PRINT: ISSN 0970-9274 ONLINE: ISSN 2456-6608
J Hum Ecol, 56(1,2): 31-41 (2016)
DOI: 10.31901/24566608.2016/56.1-2.04

Isolation and Identification of Potential Antibiotic Producing Rare Actinomycetes from Rhizospheric Soils

Mobolaji Felicia Adegboye and Olubukola Oluranti Babalola*

Food Safety and Safety Niche Area, Faculty of Agriculture, Science and Technology, North-West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa

KEYWORDS Antibacterial Activity. Antibiotic. Characterization. Phylogenetic. Rare Actinomycetes. Rhizospheric Soil

ABSTRACT The search for novel antibiotics producers and their characterization continues to be an important objective in the discovery of novel bioactive compounds. This work was carried out to isolate and identify bioactive secondary metabolite producing rare actinomycetes and to analyse the phylogenetic relationship. The rhizospheric soil samples were collected from different localities of Ngaka Modiri Molema district of North West Province, South Africa and screened for antibacterial potential. Molecular identification of the bacterial isolates by analysis of the 16S rDNA nucleotides sequences showed that the isolates were as follows: *Actinomadura*, *Nocardiopsis*, *Promicromonospora*, *Nocardia*, *Arthrobacter*, *Pseudonocardia*, *Micrococcus*, *Nonomuraea*, *Rhodococcus*, *Streptosporangium* and *Saccharothrix* spp. Nineteen (21.6%) of the 88 isolates exhibited antibacterial activity against at least one of the test organisms. Phylogenetic analysis revealed that the bacterial isolates are the members of rare actinomycetes which are associated with the rhizosphere. Results from the phylogenetic analysis indicate that the 19 isolates could be sorted into 11 phylotypes. It was also inferred from the tree that the potent bacterial isolates clustered with other antibiotic producing rare actinomycetes reference strains retrieved from the GenBank. This study corroborates that rhizospheric soil harbours diverse actinomycetes which can be explored for antibacterial secondary metabolites.

INTRODUCTION

Microbial natural products are the frontier in the discovery of bioactive compounds of pharmaceutical importance. The majority of the bioactive compounds in use today are derived from the secondary metabolites of actinomycetes (Janardhan et al. 2014). The diversity of these bioactive secondary metabolites is unsurpassed in medicine and agriculture. Various bioactive compounds have been isolated and characterized from actinomycetes having great structural and functional diversity including antibacterial, antifungal, antiprotozoal, antiviral, anticholesterol, antihelminthic, anticancer, immunosuppressant agent, herbicides, and pesticides (Marinelli and Marcone 2011; Newman and Cragg 2012). These compounds do not only exhibit potent therapeutic activities but also possess the desirable pharmacokinetic properties. The order Actinomycetales is composed of approximately 140 genera, majority are free living organisms that are widely distributed in nature. They are aerobic, filamentous, Gram-positive bacteria and produce extensively branched substrate mycelium and aerial hyphae. Members of this order are characterized by high G+C in their genetic makeup and complex life cycle (Adegboye and Babalola 2012).

Among actinomycetes, *Streptomyces* is the most common actinomycete in terrestrial habitat, above 60 percent of the actinomycetes isolates (Sharma 2014). The results of extensive screening show that more than 70 percent of known bioactive compounds are produced by *Streptomyces* (Demain and Sanchez 2009). As more new antibiotics were discovered, the chances of finding novel bioactive compounds from *Streptomyces* have dwindled. The possibility of re-isolating already isolated compounds from *Streptomyces* is very high (Hayakawa 2008). There is a need to screen the less exploited genera of rare actinomycetes such as *Actinomadura*, *Actinoplanes*, *Amycolatopsis*, *Dactylosporangium*, *Kibdelosporangium*, *Microbispora*, *Micromonospora*, *Nocardiopsis*, *Nonomuraea*, *Promicromonospora*, *Planobispora*, *Rhodococcus*, *Saccharothrix*, *Streptosporangium* and *Planomonospora* for novel antimicrobial agents (Busti et al. 2006; Adegboye and Babalola 2013a).

*Address for correspondence:
Olubukola Oluranti Babalola
Food Safety and Safety, Niche Area,
North West University, Mafikeng Campus,
Private Bag X2046,
Mmabatho 2735 South Africa
Telephone: (+27) 18 389 2568,
Fax: (+27) 18 389 2134,
E-mail: Olubukola.Babalola@nwu.ac.za

However new approaches for the isolation of soil actinomycetes have shown that other members of this order are also of importance. New species and genera have been identified, and most of them are able to produce novel bioactive compounds (Bérdy 2012). They have produced important classes of antibiotics such as macrolides, β–lactams, aminoglycosides, glycopeptides, lipopeptides, ansamycins, polyenes, anthracyclines, nucleosides, peptides, polyethers and tetracyclines.

As part of our on-going research to find bioactive secondary metabolites that may have application in medicine, this research work is designed to isolate and identify antibiotic producing rare actinomycetes from rhizospheric soils. Actinomycetes strains were isolated, characterized and screened for their antibacterial activity. Of the actinomycetes isolated, the rare actinomycetes strains were also subjected to phylogenetic analysis using comparison of their 16S rDNA gene sequences.

MATERIAL AND METHODS

Actinomycetes Isolation

The sampling area and details of soil samples collected have been previously described (Adegboye and Babalola 2013c). The soil samples were subjected to various physical and chemical pretreatment methods in order to facilitate the isolation of actinomycetes (Hayakawa 2008). Actinomycetes were isolated by serial dilution method from soil samples (Gebreyohannes et al. 2013). Various selective media used in this study were previously described (Adegboye and Babalola 2013b). The plates were observed periodically for the growth of actinomycetes. The pure colonies were selected, isolated and maintained in starch casein agar slants at 4°C for subsequent studies.

Screening for Antibiotic Production

Antagonistic activity of isolates against Gram-negative and Gram-positive bacteria was screened by using the perpendicular streak (Duraipandiyan et al. 2010; Abioye et al. 2013). The experiment was carried out in triplicate.

Morphological Characterization

Pure bacterial isolates were characterized culturally following the protocol given by the International Streptomyces project (ISP) (Shirling and Gottlieb, 1966), growth on sterile ISP-medium 2 was recorded after incubation at 25°C for 14 days. Morphological characters such as colony characteristics, pigment production, absence or presence of aerial and substrate mycelia were observed.

The arrangement of spores and sporulating structures were examined microscopically using cover the slip culture method by inserting a sterile cover slip at an angle of 45°C in the starch casein agar medium (Gebreyohannes et al. 2013).

Biochemical and Physiological Characterization

Various biochemical and physiological tests were performed for the characterization of the bacterial isolates using standard methods previously described (Adegboye and Babalola 2013b).

Isolation of Genomic DNA

The genomic DNA was extracted from all the actinomycete isolates using the cetyltrimethylammonium bromide (CTAB) method as previously described (Adegboye and Babalola 2013b).

PCR Amplification

The 16S rDNA gene was amplified from genomic DNA obtained from bacterial cultures by Polymerase Chain Reaction (PCR) with previously described primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3') (Weisburg et al. 1991; Adegboye and Babalola 2013b).

Sequence Similarities and Phylogenetic Analysis

The chromatograms were edited using Chromas Lite version 2.4 software (Technelysium Pty Ltd 2012). Nucleotide sequences were analyzed and edited by using BioEdit software (Hall 1999). The obtained 16S rDNA sequences were compared to sequences in the NCBI GenBank database with the Basic Alignment Search Tool (BLAST) (Altschul et al. 1990). Multiple alignments of the sequences were carried out by Mafft program 7.050 (Katoh 2013) against correspond-

ing nucleotide sequences of the genus *Streptomyces* retrieved from GenBank. Phylogenetic analyses were conducted using software's in MEGA version 5.2.2 (Tamura et al. 2011). Evolutionary distance matrices were generated as described by (Jukes and Cantor 1969) and a phylogenetic tree was inferred by the neighbor-joining method (Saitou and Nei 1987). Tree topologies were evaluated by bootstrap analysis (Felsenstein 1985) based on 1000 resamplings of the neighbor-joining data set. Manipulation and tree editing were carried out using TreeView (Page 1996).

Supporting data

The 16S rDNA gene sequences determined for the bacterial isolates in this study were deposited in GenBank database and assigned accession numbers (Table 6).

RESULTS

Isolation of Rare Actinomycetes

Actinomycetes were isolated from the 17 rhizospheric soil samples plated based on the morphological appearance of the isolates: tough, leathery, powdery, often whitish to greyish colonies and some with pigmentation. Out of 341 isolates, 88 (26.6%) were identified as rare actinomycetes.

Screening for Antibiotic Producing Actinomycetes

Among the 88 rare actinomycetes isolated from the different soil samples, 19 (22%) isolates exhibited antibacterial activities against at least one of the test organisms (Table 1). Most of the isolates exhibited broad spectrum activities against test organisms. The following isolates were exhibited an inhibitory effect against the test organisms including: *Staph. aureus* (100%), *Strep. pyogenes* (89%), *Camp. coli* (79%), *B. subtilis* (57%), *B. cereus* (73%), *Pr. mirabilis* (73%), *Ent. faecalis* (42%), *Sh. boydii* (16%), *Kl. pneumoniae* (47%), *Ps. aeruginosa* (10%) and *Salm. typhimurium* (5%).

Morphological Characteristics of Selected Isolates

Results of morphological characteristics of the isolates revealed that the growth of the isolates was few to good on the ISP-medium 2. The following isolates NWU60, NWU80, NWU98, NWU146, NWU208, NWU255, NWU284, NWU299 and NWU336 produced aerial mycelium while only NWU73 did not produce sub-

Table 1: Antibacterial activity of selected isolates against pathogenic organisms

	Isolate Code: NWU																		
Test organism	*60*	*73*	*80*	*88*	*98*	*101*	*121*	*126*	*146*	*166*	*183*	*208*	*239*	*252*	*255*	*284*	*296*	*299*	*336*
Staph. aureus ATCC 29213	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Strep. pyogenes ATCC 12344	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+
Camp. coli ATCC 43478	+	-	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+
B. subtilis ATCC 11774	+	+	+	-	+	-	-	-	+	+	-	+	+	+	-	-	+	+	-
B. cereus ATCC 11778	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	-	+	+	+
Pr. mirabilis ATCC 49132	+	+	+	-	-	-	+	+	+	+	-	+	+	-	+	+	+	+	+
Ent. faecalis ATCC 14506	-	-	+	-	+	-	+	-	+	-	+	-	-	+	+	+	-	-	-
Sh. boydii ATCC 9207	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-
Kl. pneumoniae ATCC 8308	+	-	+	-	+	+	+	+	-	-	-	-	-	+	-	+	-	+	-
Ps. aeruginosa ATCC 10145	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
Salm. typhimurium ATCC 14208	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+= Inhibition of growth; -= No inhibition of growth

strate mycelium. Isolates NWU73 and NWU166 were observed with yellowish pigment and NWU282 with brown pigment (Table 2). The reverse colony color observed ranges from cream (NWU183), yellowish cream (NWU60, NWU73, NWU80, NWU98, NWU166, NWU239, NWU284 and NWU299), peach (NWU88), orange (NWU146), light brown (NWU126, NWU255 and NWU336) to brown (NWU101, NWU121, NWU208, NWU252 and NWU296).

Biochemical and Physiological Characteristic of Selected Isolates

Biochemical characteristics results indicate that all isolates are Gram positive, catalase positive and have the ability to hydrolyze starch. Isolates NWU98, NWU121, NWU126, NWU166, NWU208, NWU239, NWU252, NWU255 and NWU296 were able to produce the enzyme catalase. None of the isolates were able to liquefy gelatin. Isolates NWU166, NWU183, NWU299 and NWU336 were able to utilize citrate; NWU60 and NWU239 were able to utilize urea; and NWU60, NWU88, NWU239, NWU284 and NWU336 were able to hydrolyze casein. The positive utilization of esculin degradation was recorded in NWU73, NWU80, NWU98, NWU101, NWU166, NWU183, NWU208, NWU255, NWU284 and NWU336. Isolates NWU60, NWU88, NWU101, NWU126, NWU252, NWU255, NWU284, NWU296, NWU299 and NWU336 were able to reduce nitrate. Isolates NWU73 and NWU239 were able to produce hydrogen sulphide (Table 3). The isolates were able to utilize a wide range of carbon sources (Table 4). All the isolates were able to utilize glucose; sucrose, mannitol, fructose, rhamnose, mannose inositol and maltose while sorbitol, lactose, galactose, cellulose, raffinose and xylose were utilized by few of the isolates. It is also evident that different physiological characteristics influenced the growth rate of the isolates. The optimum temperature for the growth of the isolates ranged between 25-30°C. The optimum pH growth ranged between 7.0 and 8.0 which are neutral to slightly alkaline. The isolates were able to tolerate sodium chloride up to 5-7 percent concentration. Isolates NWU121, NWU146, and NWU166 were not able to grow under anaerobic conditions. Isolates NWU126 and NWU284 were able to grow on MacConkey agar (Table 5).

Molecular Identification of Selected Isolates

A 1.5 kb fragment was amplified from the genomic DNA with the bacterial universal primers (F1R5) (Fig. 1). The identification of rare actinomycetes was performed by analysis of partial sequences of their 16S rDNA gene. The partial nucleotide sequences of the 16S rDNA gene of the isolates were compared with the nucleotide database of NCBI web server through the

Table 2: Morphological characteristics of selected isolates on isolation medium

Isolate code	*Growth*	*Aerial mycelium*	*Substrate mycelium*	*Pigmentation*	*Reverse colony color*
NWU60	Good	Dull white	Grey	None	Yellowish cream
NWU73	Moderate	None	None	Yellow	Yellowish cream
NWU80	Good	White	Yellow	None	Yellowish cream
NWU88	Good	None	Pink	None	Peach
NWU98	Good	White	White	None	Yellowish cream
NWU101	Good	None	White	None	Brown
NWU121	Moderate	None	Army green	None	Brown
NWU126	Moderate	None	Light yellow	None	Light brown
NWU146	Good	White	Pink	None	Orange
NWU166	Few	None	White	Yellow	Yellowish cream
NWU183	Good	None	White	None	Cream
NWU208	Good	White	White	None	Brown
NWU239	Moderate	None	White	None	Yellowish cream
NWU252	Good	None	White	Brown	Brown
NWU255	Moderate	White	White	None	Light brown
NWU284	Good	White	White	None	Yellowish cream
NWU296	Good	None	White	None	Brown
NWU299	Good	White	Creamish white	None	Yellowish cream
NWU336	Good	White	Dull white	None	Light brown

Table 3: Biochemical characteristics of selected isolates

	Isolate Code (NWU)																		
Test	*60*	*73*	*80*	*88*	*98*	*101*	*121*	*126*	*146*	*166*	*183*	*208*	*239*	*252*	*255*	*284*	*296*	*299*	*336*
Gram Staining	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	+	-	+	+	-	+	-	+	+	+	+	-	+	-	-
Citrate utilization	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+
Gelatin liquefication	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin degradation	-	+	+	-	+	+	-	-	-	+	+	+	-	-	+	+	-	-	+
Casein hydrolysis	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+
Nitrate reduction	+	-	-	+	-	+	-	+	-	-	-	-	-	+	+	+	+	+	+
H_2S production	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Urea utilization	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Methyl red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Voges Proskauer	-	+	+	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	+
Tween 20	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+	-	-	-	+
Tween 60	+	+	+	+	+	-	+	-	-	-	+	+	+	-	+	-	-	-	+
Tween 80	+	+	+	+	+	-	+	-	-	-	+	+	+	-	+	-	-	-	+

+ = Positive; - = Negative

Table 4: Carbon source utilization of selected isolates

	Isolate Code: NWU																		
Carbon source	*60*	*73*	*80*	*88*	*98*	*101*	*121*	*126*	*146*	*166*	*183*	*208*	*239*	*252*	*255*	*284*	*296*	*299*	*336*
Glucose	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Sucrose	++	++	++	++	±	-	++	-	-	++	±	++	++	+	++	++	++	++	++
Sorbitol	-	±	-	±	-	-	±	-	+	+	+	-	-	-	-	+	++	+	-
Mannitol	++	++	++	+	++	±	+	-	-	+	±	-	++	-	±	++	+	++	-
Fructose	+	++	++	++	-	++	++	+	++	++	++	++	+	+	++	++	+	++	+
Lactose	-	+	+	++	++	-	-	±	-	-	±	-	+	+	+	++	+	-	+
Galactose	-	++	+	++	+	-	-	-	+	++	±	-	++	-	±	++	-	-	-
Rhamnose	+	++	±	++	+	-	+	+	+	++	±	-	+	+	-	+	-	-	++
Cellulose	±	-	±	-	-	-	±	-	-	±	-	-	-	-	-	±	-	±	±
Mannose	-	++	+	+	+	+	++	+	+	++	+	+	++	±	++	++	++	+	+
Inositol	-	-	++	-	+	+	++	+	+	+	+	±	±	-	=	++	++	-	±
Raffinose	++	+	++	++	+	-	+	-	-	+	-	-	+	+	-	+	+	-	+
Maltose	++	++	++	++	+	-	++	-	+	++	+	+	++	+	++	++	+	+	-
Xylose	+	+	+	++	-	-	++	-	-	-	+	-	+	-	-	+	+	-	-

Results recorded by the method of the International Streptomyces Project (ISP)**:** ++, strongly positive utilization, i.e., growth on the tested carbohydrate in basal medium is equal to or greater than the growth on basal medium plus glucose; +, positive utilization, i.e., growth on the tested carbohydrate is significantly better than that on the basal medium without carbon source but somewhat less than that on glucose; ±, utilization doubtful, i.e., growth on the tested carbon source is only slightly better than that on the basal medium without carbon source and significantly less than that with glucose; -, utilization negative, i.e., growth is similar to or less than the growth on basal medium without carbon source

BLAST tool. The BLAST search inferred that the isolates were members of the GC-rich actinomycetes. The 16S rDNA gene nucleotide sequence of different actinomycetes was obtained by BLASTn search; however 58 strains of actinomycetes were selected based on high identity percentage (%) with good E value. Table 6 results show that query sequences were best pairwise aligned with 16S rDNA gene sequence of actinomycetes with sequence similarity, and identity ranged between 79-100 percent, with E value of 0.

Phylogenetic Analysis of Selected Isolates

Nineteen (19) isolates were subjected to sequencing and phylogenetic analysis. The 16S rDNA nucloetide sequences of the 19 isolates

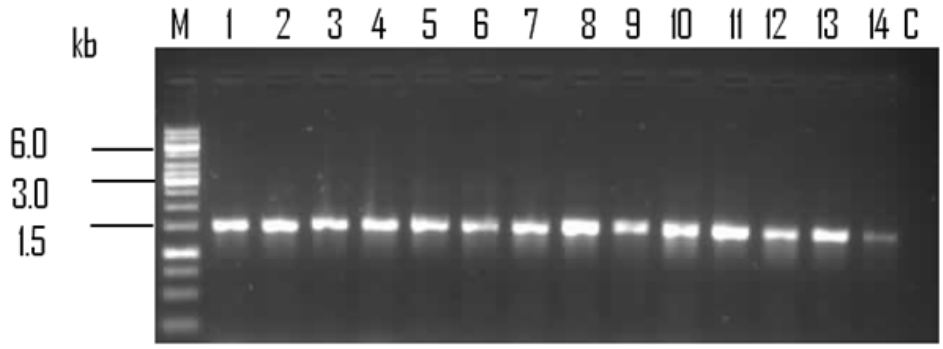


Fig. 1. Agarose gel photograph indicating the positive band of approximately 1.5 kb for 16S rDNA gene amplification from actinomycete isolates. M= 1 kb DNA Marker, 1-14= PCR amplification of isolates, C= Nuclease free water
Source: Author

were aligned with 58 actinomycetes sequences obtained from GenBank; and *Bacillus* spp. as the out-group. The phylogenetic position of the isolates was evaluated by constructing a phylogenetic tree using neighbor-joining methods (Fig. 2). This method placed the bacterial isolates in different clades encompassing members of the order Actinomycetales with bootstrap support. Bootstrap values based on 1000 replications were listed as percentages at the branching points. The tree shows completely resolved, well-supported phylogeny of the 19 bacterial isolates with high resolution of all inner branches.

DISCUSSION

The discovery of novel bioactive compounds from actinomycetes has marked an era in antibiotic research and succeeding developments in antibiotic chemotherapy. In the course of searching for possible novel antimicrobial agents against the spread of antibiotic resistant pathogens, antibiotic producing actinomycetes were isolated from rhizospheric soil samples collected from Ngaka Modiri Molema district in the North West Province, South Africa. Actinomycetes have been explored for many bioactive compounds of high clinical importance and are still routinely screened for novel bioactive compounds (Gebreyohannes et al. 2013). Considerable attention is currently given to the isolation and characterization of rare actinomycetes, due to evidence that screening such organisms raises the prospect of discovering novel bioactive compounds that can be developed as a resource for biotechnology (Hong et al. 2009). Although several research works have reported that soil is

Table 5: Physiological characteristics of selected isolates

Growth condition	*Isolate Code: NWU*																		
	60	*73*	*80*	*88*	*98*	*101*	*121*	*126*	*146*	*166*	*183*	*208*	*239*	*252*	*255*	*284*	*296*	*299*	*336*
Optimum temperature for growth (°C)	25	25	30	30	25	25	30	30	30	25	25	30	25	25	25	30	30	25	25
Optimum pH for growth	7	7	8	8	7	7	7	7	7	8	7	8	7	7	7	8	7	7	7
NaCl tolerance (%)	7	7	5	7	5	5	7	7	7	7	5	5	5	5	7	6	5	7	5
Growth under anaerobic conditions	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+
Growth on MacConkey agar	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-

+ = Positive; - = Negative

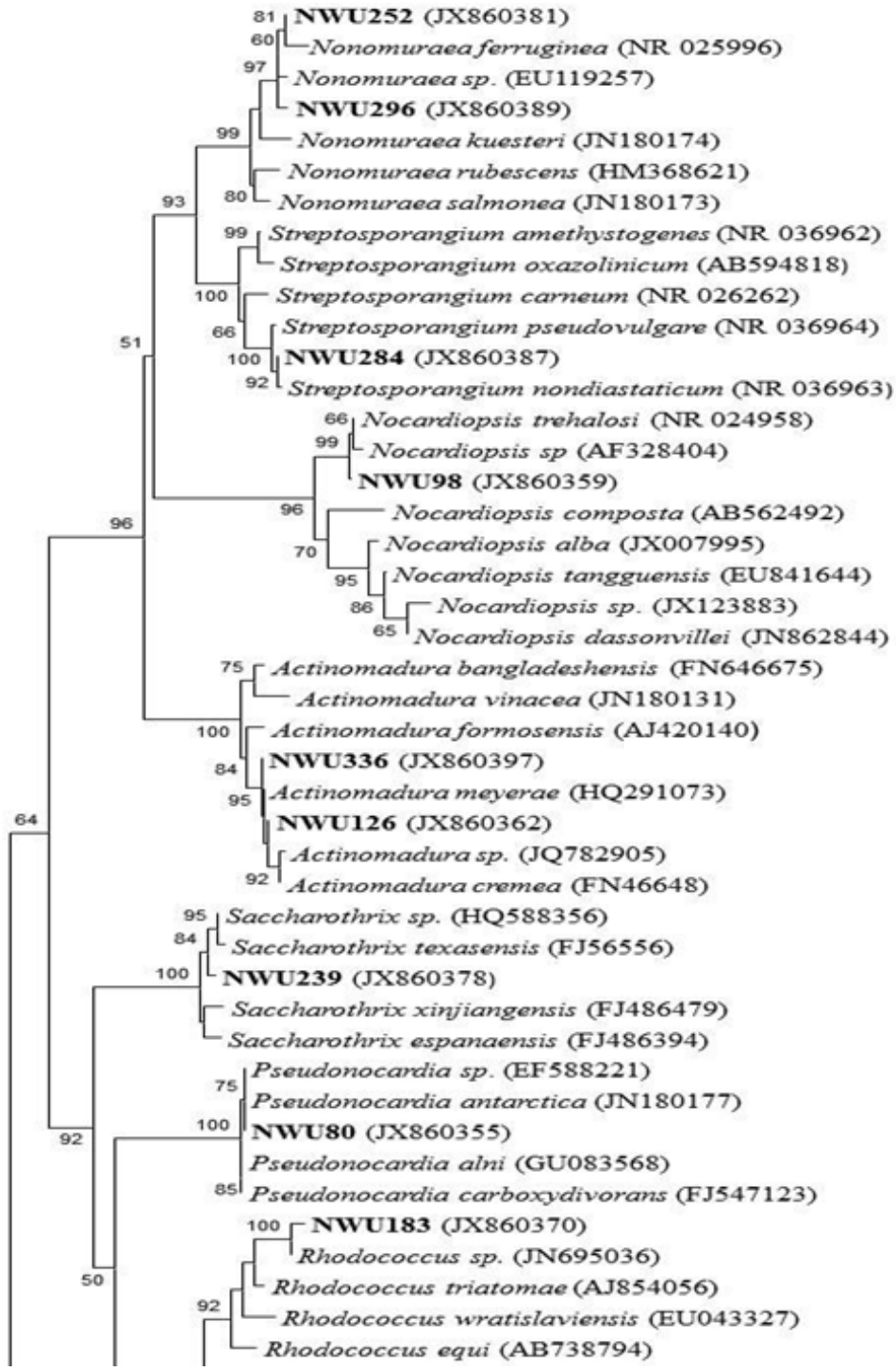
Rathod 2011). In one of the studies, research was carried out by Hayakawa and co-workers to isolate *Streptosporangium* spp from soil samples. They reported that the soil samples were subjected to both physical and chemical pretreatments, and cultured on selective medium supplemented with specific antibiotics. These treatments drastically eliminate unicellular bacteria and unwanted actinomycetes, including *Streptomyces* spp. from the isolation plates, thereby facilitating the selective isolation of *Streptosporangium* spp. For isolations from various soils, this method achieved 20 percent selective isolation of *Streptosporangium* spp. . The specific isolation of *Actinomadura* spp was achieved by air-drying, heating soil and using selective media supplemented with antibiotics such as streptomycin and rifamycin. More than 250 known antibiotics have been produced by *Actinomadura* strains . Other methods such as the enrichment method have also been reported to increase the discovery of rare actinomycetes such as *Actinoplanes*, *Amycolatopsis*, *Planobispora, Dactylosporangium, Catenuloplanes* and *Virgosporangium*. This study indicates that rare actinomycetes can be successfully recovered from soil samples, once pretreatment and selective isolation methods are applied.

The combination of pretreatment methods and selective media supplemented with specific antibiotics, have been found to stimulate the isolation of diverse rare actinomycete genera that were only recovered incidentally by conventional methods. This approach helps to answer the question: are these less exploited actinomycetes less abundant in the environment or are they just more difficult to isolate and cultivate? The appropriate pretreatment methods and various selective isolation media that met several nutritional requirements with specific antibiotics increased the range of strains isolated. The pretreatment methods will contribute to isolating novel rare actinomycetes which will help to improve knowledge concerning the occurrence, distribution, ecology, taxonomy and evolution of rare actinomycetes. The isolation of rare actinomycetes is valuable for the discovery of novel bioactive secondary metabolites. The development of selective isolation methods, have led to the discovery of novel bioactive secondary metabolites of industrial importance.

The rhizosphere is a unique biological niche that supports an abundant diversity of microorganisms, and the combination of strains found in the rhizosphere is governed by physical and chemical characteristics of the soil and by plant species (Zhao et al. 2012). The presence of relatively large populations of rare actinomycetes in the rhizospheric soil samples indicates that it is a suitable ecosystem that promotes the growth of the microorganisms. Rare actinomycetes from the rhizosphere produce secondary metabolites with novel molecules and pharmaceutical properties suggesting that the rhizosphere can be an interesting source for bioprospecting (Qin et al. 2009). The presence of actinomycetes in the rhizosphere is consistent with other reports which showed that actinomycetes are prominent in the plant root system (Yilmaz et al. 2008). Hong and co-workers reported a higher number of bioactive compounds producing actinomycetes from the rhizosphere than from corresponding soil, this might be due to the secretion of exudates from the plant root that can serve as food for the organisms (Babalola 2010).

As the aim of this study was to isolate rare actinomycete genera, the focus was on the isolates that according to the morphological criteria did not seem to belong to the genus *Streptomyces*. Eighty-eight (88) of such isolates were analysed by macro- and micromorphology, biochemical, and physiological criteria in order to preliminarily identify the genera. These characteristics can serve as markers by which individual strains can be distinguished. The morphological, biochemical and physiological characteristics varied from one isolate to another depending on the required growth conditions. The isolates utilized a wide range of carbon sources due to their ability to produce extracellular enzymes that metabolised the polymeric components of the nutrient mixture to monomeric forms for their growth. The utilization of various carbon sources serve as additional criteria for classification of actinomycetes.

The 16S rDNA gene sequence analysis by molecular methods resulted, in identification of the genera. A good agreement between preliminary genera identification of the isolates based on their morphology and characteristics and subsequent identification based on 16S rDNA gene sequence analysis was observed. The 16S rDNA sequencing analysis is routinely used to identify actinomycete isolates after comparing with reference organisms in nucleotide sequence


NWU252 (JX860381)
Nonomuraea ferruginea (NR 025996)
Nonomuraea sp. (EU119257)
NWU296 (JX860389)
Nonomuraea kuesteri (JN180174)
Nonomuraea rubescens (HM368621)
Nonomuraea salmonea (JN180173)
Streptosporangium amethystogenes (NR 036962)
Streptosporangium oxazolinicum (AB594818)
Streptosporangium carneum (NR 026262)
Streptosporangium pseudovulgare (NR 036964)
NWU284 (JX860387)
Streptosporangium nondiastaticum (NR 036963)
Nocardiopsis trehalosi (NR 024958)
Nocardiopsis sp (AF328404)
NWU98 (JX860359)
Nocardiopsis composta (AB562492)
Nocardiopsis alba (JX007995)
Nocardiopsis tangguensis (EU841644)
Nocardiopsis sp. (JX123883)
Nocardiopsis dassonvillei (JN862844)
Actinomadura bangladeshensis (FN646675)
Actinomadura vinacea (JN180131)
Actinomadura formosensis (AJ420140)
NWU336 (JX860397)
Actinomadura meyerae (HQ291073)
NWU126 (JX860362)
Actinomadura sp. (JQ782905)
Actinomadura cremea (FN46648)
Saccharothrix sp. (HQ588356)
Saccharothrix texasensis (FJ56556)
NWU239 (JX860378)
Saccharothrix xinjiangensis (FJ486479)
Saccharothrix espanaensis (FJ486394)
Pseudonocardia sp. (EF588221)
Pseudonocardia antarctica (JN180177)
NWU80 (JX860355)
Pseudonocardia alni (GU083568)
Pseudonocardia carboxydivorans (FJ547123)
NWU183 (JX860370)
Rhodococcus sp. (JN695036)
Rhodococcus triatomae (AJ854056)
Rhodococcus wratislaviensis (EU043327)
Rhodococcus equi (AB738794)

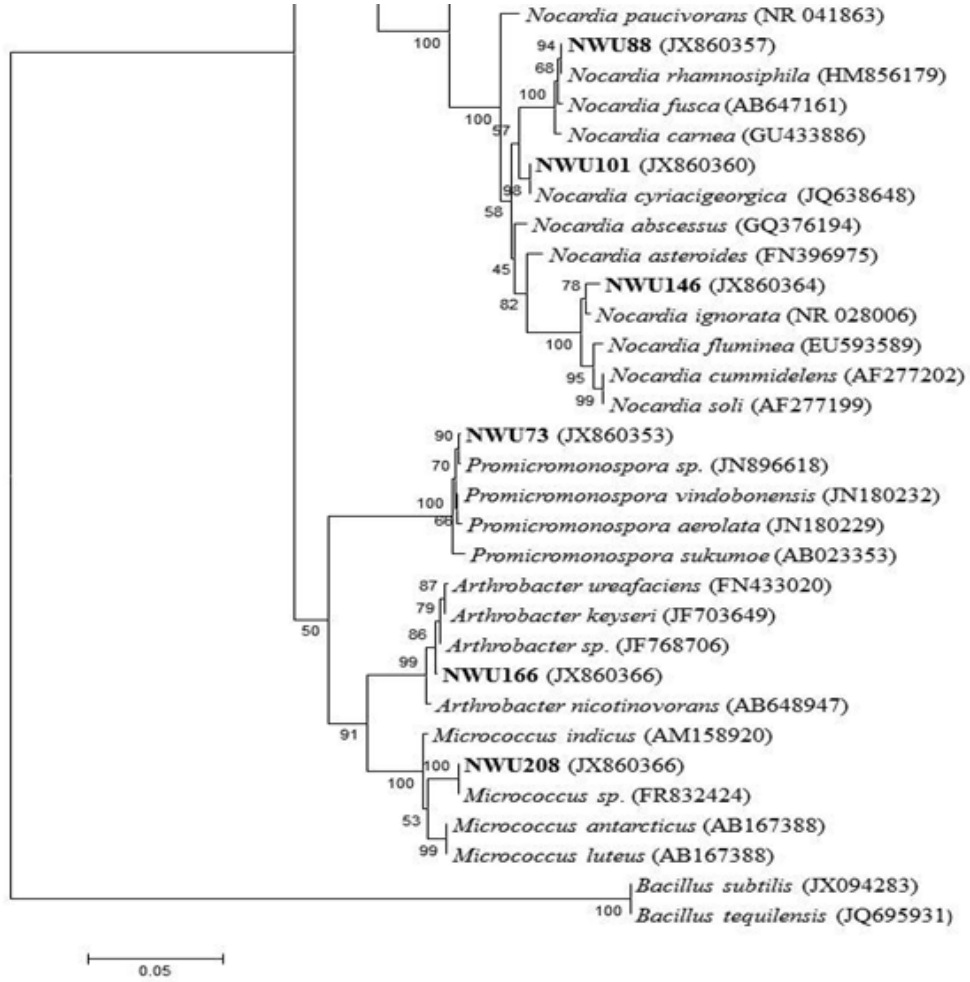


Fig. 2. Neighbour-joining tree of the selected isolates and representative species of actinomycetes based on partial 16S rDNA gene sequences. Numbers at the nodes indicate the levels of bootstrap support based on 1000 resampled data sets. Only values greater than 50% are shown. The scale bar indicates 0.05 substitutions per nucleotide position.
Source: Author

an excellent source of actinomycetes with diverse potential, it has not been fully explored, and there is tremendous potential to isolate rare actinomycetes with biological activities. Rare actinomycetes are difficult to isolate by conventional culturing methods due to their very slow growth on culture plates, and hence their inability to compete with fastidious microorganisms. Because actinomycetes are at a competitive disadvantage when grown on agar media in association with other soil inhabiting microbes, isolation media must be designed to reduce the development of competing microbes without adversely affecting actinomycetes.

In this study, 88 rare actinomycetes were isolated by pretreatment of soil samples, which stimulates the isolation of actinomycetes by eliminating most unwanted organisms. It was previously reported that pretreatment of the soil samples decreased the growth of fast growing bacteria and fungi, allowing the rare actinomycetes to dominate the culture plate (Naikpatil and da-

tabase (Franco-Correa et al. 2010). The phylogenetic relationship between isolates was determined by 16S rDNA nucleotide sequence analysis of representative strains of each identified genus. As shown on the phylogenetic tree, depicting also bootstrap values, the 19 selected isolates were sorted into 11 clusters with highest similarity to the genera *Nocardia* spp., *Nocardiopsis, Streptosporangium, Rhodococcus, Actinomadura, Promicromonospora, Arthrobacter, Micrococcus, Saccharothrix, Pseudonocardia* and *Nonomuraea*. A good agreement between the BLAST search and clustering in the phylogenetic tree of the selected isolates was observed. These isolates clustered with known strains that are producers of bioactive compounds. Some of the closest neighbors of the isolates were found to produce bioactive compounds. This data indicates a considerable biodiversity of actinomycetes in the soil samples. Even though these isolates may be strains of known species, they are still considered to be important sources of novel bioactive compounds, as it has been reported that strains of the same species might produce different secondary metabolites depending on their isolation sources (Jensen et al. 2007).

Actinomycetes are known as prolific producers of secondary metabolites that are of importance in medicine and agriculture. The rare actinomycetes isolates obtained in this study were screened for their antibiotic production potential. Out of the 88 rare actinomycetes tested for activity against pathogens, 19 (21.6%) exhibited broad spectrum antibacterial activity suggesting their ability to produce bioactive secondary metabolites. The fact that the isolates exhibited broad spectrum antimicrobial activity, this signifies possible production of several antimicrobial compounds and/or production of compounds with multiple microbial targets. Isolates NWU60, NWU80, NWU121, NWU146, NWU252 and NWU284 exhibited broad spectrum antibacterial activities against the test organisms. These isolates showed potential as sources of antimicrobial agents, and there is a need to explore them. These isolates have been identified as *Nocardiopsis sp* (NWU60), *Pseudonocardia sp* (NWU80), *Actinomadura sp* (NWU121), *Nocardia sp* (NWU146), *Nonomuraea sp* (NWU252) and *Streptosporangium sp* (NWU284), all of which have been reported as novel producers of bioactive compounds (Liu et al. 2011).

Several researchers reported that rare actinomycetes have biocontrol activity against pathogenic organisms (Bredholdt et al. 2007; Gebreyohannes et al. 2013). Many of the rare actinomycetes produced antibiotic complex ranging from two to ten structurally related components (Gastaldo and Marinelli 2003). These bioactive compounds exhibit broad spectrum of diverse chemical classes making the chemotherapy potential expressed by members of this group very attractive to industrial screening programmes (Vaishnav and Demain 2011). It is possible that the activities observed are due to already known antibiotics such as aminoglycosides, polyenes, macrolides, tetracyclines and glycopeptides, which are commonly produced by many actinomycetes.

CONCLUSION

This present study provides further evidence of significant biodiversity of actinomycetes in rhizospheric soils that present strains that can be a valuable source of bioactive compounds with antibiotic activity. The rare actinomycetes are targeted as they have proven to be a valuable source of bioactive secondary metabolites, notably antibiotics, even if a relatively low number of strains have been exploited in comparison with *Streptomyces.* Fermentation and chemical analysis of the extracts is concurrently being carried out in order to identify active compounds and to assess their novelty.

ACKNOWLEDGEMENTS

This research work was supported by International Foundation for Science (grant no: F/5330-1), North-West University and OOB would like to thank the National Research Foundation for grant (UID:81192) that has supported research in her laboratory.

REFERENCES

Abioye E, Akinpelu D, Aiyegoro O, Adegboye M, Oni M, Okoh A 2013. Preliminary phytochemical screening and antibacterial properties of crude stem bark extracts and fractions of *Parkia biglobosa* (Jacq.). *Molecules,* 18**:** 8485-8499.

Adegboye MF, Babalola OO 2012. Taxonomy and ecology of antibiotic producing actinomycetes. *Afr J Agricult Res,* 7**:** 2255-2261.

Adegboye MF, Babalola OO 2013a. Actinomycetes: a yet inexhaustive source of bioactive secondary me-

tabolites. In: A Méndez Vilas (Ed.): *Microbial Pathogens and Strategies for Combating Them: Science, Technology And Education*. Badajoz, Spain: Formatex Research Center, pp. 786-795.

Adegboye MF, Babalola OO 2013b. Isolation, characterization and antibacterial activity of Streptomycetes from rhizosphere soils in North West Province, South Africa. *Asia Life Sci*, 9: 403-421.

Adegboye MF, Babalola OO 2013c. Phylogenetic characterization of culturable antibiotic producing *Streptomyces* from rhizospheric soils. *Mol Biol*, 1: 2.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ 1990. Basic local alignment search tool. *J Mol Biol*, 215: 403-410.

Babalola OO 2010. Beneficial bacteria of agricultural importance. *Biotechnol Lett*, 32: 1559-1570.

Bérdy J 2012. Thoughts and facts about antibiotics: where we are now and where we are heading. *J Antibiot*, 65**:** 385-395.

Bredholdt H, Galatenko OA, Engelhardt K, Fjaervik E, Terekhove LP, Johsen G, Ztchev SB 2007. Rare actinomycete bacteria from the shallow water sediments in Trondheim Fjord Norway: Isolation, diversity and biological activity. *Environ Microbiol*, 9: 2756-2764.

Busti E, Monciardini P, Cavaletti L, Bamonte R, Lazzarini A, Sosio M, Donadio S 2006. Antibiotic-producing ability by representatives of a newly discovered lineage of actinomycetes. *Microbiol*, 152: 675-683.

Demain AL, Sanchez S 2009. Microbial drug discovery: 80 years of progress. *J Antibiot*, 62: 5-16.

Duraipandiyan V, Sasi AH, Islam VIH, Valanarasu M, Ignacimuthu S 2010. Antimicrobial properties of actinomycetes from the soil of Himalaya. *J Med Mycol*, 20: 15-20.

Felsenstein J 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evol*, 39: 783–791.

Franco-Correa M, Quintana A, Duque C, Suarez C, Rodríguez MX, Barea J-M 2010. Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. *Appl Soil Ecol*, 45: 209-217.

Gastaldo L, Marinelli F 2003. Changes in GE2270 antibiotic production in *Planobispora rosea* through modulation of methylation metabolism. *Microbiol*, 149: 1523-1532.

Gebreyohannes G, Moges F, Sahile S, Raja N 2013. Isolation and characterization of potential antibiotic producing actinomycete from water and sediments of Lake Tana, Ethiopia. *Asian Pac J Trop Biomed*, 3: 426-435.

Hall TA 1999. BioEdit: A user-friendly biological sequence aligment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser*, 41: 95-98.

Hayakawa M 2008. Studies on the isolation and distribution of rare actinomycetes in soil. *Actinomycetologica* 22: 12-19.

Hong K, Gao A-H, Xie Q-Y, Gao HG, Zhuang L, Lin H-P, Yu H-P, Li J, Yao X-S, Goodfellow M 2009. Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. *Mar drugs*, 7: 24-44.

Janardhan A, Kumar AP, Viswanath B, Saigopal D, Narasimha G 2014. Production of bioactive compounds by actinomycetes and their antioxidant properties. *Biotechnol Res Int*, 2014.

Jensen PR, Williams PG, Oh D-C, Zeigler L, Fenical W 2007. Species-specific secondary metabolite production in marine actinomycetes of the genus *Salinispora*. *Appl Environ Microbiol*, 73: 1146-1152.

Jukes TH, Cantor CR 1969. Evolution of protein molecules. In: HN Munro (Ed.): *Mammalian Protein Metabolism*. New York: Academic Press, pp. 21-132.

Katoh S 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*, 30: 772-780.

Liu C, Yang X-Q, Ding Z-T, Zhao L-X, Cao Y-R, Xu L-H, Yang Y-B 2011. Cyclodipeptides from the secondary metabolites of two novel actinomycetes. *Chin J Nat Med*, 9: 78-80.

Marinelli F, Marcone GL. 2011. *Microbial Secondary Metabolites Comprehensive Biotechnology.* 2nd Edition. Burlington, American: Academic Press.

Naikpatil SV, Rathod J 2011. Selective isolation and antimicrobial activity of rare actinomycetes from mangrove sediment of Karwar. *J Ecobiotechnol*, 3: 48-53.

Newman DJ, Cragg GM 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*, 75: 311-335.

Page RDM 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput Appl Biosci*, 12: 357-358.

Qin S, Li J, Chen H-H, Zhao G-Z, Zhu W-Y, Jiang C-L, Xu L-H, Li W-J 2009. Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. *Appl Environ Microbiol*, 75: 6176-6186.

Saitou N, Nei M 1987. The Neighbor-Joining Method—a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, 4: 406–425.

Sharma M 2014. Actinomycetes: source, identification, and their applications. *Int J Curr Microbiol Appl Sci*, 3**:** 801-832.

Shirling EB, Gottlieb D 1966. Methods for characterization of *Streptomyces species. Int J Syst Evol Microbiol*, 16: 313-340.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*, 28: 2731-2739.

Vaishnav P, Demain AL 2011. Unexpected applications of secondary metabolites. *Biotechnol Adv*, 29: 223-229.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ 1991. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*, 173: 697-703.

Yilmaz EI, Yavuz M, Kizil M 2008. Molecular characterization of rhizospheric soil streptomycetes isolated from indigenous Turkish plants and their antimicrobial activity. *World J Microbiol Biotechnol*, 24: 1461-1470.

Zhao K, Penttinen P, Chen Q, Guan T, Lindström K, Ao X, Zhang L, Zhang X 2012. The rhizospheres of traditional medicinal plants in Panxi, China, host a diverse selection of actinobacteria with antimicrobial properties. *Appl Microbiol Biotechnol*, 94: 1321-1335.