MIKAILALSYS
Journal of Multidisciplinary Sciences

e-ISSN : 2987-2286
p-ISSN : 2987-3924

Index : Dimensions, Scilit, Crossref, Garuda, Semantic, Lens,Google Scholar,Base, etc

https://doi.org/10.58578/mikailalsys.v2i1.2659

Assessment of Lipase Enzyme Production Potential of Bacteria Isolated from Oryza Sativa (Rice) Rhizosphere in Sokoto, Nigeria

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Jan 15, 2024	Jan 20, 2024	Jan 25, 2024	Jan 28, 2024

Abstract

The work analyzed a total five (5) different soil samples in the rhizosphere of rice from Sokoto metropolis. Therewith, the potentially of bacterial isolate was screened for the lipase production using standard methods and procedures, and chemicals of analytical grade. Bacillus spp had the highest percentage of occurrence 40%. The next was E. coli with percentage of 30%. Followed by, Flavobacterium with percentage of 20% which also show that flavobacterium appears in two locations. S.aureus had a percentage occurrence of 10% respectively. The results of this study outlined the potential of synthesizing lipase enzyme from bacteria (especially the Bacillus spp which had a highest lipase activity). In turn, microbes examined could be utilized as for industrial production and other benefits.

Keywords: Lipase, bacteria, rice, E.coli, Rhizosphere

INTRODUCTION

Oryza sativa (*Rice*) is a major staple foodstuff consumed by about half of the inhabitants on earth (Ayoola, 2020, Chumphon *et al*, 2022). In terms of size of area utilized for production, rice remained the largest crop ever in the world. It is a predominant diet in many parts of the world, let alone in Nigeria. Due to the current population increase in the world, more rice production is expected to be seen in the years to come (Chumphon *et al.* 2022).

The rice has a term known as rhizosphere referring to an area on the soil compartments serving as habitat to microorganisms in the ecosystem. It lies within the roots, influenced by the plants and acts as an interface to the plant, soil, and microbial interactions as influenced by the prevailing environment (Umar *et al.*, 2019; Ayoola, 2020, Chumphon *et al.,* 2022). Rhizosphere definitely has a rhizobacteria that helps in improving plant growth through intervention such as nutrients, phosphate solubilization, pathogens resistance, nitrogen fixation etc (Patel and Desai, 2015, Tsegaye *et al.,* 2019).

Moreover, the rhizobacteria have the tendency to multiply and colonize all the niches of roots despite the other competing microbes (Resti and Liswani, 2020). These bacteria are considered vastly applicable in ways that also benefit humans directly by producing useful chemicals needed for normal biochemical metabolic activities in humans and at industrial or health areas. Among the important materials obtained from bacterial metabolism are the enzymes. Enzymes are organic catalysts made by organism to speed up the rate of chemical reactions (Gopalakrishnan *et al.,* 2013). These enzymes are engaged in a diverse array of processes in the biological system and are championed for several industrial activities (Ayoola, 2020).

Nevertheless, lipases are typical enzymes that are produced by bacteria. They are responsible for hydrolysis of fats and oils to release fats, diacyl glycerols, monoacyl glycerols, and glycerols. They can also be useful in esterification, transesterification, amino lysis, and lactonization. Therefore, very beneficial at industrial level (Ayoola, 2020). Therefore, the need to intensify lipase production from rhizobacteria intensifies (Tsegaye *et al.,* 2019). Microorganism such as bacteria synthesize highly Valuable substances like lipase enzymes that are applicable in industries, and scientific innovations (Ayoola, 2020). Enzymes are more important when coming from microbes like bacteria, because they are more accommodating, more consistent, more synergistic, more productive, more consistent and logically safer and useful (Lawal *et al.*, 2017; Tsegaye *et al.,* 2019; Sarkingobir *et al.*, 2022a). They can be subjected to manipulation to increase yield or confer other useful unique properties (Resti and Liswani, 2020). In this vein, lipase is used in food companies, dairy production, oil sector, medicine, pharmaceuticals, detergents, perfumes, cosmetics, and agriculture, among other (Ayoola, 2020). When originating from microbes, they are more extraordinary, more solid, expensive, and selective as well (Resti and Liswani, 2020). Noteworthy**,** enzymes produced from other sources other than microbes are more expensive, less productive, less manipulative, and less effective among others defects. Other methods are less safe, and produced much pollution in the course of extensive downstream processes that also add to the production cost (Gopalkrishnan *et al.,* 2013; Umar *et al*., 2016). The aim of this research is to isolate and identify lipase producing from rhizosphere of rice (*Oryza sativa*).

METHODS

Sample Collection

Soil samples from rice rhizosphere in Sokoto State were collected with the guide of sterile spatula from the profundity of 0.5 to 1.0 cm inside a sterile 100 ml glass bottles and immediately transferred to the laboratory analysis (Cavalho *et al.,* 2023).

Isolation and Screening of Lipase Producing Bacteria

Soil samples were diluted serially from 10^{-1} up to 10^{-6} in sterile distilled water, each dilution was cultured on nutrient agar plates by spread plate method to obtain isolated colonies after 24 hours of incubation (Cavalho *et al.,* 2023). The pure bacterial isolates were screened for lipase production by streaking the Bacterial colonies on Tributyrin agar Plate ((0.5% peptone, 0.3% yeast extract, 1% tributyrin and 2% agar, pH 7.0) and incubated at 37˚C for 48 hours. The isolates were observed for zone of clearance and measured accordingly (Cavalho *et al.,* 2023).

Identification of the Selected isolate

The selected isolate was identified using morphological and biochemical characteristics.

Morphological Characterization

Morphological characterization of a pure culture of the best isolate on tributyrin agar was done using colonial, cellular and pigment appearances on culture plates (Cavalho *et al.,* 2023).

Biochemical Characterization

The biochemical test such as: sugar utilization test, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, starch hydrolysis test, catalase test, sporulation test, oxidase test, were studied and characterization (Cavalho *et al.,* 2023).

a. Gram Staining Technique

A smear of the chosen strain was prepared on a clean glass slide and therefore the smear was allowed to air-dry then heat fixed, the warmth fixed smear was flooded with crystal violet and washed under water after 1 minute, it had been flooded with mordant (Iodine), The smear was decolorized with 95 % ethyl alcohol and washed with water then counter stained with safranin for 45 seconds so the smear was allowed to dry and examined under oil immersion

(100x) (Patel *et al.*, 2016).

b. Sugar Utilization Test

A loopful culture of chosen potential isolates was inoculated to the sugar stock (10 % watery test sugar arrangement (glucose) in 10 ml basal medium (supplement stock or 1 % peptone water), 1 ml and was incubated at 37^0C for overnight. Corrosive creation change the shade of medium to pink and gas creation was closed from a little air pocket in modified Durham's cylinder kept in the test tube (Patel *et al.*, 2016).

c. Indole Production Test

A loopful culture of chosen selected isolates was inoculated into tryptone broth (1% tryptone water, 0.5 gm NaCl, 100 ml water) and incubated at 37^0 C for overnight. 3-4 drops of xylene were included medium after brooding and shaken overwhelmingly. The two layers could separate and 1 ml of Kovac's reagent was added gradually. The development of pink shading ring showed positive test (Cavalho *et al.,* 2023).

d. Methyl Red Test

A loopful culture of chosen potential isolate was innoculated into glucose phosphate stock (5 gm glucose, 5 gm K_2HPO_4, 5 gm peptone, 1000 ml water) and incubated at 37^0 C for 48-72 hours, 5 drops of methyl red pointer were added inside the medium after brooding. The occasion of red shading showed positive test (Cavalho *et al.,* 2023).

e. Voges-Proskauer Test

A loopful culture of chosen potential disconnects were immunized into glucose phosphate stock (5gm glucose, 5 gm peptone, 1000 mL water) and incubated at 37^0 C for 48-72 hours, 0.6 ml of a-naphthol and 0.2 ml KOH arrangement was added after brooding and shaken well. The occurence of red tone shows positive test (Cavalho *et al.,* 2023).

f. Citrate Utilization Test

The bacterial isolates were streaked on the outside of Simon's citrate agar incline (0.2 g sodium citrate, 0.02 g $MgSO_4$, 0.5 gm NaCl, 0.1 gm ammonium dihydrogen orthophosphate, 0.005 g bromothymol blue, 100 mL H_2O, 4g agar,) vigorously and in this manner the inclination was brooded at 37^0 C for 48-72 hours. The occasion of dark blue tone inside 24-48 hours demonstrated positive outcome (Cavalho *et al.,* 2023).

g. Catalase Test

The bacterial isolates were streaked on the outside of supplement agar incline intensely and brooded at 37^0 C for 24 hours, 1 ml of hydrogen peroxide was added over the development on agar incline after hatching. The fast appearance and support creation of gas bubbles showed positive outcome (Patel *et al*., 2016).

h. Oxidase test

All bacterial isolates were streaked on the outside of supplement agar incline (3 g meat separate, 5 g peptone, 15 g agar, 1000 ml refined water) intensely and incubated at 37^oC for 24 hours, after brooding, a settlement was picked and a smear was set up on a channel paper saturated with 1 % tetraethyl-phenylenediamine dihydrochloride arrangements. The arrangement of violet tone with 45-60 seconds showed positive outcome (Cavalho *et al.,* 2023).

Screening of Microbial Lipases Production on the Agar Solid Surface

The bacterial single colonies were screened for their ability to produce lipases by using solid media containing different substrates, including olive oil with phenol red. The screening assays were performed using solid media due to difficulty in the determination of lipolytic activity as the water soluble lipases catalyze reaction of only the water insoluble substrates. The relative enzymatic activity was identified based on visual observation and measuring the formation of a clearance zone on the agar surface.

RESULTS

The results for this study were revealed in Tables 1-4. The results of morphological characteristic of soil sample of different locations in Sokoto metropolis and biochemical analysis of the soil sample in the rhizosphere of rice of different locations in Sokoto metropolis are presented in Tables 1 and 2.

Table 1: Morphological Characteristic

Sample	Colour	Shape	Elevation	Size	Texture
A1	Milky	Circular	Raise	11	Mucoid
A2	Milky	Irregular	Raise	6	Watery
B1	Milky	Circular	Flat	15	Mucoid
B2	Milky	Circular	Flat	13	Mucoid
C1	Milky	Irregular	Flat	5	Mucoid
C2	Milky	Irregular	Flat	7	Mucoid
D1	Milky	Irregular	Raise	7	Watery
D2	Milky	Irregular	Flat	15	Mucoid
E1	Milky	Irregular	Raise	18	Mucoid
E2	Milky	Irregular	Raise	8	Mucoid

Table 2: Biochemical Characterization of Bacteria Isolation from Samples

Sample	Gram	Shapes	Cat	Oxidase	Glu	Suc	Lac	H_2s	Mrvp	Indol	Citrate	Gas	Species
A1	_	Rod	+	_	+	+	+	_	+/-	+	-	+	*E.coli*
A2	_	Rod	+	_	+	+	+	_	-/+	-	+	+	*Flavobacterium*
B1	_	Rod	+	_	+	+	+	_	+/-	+	-	+	*E. coli*
B2	+	Cocci	+	+	+	+	+	_	+/-	-	+	+	*Bacillus spp*
C1	+	Cocci	+	+	+	+	+	_	+/-	-	+	+	*Bacillus spp*
C2	_	Rod	+	_	+	+	+	_	-/+	-	+	+	*Flavobacterium*
D1	_	Rod	+	_	+	+	+	_	+/-	+	-	+	*E. coli*
D2	+	Cocci	+	+	+	+	+	_	+/-	-	-	-	*S. aureus*
E1	+	Cocci	+	+	+	+	+	_	+/-	-	-	+	*Bacillus spp*
E2	+	cocci	+	+	+	+	+	_	+/-	-	-	+	*Bacillus spp*

Table 3: Percentage and Frequency of Occurrence Of Bacteria Isolates

s/n	Bacterial isolates	Frequency	Percentages (%)
1	*Escherichia coli*	3	30
2	*Flavobacterium*	2	20
3	*Bacillus spp*	4	40
4	*S. aureus*	1	10
Total		10	100%

Table 4: Lipase Production by the Bacteria Isolates Often From Sample

S/N	Bacteria	Lipases production
1	*E. coli*	Positive (+)
2	*Flavobacterium*	Positive (+)
3	*Bacillus spp*	Positive (+)
4	*S. aureus*	Positive (+)

Keys: + = positive, - = gram negative, - ve = Gram negative, Cat = catalase, Coa = coagulase, Mot = motility, Mr = methyl red, Vp = voges proskeus, Urea = Urease base, Cit = Citrate, Glu = glucose, Suc = Sucrose, Lac = Lactose, H_2S = Hydrogen sulphate, Ind =Indole

DISCUSSION

This work analyzed a total of five (5) different soil samples in the rhizosphere of rice from Sokoto metropolis. Therewith, the potentially of bacterial isolate was screened for the lipase production.

Bacillus spp had the highest percentage of occurrence 40%. This indicates the *Bacillus spp* was present in most samples that were analyzed. The next was *E.coli* with percentage of 30% (indicating *Bacillus spp* separated of five locations). Followed by, *Flavobacterium* with percentage of 20% which also show that *flavobacterium* appears in two locations. *S. aureus* had a percentage occurrence of 10% respectively. Bacterial organisms isolated were identical to those earlier reported by Cavalho *et al,* (2023).

However, microbes (including the bacteria species) in the soil are important in many respects, such as participation in ensuring the biochemical cycles required for ecosystem sustainability. Likewise, those microbes (bacteria for example) due to their enzymes producing capacity could invariably help in getting rid of environmental wastes through the enzymes they produced (Fakorode *et al*., 2019; Mathew and Izomor, 2023). Bacteria producing enzyme could be used as a tool for making larger amount of enzymes needed for various industrial applications (Mathew and Izomor, 2023). The study is similar to the reports of Fakorode *et al*., (2019) that show *Bacillus spp, E. coli* among other bacteria showing lipase activity producing potential. Mathew & Izomor (2023) also demonstrated that *Bacillus spp* microbe shoe lipase enzyme activity in their study.

Certainly, the four bacterial species isolated were found to produce varying zones of clearance. The appearance of a zone of clearance and visible precipitate as a result of deposition of phenol red is an indicator for lipase production (Tsegaye, *et al*. 2019). The diameter of the zone of clearance of various isolates revealed that, *Bacillus spp* had highest, followed by *E. coli*, then *Flavobacterium,* and *S. aureus* respectively. The *Bacillus spp* had the least diameter zone of clearance. The variation in enzyme production could be attributed to species difference (Benjamin and Pandey 1996). The enormous uses of lipase in various industries cannot be over emphasized and it is on the increase in several areas of applications (Sarkingobir *et al*., 2022b). According to Mathew and Izomor (2023), extensive and persistent screening for new microorganism and their lipolytic enzyme will open new, simple routes for synthetic processes and consequently new and faster way to the application of lipase in adding value to human life including solving environment problems.

CONCLUSION

The results of this study show capability of producing lipase enzyme from bacteria. The *Bacillus spp* had a highest lipases activity; therefore, the study shows higher yielding lipases producing bacteria that could be used for industrial production.

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