PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (5) 1443-1449 May 2024

Isolation and Characterization of Arginase Producing Bacteria from Soil Compost Sites at Ogwa Community, Edo State, Nigeria

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ABSTRACT: Under typical physiological conditions, arginine plays a crucial function in the cell development and proliferation of healthy cells. Arginase has recently caught the attention of numerous researchers as a potential therapy option for auxotrophic cancer. Using standard morphological and biochemical methods, this paper isolates and characterize Arginase producing bacteria from soil compost sites collected from Ogwa community, Edo State, Nigeria. Enzymatic and physicochemical activities were assessed. The species identified to be present include *Pseudomonas* sp., *Bacillus licheniformis*, and *Bacillus subtlis*. The enzyme was active throughout a wide pH range, from 2 to 12. The optimal pH for the activity was found to be 9, and the ideal temperature was 70 °C. K_m values for Arginine and Guanidine hydrochloride were 0.21 mM and 0.25 mM, respectively, in terms of substrates. Studies on the enzyme with different cations showed that the activity of the enzyme was affected by Sn^{2+}, Hg^{2+}, Pb^{2+}, Na^{2+}, and K^{+}. This study shows the therapeutically significant arginase enzyme is present in the isolated bacteria from various compost sites.

DOI: https://dx.doi.org/10.4314/jasem.v28i5.15

Cite this Article as: OKANLAWON, K. P; OKANLAWON, T. S; ADEDIRE, S. A; AGBAJE, I. S; ADEBOMI, O. H; AKINBAMI, A. M. (2024). Isolation and Characterization of Arginase Producing Bacteria from Soil Compost Sites at Ogwa Community, Edo State, Nigeria. *J. Appl. Sci. Environ. Manage.* 28 (5) 1443-1449

Dates: Received: 21 February 2024; Revised: 22 March 2024; Accepted: 20 April 2024 Published: 19 May 2024

Keyword: Arginase; Composting**;** *Pseudomonas* species; *Bacillus* species; Therapeutics

Each region of the planet contains abundant microbes, especially dirt, thermal springs, and mountains buried no less than a few kilometers beneath the surface. The equilibrium that exists on the earth is crucially maintained by the microorganisms found in soil. In a variety of soil conditions, all soils contain various levels of bacteria, fungi, and viruses (Murugalatha *et al*., 2018). Arginase is an enzyme with manganese that catalyzes the conversion of arginine to ornithine and urea. It has been shown to have anti-cancer properties and to inhibit tumor argininosuccinate synthase. The primary metabolic hurdles may be the rapid proliferation of microbe producers and thermally decreased equilibrium for enzymes (El-Sayed *et al*., 2014). According to Hussain et al. (2017), the drawbacks of the treatment activity arginase for orthostatic carcinogenic carcinomas include its weak anti-cancer activity, poor protein tolerance, and brief blood half-life arginase, which depletes arginine, shows a potent anti-cancer effect against numerous

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cancers that induces stage G1 cell cycle arrest. Numerous arginine auxotrophic tumors, including hepatocellular carcinomas, have been successfully treated with L-arginase (Yau *et al*., 2010; Glazer *et al*., 2011). Several research has revealed that arginine deprivation therapy is a successful method for treating some types of cancer. In the successful arginine-deprivation cancer treatment approach, L-arginase is used. Other anti-cancer medications can more easily kill tumor cells as a result of this sort of cancer treatment (Patil *et al*., 2016).

Clinical investigations were performed to show that arginase therapy decreased the growth of human hepatocellular carcinoma cells that are surviving due to the presence of arginine. Patients with metastatic melanoma may be treated by selectively removing arginine using the arginase enzyme (Conners, 2016). Numerous strains of bacteria may have been identified isolated, described, and could have produced enzymes. Since different microbial strains can produce different kinds of enzymes with different psychological issues and biochemical, catalytic, and immune function characteristics, continuous screening programs are used to find new microbe that could potentially produce useful enzymes with few restrictions on their application (Unissa *et al*., 2015). Animals, organisms such as bacteria, fungi, and plants all contain arginase (Parveen and Ankala, 2020). For the isolation and characterization of arginase, numerous researchers have used a variety of bacteria, including *Pseudomonas sp., Bacillus caldovelox, Helicobacter pylori, Bacillus thuringiensis*, and *Bacillus subtilis* (Salwoom *et al*., 2019). Hence, the objective of this paper was to isolation and characterize Arginase producing bacteria collected from soil compost sites at Ogwa Community, Edo State, Nigeria.

MATERIALS AND METHODS

Sample Collection: Soil samples were collected from compost sites at Ogwa Community, Edo State, Nigeria in sterile containers and transported to the laboratory for microbiological evaluation. The research work was conducted in Microbiology Laboratory, Glorious Vision University, Ogwa, Esan West local government area of Edo State, Nigeria. It is about at latitude 6˚30 20. 16" North, and longitude 6˚12 30. 24" East.

Microbiological Analysis: Isolation of bacterial and fungi Isolates: 1ml was weighed and transferred right into a test tube containing sterile distilled water for serial dilution. Fifteen ml (15 ml) of the organized molten agar which turned left to chill turned into poured aseptically into the dishes and gently swirled in the clockwise and anticlockwise path to permit for even distribution of the colonies at the surface of the agar. The agar was allowed to be set. Nutrient and MacConkey agar were incubated in an inverted role at 37°C for 24 hours (Okanlawon *et al*., 2023).

Identification and Characterization: Bacterial isolates were characterized based totally on their colonial morphology, cell morphology, and biochemical characteristics. The identity of bacterial isolates was accomplished in line with Bergey's Manual of Determinative Bacteriology (Okanlawon *et al*., 2023).

Biochemical Identification of Isolates: Catalase, Oxidase, Methyl red, Voges Proskauer, Nitrate discount, Citrate utilization, Motility spore staining, Indole, Gelatin, Casein, Starch hydrolysis, increase at different temperatures and pH and Sugar fermentation were a number of the biochemical assessments are done to identify oorganisms (Okafor *et al*., 2023).

Physicochemical Parameters: The pH of the soil sample: Ten grams of soil sample was weighed, mixed with 90 mL of sterile distilled water, homogenized and filtered through Whatman Filter paper (No.1). The pH of the resulting filtrate was then measured using a pH meter (H19107, Hanna), after standardizing with a phosphate buffer solution at pH 4.0 and 7.0. (Corwin and Yemota, 2020).

Temperature: Soil temperature was measured by a group of curved tube thermometers, which were placed on the top of the ridge in each plot. The measurement depths were 5 cm, 15 cm, and 25cm. The measurement times were 07:00, 14:00, and 18:00. (Corwin and Yemota, 2020).

Ezyme Assay: Effect of pH: At various pH levels, the impact of pH on arginase activity was evaluated. The pH values were changed by employing buffers with various pH levels, including borate buffer 9, 0.01M citrate buffer, which has a pH range of 3.0-5.0, 0.01M phosphate buffer, which ranges from 6.0 to 7.0, and 1mM tris buffer, which has a pH range of 8.0 to 9.0. 1mM of each separate buffer, 0.33M of arginine, and 0.05ml of enzyme were all present in the reaction mixture.

Effect of Metals: A metallo-enzyme known as arginase functions as both an activator and a co-factor (Jenkinson *et al*., 1996; Dabir *et al*., 2005). It has been demonstrated that other metals can also activate arginine. Using the standard enzyme assay, the impact of the divalent cations Na^{+}, Hg^{2+}, Mn^{2+}, and Fe^{3+} on the enzyme was determined. 1.0 mM Tris buffer, pH 9.5, 1.0 mM $MnCl_2$, 30 mM arginine, 25 l of the metals, and 50 l of the enzyme were added to the reaction mixture. After being incubated at room

temperature for 10 minutes, the reaction mixture was stopped by adding Ehrlich's reagent. The activity was then calculated from the urea curve using the absorbance measured at 450 nm.

Effect of Temperature: In order to examine how temperature affects the enzyme's activity and identify the ideal temperature for the enzyme, the activity of arginase was measured at various temperatures between 30 °C and 100 °C. The reaction was started by adding 50 l of enzyme that had previously been incubated at the chosen temperature after the assay mixture had first been incubated at that temperature for 10 minutes.

Determination of Kinetic Parameters: The kinetic parameters V_{max} and K_m were determined by varying the concentrations of arginine between 25 mM- 225 mM. The kinetic parameters were determined from the double reciprocal plot of Lineweaver-Burk (Line-weaver-Burker 1934)

RESULTS AND DISCUSSION

The mean microbial load of bacterial isolates from soil sample is presented in table 1 and ranges from 2.0 x 10^2 cfu/g to 3.0 x 10^5 cfu/g. The prevalence of bacterial isolates in the samples showed that all the bacterial species are evenly distributed as shown in table 2. The colony morphology of bacterial isolates from sample is presented in table 3. Some of the isolates were circular, low convex, entire with wet and smooth surfaces, small in size, creamy in colour and did not produce any pigment.

Table 1: Total Heterotrophic Bacterial count

	Total Heterotrophic bacterial Count		
	A	B	C
Site 1	$3.0x10^5$	$1.2x10^3$	$5x10^2$
Site 2	$4.1x10^5$	$3.3x10^3$	$2x10^2$
Site 3	$4.7x10^5$	$2.3x10^3$	$6x10^2$

Legend: A= *Bacillus licheniformis*; B= *Bacillus subtilis;* C= *Pseudomonas* sp.

Table 2: Prevalence of bacteria Isolate

SOURCE	*Bacillus licheniformis*	*Bacillus Subtilis*	*Psuedomonas* sp.
Site 1	+	+	+
Site 2	+	+	+
Site 3	+	+	+

Legend: + = Present

Table 3: Morphology of Bacterial Isolates

Isolate	Shape	Colour	Size	Elevation	Edge	Surface	Pigment
A	Rod	Cream	Small	Flat	Entire	Wet	None
B	Rod	Cream	Big	Flat	Entire	Wet	None
C	Rod	Cream	Big	Raised	Undulate	Wet	None

LEGEND: A: *Bacillus licheniformis;* **B:** *Bacillus subtilis;* **C:** *Pseudomonas* sp.

Table 4: Biochemical Characteristics of Bacterial Isolates

Grams reaction	**Cellular**	**Catalase**	**Oxidase**	**Methyl Red**	**VP**	**Indole**	**Nitrate Red**	**Citrate**	**Starch**	**Gelatin**	**Glucose**	**Fructose**	**Maltose**	**Sucrose**	**Lactose**	**Probable identity**
+	**R**	+	+	-	-	-	-	+	+	+	+	+	-	+	+	***Bacillus subtlis***
+	**R**	+	+	+	-	-	+	-	-	+	+	+	+	+	+	***Bacillus***
-	**R**	+	+	-	-	-	+	+	-	+	-	+	-	-	-	***licheniformis***
																***Pseudomonas* sp.**

Table 5: Soil Parameters Observed in the Compost Sites

	For Site One (1)			
Parameters	A	B	C	Average
pH	6.70	7.10	7.30	7.03
Temperature	25℃	26℃	27℃	26℃
	Site Two (2)			
Parameters	A	B	C	Average
pH	7.40	7.50	7.55	7.48
Temperature	24℃	25℃	26℃	25℃
	Site Three (3)			
Parameters	A	B	C	Average
pH	7.30	7.20	7.35	7.28
Temperature	26℃	24℃	28℃	26℃

The biochemical characterization of bacteria is presented in table 4. Some of the organisms were Gram positive, the organism were not able to utilize casein, some of the organisms were able to utilize starch for growth while some were not, the organisms were able to produce the enzyme catalase, some were able to utilize citrate as a sole carbon source, some organisms were able to reduce nitrate. Organisms were able to ferment glucose with the production of gas, fructose, maltose, raffinose, rhamnose etc. Probable identity of bacteria isolates includes, *Bacillus subtilis, Psuedomonas* sp., and *Bacillus licheniformis.* The meant temperature values collected from the three soil compost sites are presented in table 5. The mean value ranges between 24 ℃ and 28 ℃ with the higest temperature recorded at the third sampling site while the lowest was recorded at the second sampling site. The effect of the heavy metals on the arginase produced by the bacterial species presented in fig 1, figure 5 and figure 9.The highest activity was recorded for the *Bacillus subtilis* while *Bacillus licheniformis* showed the lowest activity on $MnCl_2$ as represented in figure 5. The highest effect of heat stability was

recorded for the arginase produced by *Bacillus subtilis* while the lowest *Bacillus licheniformis* as presented in figure 2 and 6 respectively. The arginase produced is greatly affected by pH at 8 while the lowest was recorded for *Bacillus licheniformis* as presented in figure 3 and 7.

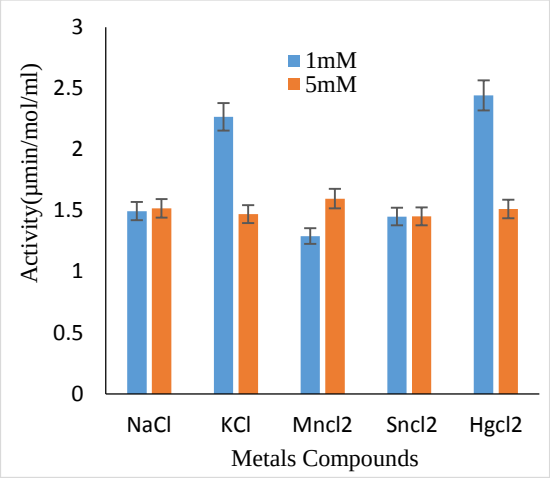

Fig 1: Effect of heavy metals on the activity of arginase obtained from *B. subtilis*

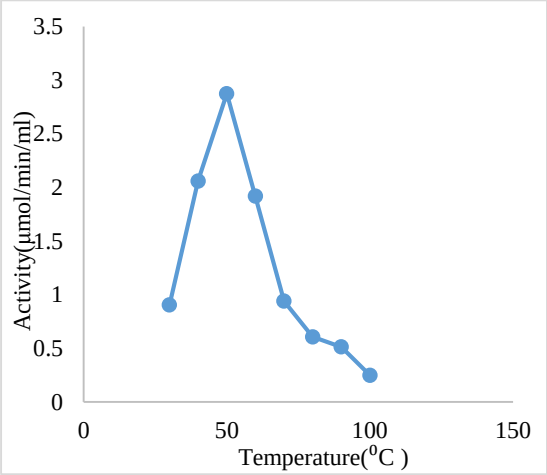

Fig 2: Effect of heat stability on the activity of arginase obtained from *B. subtilis*

A critical enzyme in pathophysiology with novel pharmacological implications, arginase is a biocatalyst of choice for the present and the future (Nadaf *et al*., 2019). Enzymes are one of the key sectors and have a large market in the fabric, medicine, confectionary, and waste disposal industries. It has been claimed that enzymes produced by microbes are more stable than plant and animal enzymes. The isolates underwent multiple biochemical characterizations as well as gram reactions. Similar to the findings of Murugalatha et al. (2018), the isolates were identified as *Bacillus subtilis*, *Bacillus licheniformis*, and *Pseudomonas* sp. based on biochemical analysis and sugar fermentation. When screening arginase-producing strains, Nadaf et al. (2019) discovered Pseudomonas sp. to be the most effective one. For the three sites, the compost soil's pH and temperature range from 7.03 to 7.48 and 25°C to 26°C, respectively.

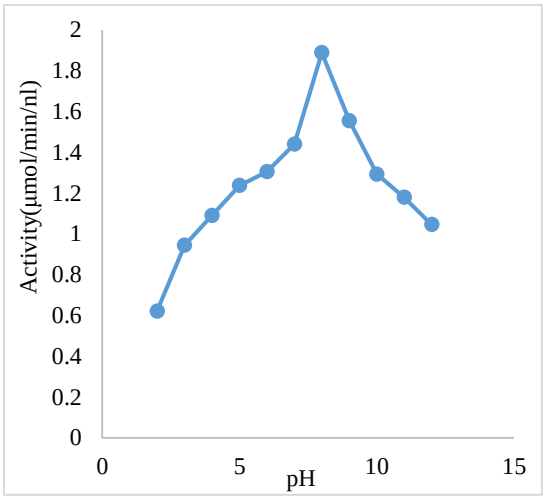

Fig 3: Effect of pH on the activity of arginase obtained from *B. subtilis*

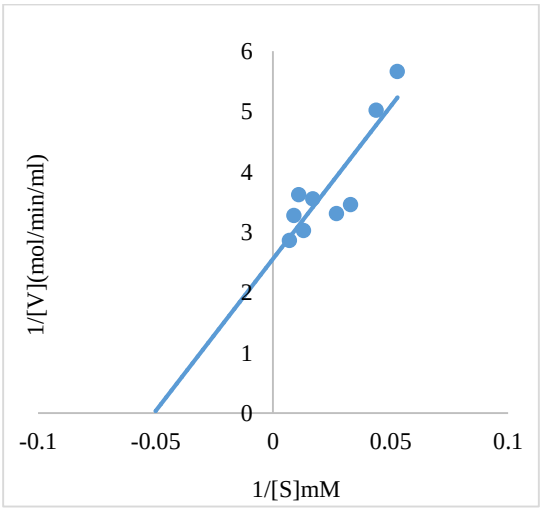

Fig 4: Lineweaver-Burk plot of 1/V against 1/S at varying concentrations on the activity of arginase obtained from *B. licheniformis*

Temperature, pH, contact duration, type and number of microorganisms, nutrient availability, microbial consortia, pollutant characteristics, and salinity are the variables that affect soil parameters (Emadian *et al*., 2017; Al-Jailawi *et al*., 2014). The examination into how metal ions can restrict enzyme activity revealed that none of the metal ions examined significantly decreased the activity of enzymes. The study suggests that because these ions may be present in the organism's environment, it may become tolerant of or resistant to them.

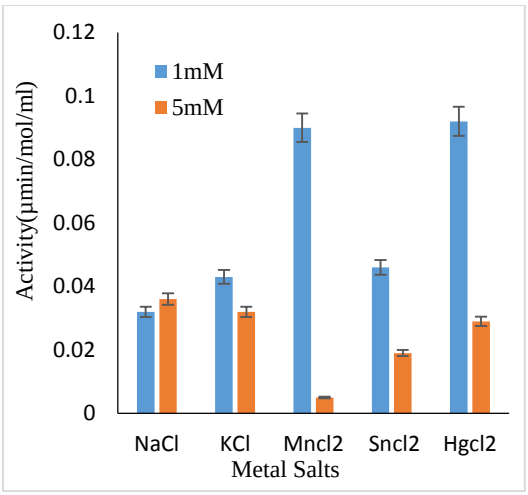

Fig 5: Effect of heavy metals on the activity of arginase obtained from *B. licheniformis*

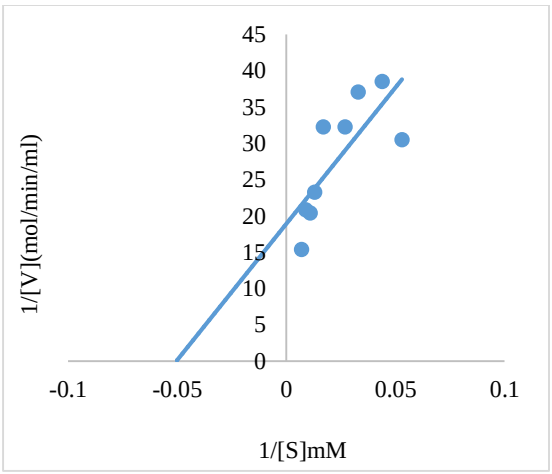

Fig 8: Lineweaver-Burk plot of 1/V against 1/S at varying concentrations on the activity of arginase obtained from *B. licheniformis*

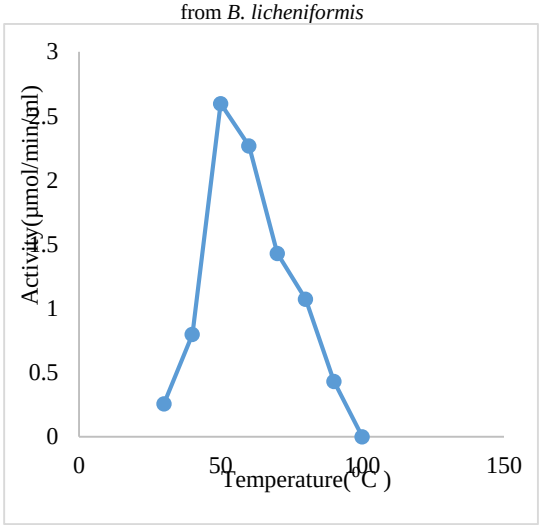

Fig 6: Effect of heat stability on the activity of arginase obtained from *B. licheniformis*

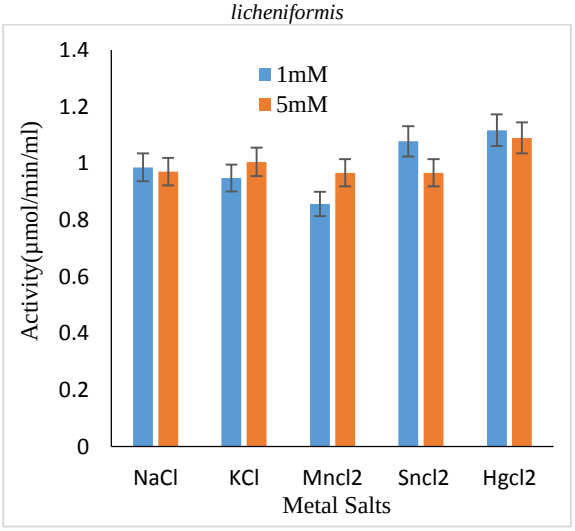

Fig 9: Effect of heavy metals on the activity of arginase obtained from *Pseudomonas* sp.

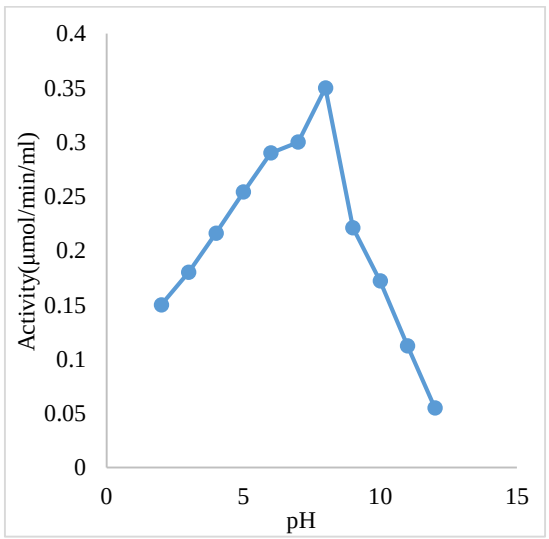

Fig 7: Effect of pH on the activity of arginase obtained from *B. licheniformis*

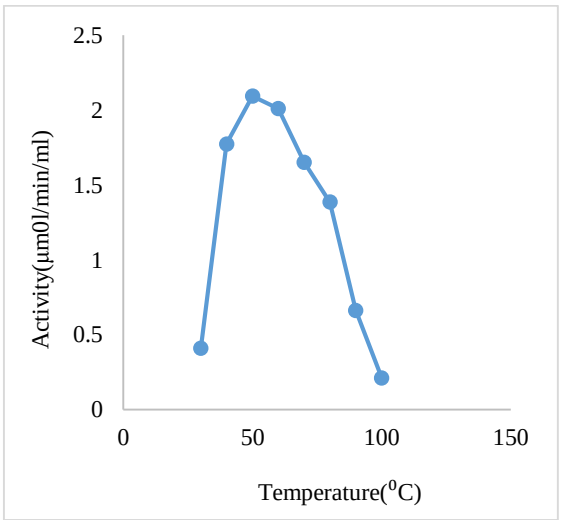

Fig 10: Effect of heat stability on the activity of arginase obtained from *Pseudomonas* sp.

The results are equivalent to those of Akinsiku et al. (2010) work. For *Bacillus licheniformis*, *Bacillus subtilis*, and *Pseudomonas* sp., the maximum values for the kinetic parameters (Km and Vmax) were 0.21 mM and 0.25 mM, respectively. This agreed with the conclusions reached by Parveen and Ankala (2020). The bacterial isolates exhibit a high affinity for these substrates, according to the results of the kinetic investigation, which suggests that the enzyme is catalytically efficient. Enzyme activity is significantly influenced by temperature. Because of its impact on microbial growth and enzyme synthesis, it is also a crucial component of every living system. Bacillus sp. displayed the highest arginase activity in the current investigation. At temperatures above 50°C, arginase activity was decreased. Most likely, a rise in temperature lowered the amount of proteins needed for physiologic and growth activities, which might have prevented bacterial growth. *Alcaligenes faecalis* displayed its highest arginase activity at 35°C, according to Ibrahim et al. (2018). For the greatest possible growth of the microorganisms and a high yield of the enzyme, the media must have the ideal pH. Although there are few studies on how arginase functions in acidic pH, the enzyme exhibits greatest activity in alkaline pH. The study revealed that alkaline pH 9 had the maximum enzyme activity. Furthermore, when we exposed the organism to greater pH, the enzyme activity reduced. This result contrasts with the arginase from *H. pylori*, which, according to Zhang et al. (2011), demonstrated an acidic pH preference and an ideal pH of 6.1. Bacteria are preferred among all of the microbes that have been discovered to produce enzymes because of their high production, simplicity in growing, and genetic modification. The present paper describes a species of bacteria that can manufacture a sizable amount of the enzyme.

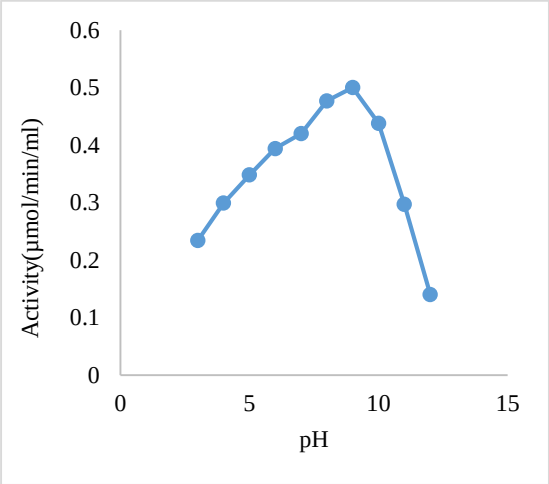

Fig 11: Effect of pH on the activity of arginase obtained from *Pseudomonas* sp.

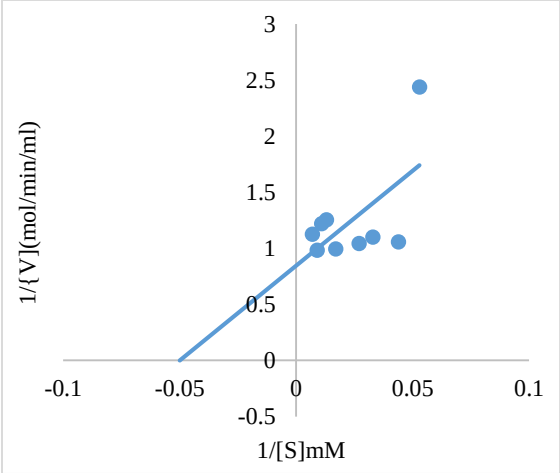

Fig 12: Lineweaver-Burk plot of 1/V against 1/S at varying concentrations on the activity of arginase obtained from *Pseudomonas* sp.

Conclusion This work has established the soil environment as a source of bacteria that are capable of producing arginase, which have a novel therapeutic role in the biopharmaceutical industries. Hence, Bacteria that can produce arginase were isolated from compost sites for this investigation and demonstrated activity under various conditions for the optimal production of the enzyme.

Conflict of Interest: Authors declared that there is no conflict of interest.

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