacillus amyloliquefaciens* FZB45 depends on inoculum concentration and P-related soil properties. 8th International PGPR workshop. Proceedings book. Portland, Oregon, OR.

Rao, A. V. and J. C. Tarafdar (1992). Seasonal changes inavailable phosphorus and different enzyme activities in arid soils. *Annals of Arid Zone,* **31** : 185–189.

Rubin, R. L., K. J. Van Groenigen and B. A. Hungate (2017). Plant Growth promoting rhizobacteria are more effective under drought : a meta-analysis. *Plant Soil*, **416** : 309–323. doi: 10.1007/s11104-017-3199-8.

Santos, V. B., A. S. F. Araújo, L. F. C. Leite, L. A. P. L. Nunes and W. J. Melo (2012). Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma*, **170** : 227-231, 2012. <http://dx.doi.org/10.1016/j.geoderma.2011.11.007>.

Schreinemachers, P., E. B. Simmons and C. S. Wopereis Marco (2018). Tapping the economic and nutritional power of vegetables. *Global Food Security*, **16** : 36-45.

Schütz, L., A. Gattinger, M. Meier, A. Müller, T. Boller, P. Mäder and N. Mathimaran (2018). Improving Crop Yield and Nutrient Use Efficiency via Biofertilization–A Global Meta-analysis. *Front. Plant Sci.*, **8** : 2204. doi: 10.3389/fpls.2017.02204

Schweinsberg-Mickan, M. S. and T. Müller (2009). Impact of effective microorganisms and other biofertilizers on soil microbial characteristics, organic-matter decomposition, and plant growth. *Journal of Plant Nutrition & Soil Science*, **172(5)** : 704-712. <http://dx.doi.org/10.1002/jpln.200800021>.

Singh, G., D. Kumar and P. Sharma (2015). Effect of organics, biofertilizers and crop residue application on soil microbial activity in rice – wheat and rice-wheat mungbean cropping systems in the Indo-Gangetic plains. *Cogent Geoscience*, **1** : 1085296.

http://dx.doi.org/10.1080/23312041.2015.1085296

WHO (2015). Promoting Fruit and Vegetable Consumption around the World. World Health Organization, Geneva.

Zlatev, Z. and V. Popov (2013). Effect of organic fertilizers on photosynthesis of young tomato plants (*Lycopersicon esculentum* Mill.). *Agricultural Science and Technology*, **5(1)** : 35-38.