



RESEARCH ARTICLE

Enzymatic Activity of Endophytic Bacterial Isolates from Selected Mangrove Plants in Kenya

Regina M. Ntabo^{1,*}, Anthony Kebira Nyamache¹, Wilber Lwande², James Kabii² and James Nonoh¹

¹Department of Microbiology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya

²International Center of Insect Physiology and Ecology, P.O. Box 30772-00100 Nairobi, Kenya

Received: July 31, 2018

Revised: October 19, 2018

Accepted: October 20, 2018

Abstract:

Introduction:

Microorganisms are a preferred source of enzyme production due to their high production capability and low cost of production. Bacterial endophytes occupy unexplored sites hence they represent a new source of enzymes with diverse applications. Mangrove plants in Kenya have traditionally been used as medicinal plants due to their bioactive metabolites. However the enzymatic activity of mangrove plants associated endophytes has not been studied.

Aims & Objectives:

The study is aimed at bioprospecting for enzymes with potential biotechnological applications from mangrove ecosystems.

Methods & Materials:

Forty-two bacterial isolates were cultured and isolated from the leaves and roots of six mangrove plants sampled from Mida Creek and Gazi Bay in the coastal region of Kenya. The isolates were screened for pectinases, chitinases, cellulases, proteases, and amylases. The isolates were identified based on morphology and 16S rRNA gene sequences analysis.

Results:

The study showed bacterial isolates had enzymatic activity as follows; pectinases activity (69% of the isolates), Proteases (95% of the isolates), amylases activity (88% of the isolates), cellulases and chitinases (92% of the isolates each). Bacterial endophytes from leaves showed a higher enzymatic index of cellulases suggesting a potential role in degrading cellulose in the leaves of plants. The enzymes amylases and proteases were mostly exhibited by endophytes in roots suggesting a potential role in metabolizing sugar and amino acids in the roots. Isolates from the mangrove plant *Sonneratia alba* showed highest enzymatic indices. The study also observed that isolates from mangrove plants sampled from Gazi bay had high means of enzymatic indices. Molecular identification showed the isolates were closely related to *Bacillus*, *Streptomyces*, *Myroides*, and *Staphylococcus* species. Their respective enzymatic activities have been provided in this study.

Conclusion:

The study showed that Kenyan Mangrove plant-associated bacterial endophytes provide a good reservoir of enzymes with potential industrial applications.

Keywords: Endophytic bacteria, Mangrove plants, Enzymes, Mangrove plant, *Sonneratia alba*, Enzymatic index.

1. INTRODUCTION

Endophytes are microbes that inhabit asymptotically in the living tissues of plants without causing any

* Address correspondence to this author at the Department of Microbiology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya; Tel: +254 20870; E-mail: rntabo@gmail.com

substantive negative effect [1]. They colonize the plant tissue both inter- and intracellularly where they exhibit complex interactions with their host [2]. Each plant that exists on earth is considered to be a host for one or more of these endophytes. These endophytes vary from one plant species to another and their diversity depends on the climatic condition of a particular region and age of the plant [3]. Mangrove ecosystems are intertidal productive wetland forest that mostly constitutes halophytic plants. This environment is unique with its highly characterized organic matter, sulfur and nitrogen that are very important for bacterial growth [4]. Mangrove plants have been shown to have medicinal properties with over two hundred natural products whose antibacterial, antiviral and antidiarrheal activities have been potentially confirmed [5]. Plant-associated endophytes are a potential source of enzymes such as cellulases that can be used in biotechnological and pharmaceutical processes [6]. These hydrolytic enzymes can also be used in bioremediation process since they can break down complex biopolymers into sugars [7]. Studies have also shown that endophytic microbial enzymes such as pectinolytic and cellulolytic enzymes are involved in the infection process [8]. Endophytes in mangrove plants occupy a relatively unexplored site in microorganism isolation and can be a source for enzymes that can be utilized in food processing, detergent industries and pharmaceutical companies [9]. The intimate and non-detrimental association between endophyte, their host plant and the complex substrates involved may provide a unique habitat causing the endophytes to develop various mechanisms producing a variety of bioactive compounds [10] or bioprospecting for potential industrial enzymes. While most of the investigations on endophytes have been focused on terrestrial plants, the potential of bacterial endophytes of mangrove plants especially those isolated from Kenya's mangrove ecosystem as a source of beneficial enzymes has not been investigated. The study hypothesis states that mangrove plants in Kenya have bacterial endophytes with potential enzymes. The objectives of the study were to isolate, identify and enzymes screening of bacterial endophytes from leaves and roots of selected mangrove plants found in at Mida Creek and Gazi Bay in Kenya coastal region.

2. MATERIALS AND METHODS

2.1. Sample Collection

Mangroves plants were collected from two sites namely Mida Creek which is located in Malindi County on the North Coast of Kenya and Gazi Bay which is located 55 km South of Kenya's coast. The Leaves and roots samples were collected from six mangrove plant namely; *Rhizophora mucronata*, *Avicennia marina*, *Sonneratia alba*, *Lumnitzera racemosa*, *Bruguiera gymnorhiza* and *Xylocarpus granatum*. The samples were transported in sealed bags and kept in a cold room at 4 °C and processed within 24 hours.

2.2. Isolation and Cultivation of Bacterial Endophytes

Approximately, 1g of roots and leaves of the mangrove plants were surface sterilized with 70% ethanol for a few seconds and further disinfected in 3.8% sodium hypochlorite for three minutes to eliminate surface contaminating microbes. The samples were then rinsed in sterile distilled water and air dried in the laminar flow hood to eliminate residual disinfectants. The samples were excised and macerated in sterile distilled water using mortar and pestle. Approximately 1ml of the sample was serially diluted in normal saline and 0.1 ml aliquot was spread on different media. The study utilized various unconventional media with low nutrient and supplemented with sea water to assimilate the natural ecological niche of the endophytes. The media were as follows; M1 media contained 12 g of starch, 4 g of yeast extract, 2 g of peptone, 18 g of agar and 1000 mL of distilled water; NASEA media contained 23 g of nutrient agar and 1000 mL of seawater, NaCl media contained 23 g of nutrient agar 20 g of NaCl and 1000 mL of distilled water; Differential media contained 12.5 g of glycerol 1.0 g of Arginine, 1 g of NaCl, 1g of K_2HPO_4 , 0.5 g of $MgSO_4 \cdot H_2O$, 0.01 g of $FeSO_4 \cdot H_2O$, 0.001 g of $CUSO_4 \cdot H_2O$, 0.001 g of $MNSO_4 \cdot H_2O$ 20 g of agar and 1000 mL of distilled water. DNBA media contained 0.08 g of nutrient broth, 20 g of agar and 1 L of distilled water. D.D (Modified DNBA) media contained 0.08 g of nutrient broth, 20 g of agar and 1 L of seawater [11]. The plates were incubated at 30°C for up to seven days. Based on distinct morphological characteristics, individual colonies of the cultures were isolated from the primary culture and streaked on fresh respective media in which they had grown to obtain pure cultures were isolated.

2.3. Identification of the Isolates

The morphological and gram stain characteristics of the isolates were determined using standard procedures. The cultures were characterized based on colony morphology and shape of bacterial colonies under a compound microscope at $\times 1000$ magnification [12]. Molecular characterization was further performed to determine the identity of the isolates

using the 16S rRNA gene sequencing analysis. The endophytic bacterial DNA was extracted using FastDNA™ SPIN KIT for soil (MP Biomedicals, Santa ANA, CA) using manufacturer protocol. The 16S rRNA gene was amplified using eubacterial universal primers targeting position 27F to 1491R and HotStarTaq® Mix (QIAGEN) under the following conditions initial activation step at 95°C for 15 minute, denaturation temperature of 95°C for 1 minute, annealing temperature of 56°C and extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. The amplified 16SrRNA gene was purified using QUICK clean II Gel kit (Genscript USA inc.) and sequenced directly. The generated sequences obtained were aligned together with reference sequences generated through the blast program (<http://ncbi.nlm.nih.gov>). Sequences have been deposited in DNA data bank of Japan

GenBank accession numbers can be obtained from the following link

<https://submit.ncbi.nlm.nih.gov/subs/?search=SUB3843120>

Genbank MH136813-MH136822

Genebank MH135805-MH135847

2.4. Determination of Enzymatic Activity

The ability of the identified bacterial endophytic isolates to produce enzymes that hydrolyzes starch, casein, chitin, cellulose, and pectin was performed by streaking the isolate on solid media containing respective substrate. After incubation, observing clearance around the bacterial growth indicted presence of the enzyme. [28].

2.4.1. Determination of Amylases

Presence of amylase enzyme was determined using starch agar containing 0.2% soluble starch and 1.5% agar. Freshly prepared isolates were inoculated by streaking using a sterile wire loop and incubated at 30°C for 18-24 hours to allow bacterial growth. The hydrolytic activity of the isolate was then confirmed by addition of iodine according to the method described by Bibi *et al.* [13]. A clear zone around the colonies confirmed the presence of amylases. The enzymatic index was expressed by the relationship between the average diameter of the halo and average diameter of the colony growth [9].

2.4.2. Determination of the Presence of Proteases

The presence of proteolytic enzyme was determined using skimmed milk agar for protein hydrolysis. Freshly prepared isolates were inoculated by streak method using a sterile loop on milk agar and incubated at 30 °C for 24 hours. Detection of the hydrolytic activity was determined by a clear zone around the colonies according to the method described by Jones and Jack [14]. A translucent zone around the colonies was an indication of degradation of the substrate by proteases. The enzymatic index was expressed by the relationship between the average diameter of the halo and average diameter of the colony growth [15].

2.4.3. Determination of the Presence of Cellulases

The presence of cellulases was determined by inoculating the bacterial isolates on media that contained the following; 3.0 g NaNO₃, 1.0 g K₂HPO₄, 0.5 g MgSO₄·7H₂O, 0.5 g KCl, 0.01 g FeSO₄·7H₂O, 1.0 g cellulose powder and 15 g agar, pH 7.0. The plates were incubated at 30 °C for 48 hrs. After incubation, the plates were flooded with 0.1% Congo red for 20 minutes. A translucent zone around the colonies indicated the presence of cellulases according to the method described by Hung *et al.* [16]. The enzymatic index was expressed by the relationship between the average diameter of the halo and average diameter of the colony growth.

2.4.4. Determination of the Presence of Pectinases

Pectinases activity was detected by streaking isolates on media containing trypticase soy agar supplemented with 1% apple pectin (Galacturonic acid 74% dry powder) and incubated at 30°C. After 48 hours of incubation, Lugols Iodine solution was flooded on the colonies. The presence of a translucent zone around the colonies indicated the presence of pectinases enzymes according to the method described by Hung *et al.* [16]. The enzymatic index was expressed by the relationship between the average diameter of the halo and average diameter of the colony growth [9].

2.4.5. Determination of Chitinases

2.4.5.1. Preparation of Colloidal Chitin

Colloidal chitin was prepared from chitin flakes which were ground. The chitin was slowly added into 600 ml of concentrated HCl and kept for 60 minutes at 30 °C with vigorous stirring in a chemical fume hood. This mixture was then passed through layers of muslin cloth to remove the large chunks of chitin. The clear filtrate obtained was then treated with 2 L of distilled water to allow precipitation of colloidal chitin. This was incubated overnight under static conditions at 4 °C to facilitate better precipitation of colloidal chitin. This was later pressed through 2 layers of coarse filter paper. Approximately 3 L of tap water was passed through the colloidal chitin cake until the pH rose to 7. The colloidal chitin was autoclaved and stored at 4 °C until further use [17].

2.4.5.2. Screening for Chitinases

The screening was performed by single line streak of bacterial inoculum in the center of Starch casein agar and nutrient agar containing 0.1% colloidal chitin and incubated at room temperature. The zone of clearance due to chitin hydrolysis was observed after five days of incubation according to the method described by Priya *et al.*, 2011 [18].

3. RESULTS

3.1. Endophytic Isolates

A total of forty-two bacteria were isolated from the leaves and roots of mangrove plants and were distributed as shown in Table 1.

Table 1. Distribution of bacterial endophytes obtained from mangrove plants.

Mangrove plants	Roots	Leaves	Number of Isolates
<i>Xylocarpus granatum</i>	6	10	16(38.09%)
<i>Avicennia marina</i>	6	5	11(26%)
<i>Sonneratia alba</i>	1	1	2(4.76%)
<i>Bruguiera gymnorhiza</i>	3	3	6(14.28%)
<i>Lumnitzera racemosa</i>	2	3	5(11.90%)
<i>Rhizophora mucronata</i>	1	3	4(9.52%)
Total	19	23	42(100%)

3.2. Morphological Characteristics

Morphological studies showed that of the twenty-three isolates from the leaves of mangrove plants, 61% were Gram-positive bacteria while 39% were Gram-negative as shown in Table 2. The Gram-positive organisms were identified as *Bacillus* species and *Streptomyces* species while the Gram-negative isolates were identified as shown in Tables 2 and 3.

Table 2. Molecular identification and morphological characteristics of the bacterial isolates from the leaf as observed under a compound microscope (x100).

Isolate	Bacteria Shape/Spore	Gram	Identity with Accession Number
MA3XGLeaf	Spiral spore chain	+	<i>Streptomyces</i> MH136816.1
MACT5AMLeaf	Rod/with endospore	+	<i>Bacillus cereus</i> MH136814.1
MA4XGLeaf	short rod- chain like	-	<i>Klebsiella</i> MH135815.1
MGA8XGLeaf	Long rods that form chain	-	<i>Myroides</i> MH135834.1
GMD1XGAMLeaf	Rod with endospore	+	<i>Bacillus cereus</i> MH135807.1
MGN1N5AM	Rod with endospore	+	<i>Bacillus</i> MH135809.1
GMN3AMBGLLeaf	Rod with central spore	+	<i>Bacillus sp.</i> MH135810.1
MRMR27leaf	Rod with endospore	+	<i>Serratia sp.</i> MH135841.1
MS9SAleaf	Rod with central spore	+	<i>Myroides profundi</i> MH135838.1
MA4XG2L2leaf	Rod shaped with endospore	+	<i>Bacillus sp.</i> MH135831.1
MA7XG2leaf	short rod-shaped, arranged chain like	-	<i>Serratia</i> MH135840.1
MA8XG3Leaf	Spiral spore chain	+	<i>Streptomyces</i> MH135817.1
GB11XGLeaf	Rod shaped with endospore	+	<i>Bacillus cereus</i> MH135820.1

(Table 2) contd....

Isolate	Bacteria Shape/Spore	Gram	Identity with Accession Number
GD4SALeaf	Spiral spore chain	+	<i>Streptomyces Krainskii</i> MH136820.1
MRE5AMLeaf	Rod with central spore	+	<i>Bacillus cereus</i> MH136818.1
MAMSAM21Leaf	Rod	-	<i>Alcaligenes</i> MH135842.1
MRM18Leaf	Rod	-	<i>Pseudochrobactrum</i> MH135843.1
MLR1Leaf	Rod /endospores	+	<i>Lysinbacillus</i> MH135828.1
GBGN6Leaf	Cocci	+	<i>Staphylococcus xylosus</i> MH135829.1
MXGN9Leaf	Rod	-	<i>Stenotrophomonas</i> MH135844.1
MRM19Leaf	Long rods form chain	-	<i>Myroides</i> MH135845.1
MXG2Leaf	Straight rods	-	<i>Achromobacter</i> MH135846.1

Key for identifying the codes: Site: G- Gazi bay and M-Mida creek. Media: Act/Ac- M1 media, N- Nutrient agar mixed with NaCl, R-M1 media, A – Differential media, S- NASEA media, B-DNBA plus sea water and D- DNBA plus distilled water. Numeral-obtained from the primary culture; Source plant; XG-*Xylocarpus granatum*, AM-*Avicennia marina*, SA- *Sonneratia alba*, BG- *Bruguiera gymnorrhiza*, LR/ *Lumn-Lumnitzera racemosa* and RM-*Rhizophora mucronata*.

Table 3. Molecular identification and morphological characteristics of the isolates from roots as observed under a dissecting microscope (×160) and a compound microscope (x100).

Isolate	Bacteria Shape/Spore	Gram	Identity with Accession Numbers
MAct29LUMNRoot	Rod with endospore	+	<i>Bacillus sp.</i> MH135805.1
MSN7AMRoot	Rod with endospore	+	<i>Bacillus sp.</i> MH135806.1
MGAC128BGRMRoot	Rod with endospore	+	<i>Bacillus cereus</i> MH136815.1
GACTAM3Root	Long rods form chain	-	<i>Myroides sp.</i> MH135835.1
MACTBG23Root	Long rods that form chain	-	<i>Myroides sp.</i> MH135836.1
GR1XGAMRoot	Rod with endospore	+	<i>Bacillus</i> MH135808.1
NS5AMRoot	Rod with endospore	+	<i>Bacillus thuringiensis</i> MH135811.1
MAXG12root	Rod with endospore	+	<i>Bacillus cereus</i> MH135830.1
MA25RMRoot	Spore chain	+	<i>Streptomyces misionensis</i> MH135818.1
GAC28BGRMRoot	Rod with endospore	+	<i>Bacillus cereus</i> MH135819.1
GNS1XGRoot	Cocci	+	<i>Staphylococcus</i> MH135821.1
GR3XGRoot	Rod shaped	-	<i>Pseudomonas</i> MH136821.1
GR4XGRoot	Rod shaped with central spore	+	<i>Bacillus</i> MH135822.1
GR8AMRoot	Rod with endospore	+	<i>Bacillus safensis</i> MH136819.1
GSNSAMLRRoot	Rod with spore	+	<i>Bacillus thuringiensis</i> MH135823.1
MAAM22Root	Aerial mycelia	+	<i>Streptomyces krainskii</i> MH136822.1
MBGM4Root	Rod with endospore	+	<i>Bacillus sp.</i> MH135826.1
MD12BGRoot	Rod with endospore	+	<i>Bacillus cereus</i> MH135827.1
MAXG12R2 root	Rod with endospore	+	<i>Bacillus sp.</i> MH135830.1

Key for identifying the codes: Site: G- Gazi bay and M-Mida creek. Media: Act/Ac- M1 media, N- Nutrient agar mixed with NaCl, R-M1 media, A – Differential media, SN- NASEA media, B-DNBA plus sea water and D- DNBA plus distilled water. Numeral; obtained from the primary culture-. Source plant; XG-*Xylocarpus granatum*, AM-*Avicennia marina*, SA- *Sonneratia alba*, BG- *Bruguiera gymnorrhiza*, LR/ *Lumn-Lumnitzera racemosa* and RM-*Rhizophora mucronata* Plant part-root or leaf.

Out of the nineteen isolates obtained from the roots of mangrove plants, 84.3% were gram-positive bacteria while 15.7% were gram-negative as shown in Table 3. The Gram-positive organisms mainly had characteristics typical of *Bacillus* and *Streptomyces* species while the Gram-negative isolates had characteristics resembling members of *Myroides* species.

3.3. Enzymatic Activity

Enzymatic Index (EI) was tested in all the isolates from mangrove plants (Table 4). The results show that the enzyme amylase was detected in 78% of the isolates, chitinases (75% of the isolates), proteases (71% of the isolates), cellulases (68% of the isolates) and pectinases activity was detected in 61% of the isolates. The study showed that cellulase activity was mostly expressed in isolates from leaves suggesting a potential role in colonizing the leaf tissues as shown in Fig. (1).

Table 4. Enzymatic activity of the isolated bacteria from mangrove plants.

Isolate code	Genus Identity	Enzymatic indexes				
		Amylase	Proteases	Cellulases	Chitinases	Pectinases
GR8AMroot	<i>Bacillus</i>	3.30 ^c	3.0 ^a	4.3 ^a	1.6 ^a	-
GNS1XGroot	<i>Staphylococcus</i>	3.30 ^c	3.00 ^a	4.30 ^a	1.6 ^a	-
MA3XGleaf	<i>Streptomyces</i>	4.23 ^d	2.86 ^a	4.33 ^a	2.63 ^{bc}	3.86 ^c
Act 5AMleaf, MBGM4root, NS5root GMD1XGAMleaf, MRE5AMleaf	<i>Bacillus</i>	3.60 ^c	2.05 ^a	2.52 ^a	3.00 ^c	4.62
MXG12root, GB11XGleaf, MR27RMleaf, MACT29Lumnroot, GR1XGAMroot, MG,N1N5AMRMSABGXGleaf, MGA28BGRMroot,	<i>Bacillus</i>	2.43 ^b	1.08 ^a	2.50 ^a	1.325 ^a	3.20 ^c
SN7root	<i>Bacillus</i>	1.80 ^a	2.00 ^b	2.50 ^{bc}	3.00 ^c	4.20 ^d
MGA8XGleaf, GAM3root, MBG23root, MRM19L, MXG1leaf	<i>Myroides</i>	2.22 ^a	2.52 ^b	2.40 ^b	0.92 ^a	-
MXGN9leaf	<i>Stenotrophomonas</i>	3.30 ^c	3.00 ^c	4.3 ^d	1.60 ^a	-
GBGN6leaf	<i>Staphylococcus</i>	3.30 ^c	3.00 ^c	4.3 ^d	1.60 ^a	-
MAM22Rroot	<i>Streptomyces</i>	1.50 ^a	4.5 ^d	-	2.00 ^b	3.10 ^c
MLR1leaf	<i>Lysinibacillus</i>	2.50 ^b	3.30 ^c	4.00 ^d	1.30 ^a	-
MXG2leaf	<i>Achromobacter</i>	-	2.00 ^b	5.00 ^c	1.30 ^a	3.60 ^c
GR3XGR2	<i>Pseudomonas</i>	2.50 ^b	3.00	-	-	4.20 ^d
GMN3AMBGleaf, MRE5AMleaf, MS9SA leaf, GR4XGroot	<i>Bacillus</i>	4.22 ^c	2.25 ^{bc}	4.25 ^d	1.25 ^a	4.25 ^d
MD12BGroot, MA4XGleaf	<i>Bacillus</i>	-	-	4.5 ^d	1.78 ^a	3.35 ^d
MRM18leaf	<i>Pseudobacterium</i>	3.30 ^c	2.00 ^b	4.5.00 ^d	2.00 ^b	-
MA25RMroot, MGA8XGleaf, GD4SAleaf	<i>Streptomyces</i>	4.80 ^d	2.42 ^{bc}	4.75 ^d	1.92 ^{ab}	3.17 ^c
MR27RMleaf	<i>Serratia</i>	2.00 ^b	3.00 ^c	3.00 ^c	-	4.20 ^d
GSNSroot	<i>Bacillus</i>	3.30 ^c	2.60 ^{bc}	2.00 ^b	-	-
MAMSAM21leaf	<i>Alcaligenes</i>	3.30 ^c	3.00 ^c	-	3.00 ^c	-
MA4XGleaf2, MA7XGleaf,	<i>Klebsiella</i>	-	2.00 ^b	4.00 ^d	1.32 ^a	2.70 ^a
Overall		2.71 ^b	2.4 ^b	3.44 ^c	1.49 ^a	2.37 ^b

The enzymatic index represents the mean halo diameter of degradation/ diameter of bacterial colony in cm of four replicate experiments. Mean values of enzymatic indices within the same column followed by the same letter don't differ significantly among themselves (Turkey's b, p<0.05). Isolates grouped together based on morphology and biochemical tests. Key for identifying the codes: Site: G- Gazi bay and M-Mida creek Media: Act/Ac- M1 media, N- Nutrient agar mixed with NaCl, R-M1 media, A – Differential media, SN1- NASEA media, B-DNBA plus seawater and D- DNBA plus distilled water. Numeral; 1-27. Source plant; XG-*Xylocarpus granatum*, AM-*Avicenia marina*, SA- *Sonneratia alba*, BG- *Bruguiera gymnorrhiza*, LR/ Lumn- *Lumnitzera racemosa* and RM-*Rhizophora mucronata*. Plant part; leaf and root.

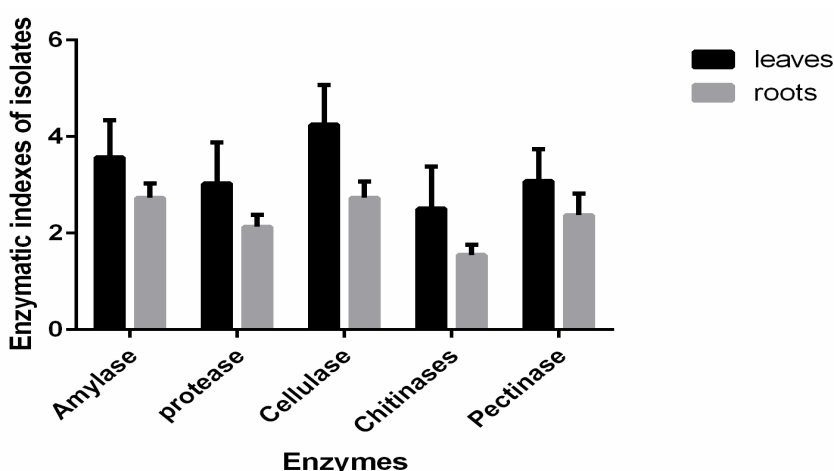


Fig. (1). Graph showing various Enzymatic indexes of isolates in leaves and roots. Two way ANOVA p=0.328 df=1.

Statistical analysis using one-way ANOVA showed that the enzymatic indexes were significant between the different enzymes at df=4 F= 13.683 and p=0.0001. One-way ANOVA showed that enzymatic index of the different isolates was significant at P=0.0001 at F=7.023, df=42 suggesting that the different species had different degradation

capacities of the substrates in their environment through the production of respective enzymes. Interaction of species and enzyme was analyzed using two way ANOVA and results showed means were significantly different at $p=0.0001$, $F=10.238$ $df=168$ suggesting a significant difference in means of enzymatic assays on different plant parts. Analysis of the enzymatic indices based on plant parts showed a significant difference at $p=.0328$ $df=1$ with leaves showing highest index. The results showed the bacterial endophytic isolates had different ability to utilize starch, cellulose, protein, chitin, and pectin and could potentially have a role to play in their environment as shown in Table 4.

Highest amylase activity was observed in isolate MA25RMroot isolated from *Rhizophora mucronata* roots sampled from Mida creek as shown in Table 4. Majority of the isolates from the leaves in all mangrove plants expressed high cellulases activity especially isolate MXG2leaf suspected to be *Achromobacter* isolated from *Xylocarpus granatum* leaves sampled from Mida creek. Least enzymatic activity was observed in chitinases by two isolates suspected to be *Serratia* and *Bacillus* isolated from *Rhizophora mucronata* and *Avicenia marina* roots. Sampled from Mida creek and Gazi bay respectively (Table 4).

The study further observed that all mangrove plants harbor bacterial endophytes with various enzymatic activities as shown in Fig. (2). High cellulases activity was observed in isolates from *Lumnitzera racemosa*. High chitinases activity was observed in isolates from *Avicenia marina* as shown in Fig. (2). Pectinase activity was highest in isolates from *Xylocarpus granatum*.

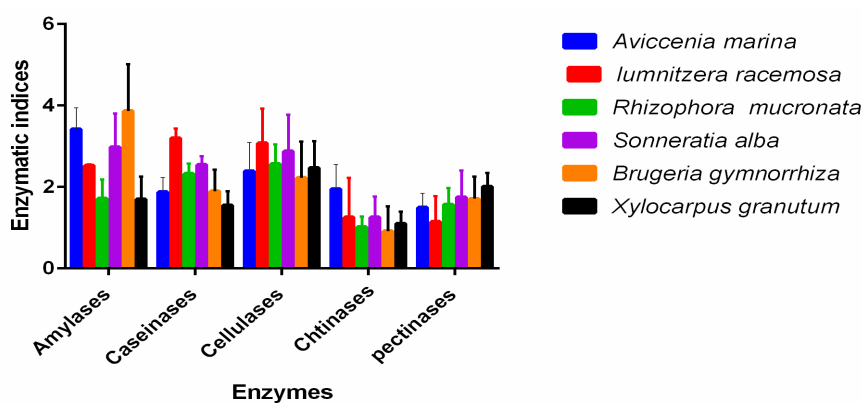


Fig. (2). Graph showing enzymatic activities of the different mangrove plants. Two way ANOVA $p=0.0006$, $F(5,24)=6.496$.

4. DISCUSSION

Endophytes are known to colonize plant tissues using enzymes involved in the infection process by degrading the plant tissues [19]. In this study, the leaves harbored more endophytic bacteria compared to the roots. This is in line with previous studies that have shown leaves of *Arabidopsis thaliana* harbored more endophytes than roots [20]. This may be attributed to high photosynthetic metabolism in the leaf hence the products could be utilized by the microbes. Most endophytic bacterial isolates were obtained from *Xylocarpus granatum* plant while least number was obtained from *Lumnitzera racemosa* this may be attributed to cultivation conditions since this plant grows in different zonation, the conditions (media) used to cultivate may have been optimal for isolates from *Xylocarpus granatum*. The study observed that all mangrove plants harbored isolates with various enzymatic activities. Similar studies on enzyme production by bacterial endophytes from leaves and stem of mangrove plants such as *Rhizophora apiculata*, *Avicennia marina*, *Excoecaria agallocha*, *Ceriops decandra* and *Aegiceras corniculatum* showed presence of chitinases, amylases, proteases, cellulases, and pectinases [21]. In addition, studies conducted in bacteria endophytes of *Rhizophora mangle* and *Avicenia nitida* mangrove found in Brazilian mangrove forest showed the presence of endoglucanases and proteases [15]. The study also revealed that members of *Bacillus* species were the most dominant in both the leaves and roots. This is in line with previous studies conducted on endophytes in mangrove plants found in Saudi Arabia that revealed the most dominant species being *Bacillus* [13]. *Bacillus* and related species have also shown presence of proteases, pectinases and cellulases in endophytic bacteria of *Paenibacillus polymyxa* [22]. The survival of these endophytes is attributed to their ability to utilize these cellulose and pectin in the plant tissues [22]. The other dominant bacterial endophytes included the *Streptomyces* which was dominantly found in the leaves of *Xylocarpus granatum* plant. Similar studies conducted in Brazil showed the presence of bacterial endophytes identified to be *Bacillus*, *Alcaligenes* and *Stenotrophomonas* in the leaves and stem of *Rhizophora mangle* and *Avicenia nitida* [15]. This may imply that the mangrove plants harbor similar microorganism however further work needs to be done in other mangrove ecosystems in

tropical Africa to confirm the same.

In this study results showed most of the isolates identified to be *Bacillus* species, for example, isolate GSN1XGroot from the roots of *Xylocarpus granatum* collected from Gazi bay expressed presence of amylases, caseinases, cellulases and chitinases suggesting a possible ecological role of these enzymes in the symbiotic relationship of the isolates and the host plants [23].

The study showed pectinases activity was present in isolates identified to be *Bacillus*, *Streptomyces*, *Achromobacter* and, *Pseudomonas* in line with previous studies that have shown bacterial endophytes having pectinases enzymes [24]. However four isolates did not show pectinases and these were identified to be *Staphylococci*, *Stenotrophomonas*, *Pseudochrobactrum* and *Alcaligenes* suggesting that the enzyme is not actively expressed or is merely lacking in the isolates. However previous have shown the presence of pectinases in *Stenotrophomonas* isolated from algae [25].

The isolates MXG2leaf and MA7XGleaf identified to be *Klebsiella* isolated from the leaves of *Xylocarpus granatum* obtained from Mida creek did not express amylases activity. Previous studies show that *Klebsiella* have amylases [25] suggesting that the enzyme is not actively expressed or is merely lacking in the isolates.

The study showed all isolates belonging to *Bacillus* showed the presence of proteolytic and amylases activity suggesting possible expression of these enzymes and their potential role in degrading starch and protein organic matter in the ecosystem. However, isolate MD12BGroot isolated from the root of *Bruigeria gymnorhiza* obtained from Mida creek and Gazi bay and isolate MA4XGleaf isolated from leaves of *Xylocarpus granatum* obtained from Mida creek did not show presence of amylases indicating the enzyme is not actively expressed. Similar studies on bacterial endophytes of *Curcuma* have shown the presence of amylases in the *Bacillus* species [26].

The presence of cellulases activity was exhibited by isolates identified to be *Bacillus*, *Myroides*, *Pseudochrobactrum*, and *Serratia*. This is in line with previous studies that have shown the presence of cellulases in bacteria endophytes cultivated from soybean [27]. However the isolates MAM22Root identified to be *Streptomyces species* isolated from the roots of *Avicenia marina* obtained from Mida Creek, GR3XGroot suspected to be *Pseudomonas* species isolated from the root of *Xylocarpus granatum* obtained from Gazi bay and MAMSAM21leaf identified to be *Alcaligenes* species isolated from the leaves of *Avicenia marina* obtained from Mida creek did not express cellulases suggesting that the enzyme is not actively expressed or is merely lacking in the isolates. These reference organisms have been reported to express cellulases enzyme [28].

The isolates identified to be *Bacillus* and *Streptomyces* showed the presence of chitinases activity suggesting the possible role of these enzymes in the isolates. Similar studies have shown the presence of chitinases in *Streptomyces* species [29] and *Pseudomonas* species [29] However isolates R3XGroot obtained from the root of *Xylocarpus granatum* identified to be *Pseudomonas*, MR27RMleaf isolated from leaves of *Rhizophora mucronata* obtained from Mida creek identified to be *Serratia* and SNSroot isolated from the root of *Xylocarpus granatum* obtained from Gazi bay identified to be *Bacillus* all did not express chitinases activity suggesting that the enzyme is not actively expressed or is merely lacking in the isolates.

CONCLUSION

The study revealed that Mangrove plants from Kenya's coastal region harbor endophytes with potential enzymes that can be applicable in industries such as detergent, textile, and agrochemical. The enzyme cellulases were dominantly present in most isolates while chitinases was least present. Most isolates from mangrove plant *Xylocarpus granatum* showed the enzymatic activity of all the enzymes tested this could be attributed to the fact that it is a medicinal plants hence its endophytes could have bioactive compounds synthesizes by these enzymes. Most of the enzymatic activity was observed in isolates from leaves when compared with those from the roots which could be attributed to the fact that the leaves have high metabolic activities due to photosynthesis. . This is the first report showing the isolation of bacterial endophytes from the leaves and roots of mangrove plants with screening for enzymatic activity. Mangrove plants in Kenya are native plants with diverse medicinal applications and the study reveals they also provide a source of endophytic bacteria community with potential beneficial applications.

AUTHORS' CONTRIBUTIONS

RN carried out carried out the isolation, screening for enzymatic activities, analyzed the data and wrote the manuscript. JN designed the study, analyzed the data and reviewed the manuscript; AKN assisted in molecular characterization and reviewed the manuscript, WL Facilitated the laboratory space and equipments for the study. JK

assisted in molecular characterization. All authors read and approved the final manuscript.

ADDITIONAL DATA

Sequences have been deposited in DNA data bank of Japan
GenBank accession numbers can be obtained from the following link
<https://submit.ncbi.nlm.nih.gov/subs/?search=SUB3843120>
Genbank MH136813-MH136822
Genebank MH135805-MH135847

AVAILABILITY OF DATA MATERIALS

Please contact author for data requests

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT OF PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGMENT

The study was funded by Kenyatta University Vice-Chancellors' Research Grant and DAAD in country/in region PHD Scholarship.

REFERENCES

- [1] Kumar A, Singh R, Yadav A, Singh D D G P K. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. 3 Biotech 2016; 6(1): 1-8.
[<http://dx.doi.org/10.1007/s13205-016-0393-y>]
- [2] Paul NC, Ji SH, Deng JX, Yu SH. Assemblages of endophytic bacteria in chili pepper (*Capsicum annum* L.) and their antifungal activity against phytopathogens in vitro. Plant Omics Journal 2013; 6(6): 441-8.
- [3] Heijden M G A, Van Der , Sessitsch A. Climate change effects on beneficial plant-microorganism interactions. 2010; 73: pp. 197-214.
[<http://dx.doi.org/10.1111/j.1574-6941.2010.00900.x>]
- [4] Hong K, Gao A, Xie Q, Gao H, Zhuang L, Lin H. Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. Marine Drugs 2009; 7: 24-44.
- [5] Saad S, Taher M, Susanti D, Qaralleh H, Rahim NABA. Antimicrobial activity of mangrove plant (*Lumnitzera littorea*). Asian Pac J Trop Med 2011; 4(7): 523-5.
[[http://dx.doi.org/10.1016/S1995-7645\(11\)60138-7](http://dx.doi.org/10.1016/S1995-7645(11)60138-7)] [PMID: 21803301]
- [6] Zhao L, Xu Y, Lai X, Shan C, Deng Z, Ji Y. Screening and characterization of endophytic Bacillus and Paenibacillus strains from medicinal plant *Lonicera japonica* for use as potential plant growth promoters. 2015; 989: pp. 977-89.
- [7] Khianngam S, Pootaeng-on Y, Techakriengkrai T, Tanasupawat S. Screening and identification of cellulase producing bacteria isolated from oil palm meal. 2014; 4: pp. (4)90-6.
[<http://dx.doi.org/10.7324/JAPS.2014.40416>]
- [8] Aliferis K A, Seguin P, Rani M, Samson R, Jabaji S. Isolation and characterization of indigenous endophytic bacteria associated with leaves of switchgrass (*Panicum virgatum* L.) cultivars. 2013.
[<http://dx.doi.org/10.1111/jam.12088>]
- [9] Jussara A, Carrim I, Barbosa EC, Gonçalves JD. Enzymatic activity of endophytic bacterial isolates of jacaranda decurrens Cham. (Carobinha-do-campo). Braz Arch Biol Technol 2006; 49(May): 353-9.

- [10] Adewale A M, Kheng G J, Ping L S, Yien T A S. Antimicrobial and enzymatic activities of endophytic bacteria isolated from *Mentha spicata* (MINT). *Malays J Microbiol* 2015; 11(2, SI): 102-8.
- [11] Janssen PH, Yates PS, Grinton BE, Taylor PM, Sait M. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. *Appl Environ Microbiol* 2002; 68(5): 2391-6. [<http://dx.doi.org/10.1128/AEM.68.5.2391-2396.2002>] [PMID: 11976113]
- [12] James G Cappuccino, Natalie Shermann. A text book of Laboratory manual. 10th Ed. Pearson: Amazon Publishers 2014.
- [13] Bibi F, Ullah I, Alvi SA, *et al.* Isolation, diversity, and biotechnological potential of rhizo- and endophytic bacteria associated with mangrove plants from Saudi Arabia. *Genet Mol Res* 2017; 16(2): 1-12. [<http://dx.doi.org/10.4238/gmr16029657>] [PMID: 28653740]
- [14] da Cruz Ramos GF, Ramos PL, Passarini MRZ, *et al.* Cellulolytic and proteolytic ability of bacteria isolated from gastrointestinal tract and composting of a hippopotamus. *AMB Express* 2016; 6(1): 17. [<http://dx.doi.org/10.1186/s13568-016-0188-x>] [PMID: 26931430]
- [15] Castro RA, Quecine MC, Lacava PT, *et al.* Isolation and enzyme bioprospection of endophytic bacteria associated with plants of Brazilian mangrove ecosystem. *Springerplus* 2014; 3(382): 382. a [<http://dx.doi.org/10.1186/2193-1801-3-382>] [PMID: 25110630]
- [16] Hung PQ, Kumar SM, Govindsamy V. Isolation and characterization of endophytic bacteria from wild and cultivated soybean varieties. *Biol Fertil Soils* 2007; 44: 155-62. [<http://dx.doi.org/10.1007/s00374-007-0189-7>]
- [17] Araújo L E. I isolation and characterisation of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgare*). *Braz J Microbiol* 2012; 0: 1562-75.
- [18] Priya CS, Jagannathan N, Kalaichelvan PT. Production of chitinases by streptomyces hygroscopicus VMCH2 by optimisation of cultral conditions. *Int J Pharma Bio Sci* 2011; 2(2): 210-9.
- [19] Miliute I, Buzaitė O, Baniulis D, Stanys V. Bacterial endophytes in agricultural crops and their role in stress tolerance : A review 2015; 102(4): 465-78.
- [20] Bodenhausen N, Horton M W, Bergelson J. Bacterial communities associated with the leaves and the roots of arabis thaliana. 2013; 8.(2)
- [21] Gayathri S, Saravanan D, Radhakrishnan M, Balagurunathan R, Kathiresan K. Bioprospecting potential of fast growing endophytic bacteria from leaves of mangrove and salt-marsh plant species. *Indian J Biotechnol* 2010; 9(4): 397-402.
- [22] Padda K, Puri A, Zeng Qingwei. Effect of GFP-tagging on nitrogen fixation and plant growth promotion of an endophytic diazotrophic strain of *Paenibacillus polymyxa*. *Canadian Science Publishers Botany* 2017.
- [23] Yang H, Puri A, Padda KP, Chanway CP, Sciences C, Centre FS. Substrate utilization by endophytic bacteria *Paenibacillus polymyxa* P2b-2R that may facilitate bacterial entrance and survival inside diverse plant hosts. *Open Access Journal* 2017; 41(90): 120-30. [<http://dx.doi.org/10.1139/facets-2016-0031>]
- [24] Elbeltagy A, Nishioka K, Suzuki H, Sato T, Sato Y, Morisaki H. Soil science and plant nutrition isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. 2012; 768.
- [25] Pradesh H. Molecular identification, production and optimization of pectinase by using *Stenotrophomonas maltophilia* P9 Isolated from Algal Biomass of Himachal Pradesh, India. 2018; 7: pp. (1)670-80.
- [26] Kumar A, Singh R, Yadav A, Singh D D G P K. Isolation and characterization of bacterial endophytes of *Curcuma longa* L . 3 *Biotech* 2016; 6(1): 1-8.
- [27] Hung PQ, Kumar SM, Govindsamy V. Isolation and characterization of endophytic bacteria from wild and cultivated soybean varieties. *Biol Fertil Soils* 2007; 44: 155-62. [<http://dx.doi.org/10.1007/s00374-007-0189-7>]
- [28] Sindhu SS, Dadarwal KR. Chitinolytic and cellulolytic *Pseudomonas* sp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* sp. Cicer in chickpea. *Microbiol Res* 2001; 156(4): 353-8. [<http://dx.doi.org/10.1078/0944-5013-00120>] [PMID: 11770853]
- [29] Priya CS, Jagannathan N, Kalaichelvan PT. Production of chitinases by streptomyces hygroscopicus VMCH2 by optimisation of cultral conditions. *Int J Pharma Bio Sci* 2011; 2(2): 210-9.