RESEARCH NOTE

A new record of Actinobacteria isolated from soil in Jerusalem and their enzymatic potential [version 1; peer review: 2 not approved]

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Abstract

Actinobacteria are well recognized for their bioactive compounds. They are considered as a promising source of wide range of important enzymes, some of which are produced on an industrial scale. In this study, 35 isolates of actinomycetes were isolated from soil samples collected in the area of Al-Aqsa Mosque in Jerusalem, Israel. To our knowledge, this is the first study of actinomycetes from this terrestrial environment. The efficiency of the isolated actinobacteria in the production of amylase, cellulase, protease, tyrosinase, lipase, catalase and phosphatase was studied. Isolates obtained showed some activity and other completely failed to produce such enzymes. From total 35 isolates, only three isolates (8.6%) showed ability to produce protease, four isolates (11.4%) for lipase, five isolates (14.3%) for tyrosinase and two isolates (5.7%) for phosphatase enzymes. However, all isolates were positive for amylase and catalase enzymes; vice versa for cellulase enzyme all isolates failed to degrade cellulose in the form of carboxymethylcellulose.

Keywords

Actinobacteria, Biotechnology, Natural products, Enzyme production, Jerusalem.

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Introduction

Actinobacteria represent one of the most diverse groups of filamentous bacteria capable of surviving in a number of ecological niches due to their bioactive potential. They are representative of terrestrial microorganisms and usually are isolated from soils. Actinobacteria have gained special importance as the most potent source of antibiotics (Kandasamy *et al.*, 2012) and other bioactive secondary metabolites (Solecka *et al.*, 2012). Their metabolic potential offers a strong area of research. Accordingly, the role of actinomycetes in biotechnology and medicine is well known and these industries are always looking for novelty bioactive compounds. While most of the studies on actinobacteria have focused on antibiotic production, only few reports have focused on their enzymatic potential.

Actinobacteria are considered as a promising source of a wide range of enzymes. Some of them are produced on an industrial scale, but many other remained to be harnessed. The bacteria have the ability to degrade a wide range of hydrocarbons, pesticides, and aliphatic and aromatic compounds (Sambasiva Rao *et al.*, 2012). They perform microbial transformations of organic compounds, a field of great commercial value. Members of many genera of actinobacteria have potential for use in the bioconversion of underutilized agricultural and urban wastes into high-value chemical products (Crawford, 1988). Some actinobacteria secrete enzymes responsible for the degradation of lignocelluloses in lignin, cellulose and hemicellulase, others may secrete enzymes that can only partially achieve this breakdown (Mason *et al.*, 2001). Here the purpose of this preliminary study was to isolate and screen new actinobacterial isolates for their ability to degrade organic compounds via the secretion of enzymes like amylase, cellulase, protease, tyrosinase, lipase, catalase, and phosphatase. Due to the high disturbance level of biodeterioration occurred in the area of Al-Aqsa mosque in Jerusalem, Israel, the soil specimens from such place may contain novel actinobacterial colonies. Meanwhile, to the author's knowledge, no previous studies concerned organisms involved in biodeterioration in this location.

Materials and methods

Sample collection and isolation technique

Soil samples were collected 5 cm below the soil surface from two different sites at Northern part of Al-Aqsa mosque in Jerusalem. The soils collected from the area around Al-Aqsa are characterized by high pH ranged from 8.15 to 8.32 with organic carbon 9.61% – 12.28%. Soil textures were clay and clay loam. No attempts have been made before to isolate actinomycetes from these areas. The soil samples collected were subjected to sieving to remove plant debris and were then pre-treated by drying in open air for 2 days. Samples of 5 g were mixed with 50 ml of sterile saline solution (0.85% NaCl) and incubated at room temperature (27±2°C) for 1 hour on orbital shaker with vigorous shaking. Soil suspension was subjected to serial dilutions and then pipetted and spread onto starch casein medium (SCM, with the following ingredients m-1Le: casein powder 1.0, starch 10.0, sodium nitrate, 3.0; agar 15.0; final pH 7.2±0.2) supplemented with antifungal cycloheximide (50 mg⁻¹L) as described by Mansour (2003). After 7 days of incubation at 30°C, actinomycete colonies were picked up and

purified onto SCM medium using streak plate techniques. The pure colonies of actinomycetes were subcultured onto starch casein slants and incubated for 3–7 days at 30°C.

Identification of actinobacterial isolates

Purified actinobacterial isolates were identified to genus level using different tools, morphological, cultural and physiological characteristics following the standard techniques as presented in Bergey's Manual of Systematic Bacteriology (Anon, 1989). For morphological characterization, sterile slide oblique technique was applied (Mansour, 2003). Actinomycete colonies were streaked onto SCM, where the slide was inserted in the agar plate with an angle of 45° and incubated at 30°C for 3 and 7 days. After each incubation period, the growth of actinomycetes was examined taking the slides out from the agar and staining the actinobacteria growth using Gram stain. The slides were then examined using a light microscope (Leica, Model DMLB). Spore orientation and their morphological types were examined. Culture characterization was carried out using different culture media: SCM (Kuster, 1959), glycerol asparagine agar (reagents g⁻¹L: L-asparagine, 1.0; dipotassium phosphate, 1.0; trace salt solution (ml) 1.0; agar 20.0; 1ml of trace salt solution contains, ferrous sulfate heptahydrate, 0.001; manganese chloride tetrahydrate, 0.001; zinc sulfate, heptahydrate 0.001; final pH 7.4±0.2 (Pridham & Lyons, 1961), glucose asparagine agar (reagents g⁻¹L: glucose, 10.0; asparagine, 0.5; di-potassium hydrogen phosphate, 0.5; agar, 15.0; pH 7.4 (Waksman, 1961), yeast extract-malt extract agar (reagents g⁻¹L: yeast extract, 3.0; malt extract, 3.0; dextrose, 4.0; agar, 20.0; pH 7.2 (Pridham *et al.*, 1956), inorganic salt-starch agar (reagents g⁻¹L: soluble starch, 10.0; di-potassium hydrogen phosphate, 1.0; magnesium sulfate, 1.0; sodium chloride, 1.0; ammonium sulfate, 2.0; calcium carbonate, 2.0; trace salts solution, 1 ml; agar, 15.0 (Kuster, 1959) and oat meal agar (reagents g⁻¹L: oat meal, 2.0 and agar, 15.0 (Kuster, 1959). All chemicals used are from Sigma Company. The color of aerial and substrate mycelia grown on the different media used were recorded in addition to pigment production. Carbon utilization, nitrate reduction, melanin production, gelatin liquefaction and H₂S production (Kuster & Williams, 1964) tests were used for physiological characterization.

Screening of strains for extracellular hydrolytic activities

In order to detect the production of extracellular hydrolases, different enzymatic agar plate assays were performed. The different assays are described below.

Determination of extracellular amylase activity

The starch agar medium was used to detect the amylase activity (Haritha *et al.*, 2010). The assay medium inoculated with each isolates was incubated at 30°C for 72 hours. After incubation, the amylolytic activity was detected by flooding the agar plates with Gram's iodine solution (2.0%). The change in color of clear zones around the growing colonies to dark blue was considered as positive.

Determination of extracellular cellulase (CMCase) activity

Cellulase production was performed in agar plates supplemented with carboxymethyl cellulose (CMC) (0.5%) as the only carbon substrate, after incubation at 30°C for 72 hours. Three replicates

were used for the each actinomycete isolates. The plates were then flooded with Congo red and NaCl. The yellow zones around colonies in respect to the red background indicated positive cellulose activity (Rathnan & Ambili, 2011).

Determination of extracellular proteolytic activity

The relative activity of protease production was detected for actinomycete isolates on milk agar plate, containing basal salt of starch casein amended with 20% of skimmed milk, following the method of Jani *et al.*, (2012). The actinomycetes were grown in the middle of the milk agar plate and incubated for 5–6 days and at an interval of 24 hours. Zones of casein hydrolysis (clear zones) indicated positive results.

Determination of extracellular tyrosinase activity

Tyrosinase activity was assessed in medium containing L-tyrosine (Sambasiva Rao *et al.*, 2012). Plates containing L-tyrosine were inoculated with each tested isolate separately and then incubated at 37°C for 72 hours. The appearance of black or brown color around the margin of colonies and diffused to the medium indicated tyrosinase activity.

Determination of extracellular lipase activity

To observe lipase production, the actinomycetes were grown on modified medium of Vishnupriya *et al.*, (2010) in which tween-20 (0.5%) was used instead of olive oil. Agar plates were inoculated and incubated at 30°C for 72 hours. The clearance zone around colonies was considered a positive evidence of tween-20 hydrolysis.

Determination of catalase activity

All isolates obtained were screened for catalase activity after 3 to 4 days of subculture on newly fresh SCM following the method of Mahon *et al.*, (2011) using the slide (drop) method. Positive reactions were evident by immediate effervescence (bubble formation).

Determination of extracellular phosphatase activity

Acid and alkaline phosphatase activities were determined according to Ghorbani-Nasrabadi *et al.*, (2013). Inoculated medium supplemented with CaHPO_4 (5 g/l) were incubated at 37°C for 48 to 72 hours. Phosphatase active isolates were recorded based on the halo-zones produced around the colonies.

Evaluation of enzymatic activity. The enzymatic activity (EA) of different tested substrates was examined. The diameter of growth was measured and the clear zone representing enzyme activity was calculated by using the formula:

$$\text{EA} = \frac{\text{Diameter of zone of tested substrate hydrolysis} - \text{Diameter of colony in cm.}}{\text{Diameter of colony in cm.}}$$

Based on the EA test, the organisms can be categorized into three groups: showing excellent activity ($\text{EA} > 2$), good ($\text{EA} < 2$) and poor ($\text{EA} < 1$).

Results and discussion

A total of 35 actinobacteria isolates were obtained from the two different soils collected from Northern part of Al-Aqsa mosque in Jerusalem. From Site 1, only 17 isolates were recovered in which four genera, *Actinomadura*, *Streptomyces*, *Elytrosporangium* and *Actinopolyspora*, were represented (Table 1). Site 2 was represented by more diverse genera of 18 isolates, *Streptosporangium*, *Actinomadura*, *Nocardiosis*, *Nocardia*, *Elytrosporangium* and *Actinopolyspora* (Table 1). Genus *Actinomadura* was represented with the highest frequency in both sites (52.95% and 33.33% for site 1 and site 2 respectively). Despite the fact that isolation methods reveal only a minor fraction of the real existing microbes (Groth *et al.*, 1999) we could demonstrate a great diversity among actinobacteria in the studied sites. To our knowledge (Thaer *et al.*, 2009), this is the first study reporting identification of actinomycetes from this terrestrial environment. Meanwhile, the genera obtained are the first to be recorded in Jerusalem or in the whole country.

The differences observed among the genera of actinobacteria identified in both sites studied, may be due to the different human activities, including construction in such area. These differences may also indicate that the methods proposed and employed for actinobacteria isolation may not be suitable for site 1, and that soil pretreatment should be sought to explore the other genera inhabiting the area. Machavariani and colleagues (2011) found that preliminary treatment of soil by chemical substances like adrenaline and heteroauxin exerts a positive influence on the germination of actinomycete spores and contributes to the natural product activity of the isolated strains. Moreover, they demonstrated that soil pretreatment with different chemical substances play a role for the most complete isolation of actinomycetes that inhabit certain soil.

When screening enzyme activities, 32 of the 35 actinobacteria isolate showed a good amylolytic activity (Figure 1A) and the other three isolates (site 2), *Streptosporangium* sp.2, *Nocardia* sp.2 and *Elytrosporangium* sp.4, had a moderate activity (Figure 1B). However, all isolates from site 1 had a good amylase activity (Table 1). For other enzymes, although actinomycetes are well known as potent degraders of cellulose, lignin, chitin and other complex polysaccharides (El-Fiky *et al.*, 2003; McCarthy & Broda, 1984; Prasad *et al.*, 2012; Wilson, 1992) none of our isolates were able to produce cellulase enzyme. Our results seem to be in contrast with previous studies, and this may be explained by the unsuitable culture conditions for cellulase production such as optimal pH as reported by (Rathnan & Ambili, 2011). In their study, they showed that cellulase enzyme production by *Streptomyces* sp. using fruit waste as substrate was the highest at alkaline pH.

Proteases represent one of the most important groups of enzymes and have been shown to play a role in many industrial and medical fields (Prakash *et al.*, 2013). They can be used in detergent, food pharmaceutical, leather, waste processing industries and silk industries. Proteolytic enzymes have already been used in various forms

Table 1. Enzymatic activity of actinomycete isolates recovered from soil (site 1 and site 2) around Al-Aqsa Mosque.

Site No.	Isolate	Enzyme detected						
		Amylase	Cellulase	Protease	Tyrosinase	Lipase	Catalase	Phosphatase
1	<i>Actinomadura sp. 1</i>	+++	-	-	-	-	++	-
	<i>Streptomyces sp. 1</i>	+++	-	-	-	-	+	-
	<i>Streptomyces sp. 2</i>	+++	-	-	-	-	+	-
	<i>Elytrosporangium sp. 1</i>	+	-	-	-	-	+	-
	<i>Streptomyces sp. 3</i>	+++	-	-	-	-	+	-
	<i>Actinomadura sp. 2</i>	+++	-	-	-	-	+	-
	<i>Actinomadura sp. 3</i>	+++	-	-	-	-	+	-
	<i>Actinomadura sp. 4</i>	+++	-	-	-	-	+	-
	<i>Elytrosporangium sp. 2</i>	+++	-	-	-	-	+	-
	<i>Actinomadura sp. 5</i>	+++	-	-	-	-	+	-
	<i>Actinopolyspora sp. 1</i>	+++	-	-	-	-	++	-
	<i>Actinomadura sp. 6</i>	+++	-	-	-	-	+	-
	<i>Actinomadura sp. 7</i>	+++	-	-	-	-	+	-
	<i>Actinomadura sp. 8</i>	+++	-	-	-	-	+	-
	<i>Actinomadura sp. 9</i>	+++	-	-	-	-	+	-
	<i>Elytrosporangium sp. 3</i>	+++	-	-	+++	-	+	-
	<i>Actinopolyspora sp. 2</i>	+++	-	-	-	-	+	-
2	<i>Streptosporangium sp. 1</i>	+++	-	+	-	-	+	++
	<i>Nocardiopsis sp. 1</i>	+++	-	-	-	++	++	-
	<i>Nocardiopsis sp. 2</i>	+++	-	-	-	+	+	++
	<i>Streptosporangium sp. 2</i>	++	-	+	+++	-	+	-
	<i>Kitasatospora</i>	+++	-	-	+++	++	+++	-
	<i>Actinomadura sp. 10</i>	+++	-	+	-	-	++	-
	<i>Nocardia sp. 1</i>	+++	-	-	-	+	++	-
	<i>Streptomyces sp. 4</i>	+++	-	-	-	-	++	-
	<i>Actinomadura sp. 11</i>	+++	-	-	-	-	++	-
	<i>Elytrosporangium sp. 4</i>	++	-	-	-	-	++	-
	<i>Actinomadura sp. 12</i>	+++	-	-	-	-	++	-
	<i>Actinopolyspora sp. 3</i>	+++	-	-	-	-	++	-
	<i>Actinomadura sp. 13</i>	+++	-	-	-	-	++	-
	<i>Nocardia sp. 2</i>	++	-	-	++	-	++	-
	<i>Actinomadura sp. 14</i>	+++	-	-	-	-	++	-
	<i>Actinomadura sp. 15</i>	+++	-	-	+++	-	++	-
	<i>Actinomadura sp. 16</i>	+++	-	-	-	-	++	-
	<i>Elytrosporangium sp. 5</i>	+++	-	-	-	-	++	-

of therapy and their use in medicine is gaining interest. Therefore, searching for new actinomycete strains still is the focus of many studies (Prakash *et al.*, 2013). Our results revealed that only three isolates, *Streptosporangium sp. 1*, *Streptosporangium sp. 2* and *Actinomadura sp. 10* were able to produce protease enzyme (Figure 1C and D). These three isolates were obtained from site 2. However, the site 1 and the rest of isolates from site 2 completely failed to show any protease activity. This rather low estimate of active strains may indicate that the method used for preliminary screening is not

accurate (Jani *et al.*, 2012). In addition, the low activity observed may suggest that our culture conditions are not optimal for such test (Jani *et al.*, 2012).

The search for novel tyrosinases is still in need due to their potential in industrial applications and medical purposes. Tyrosinases have been suggested as potential tools in treating melanoma and as potential antioxidants, antiviral agents and immunogens (Popa & Bahrim, 2011). Among the isolates, only few showed tyrosine

Figure 1. Enzymatic activities of different actinomycetes isolated from soil, Al-Aqsa Mosque, Jerusalem. (A and B) showing good to moderate amylase activity respectively; (C and D) showing moderate protease activity indicated by clear zones around colonies; (E) showing good tyrosinase activity indicated by deep brown pigments and (F) showing no activity for two different actinomycete isolates.

activity but all of these exhibited strong activity (Table 1). Only one isolate out of 17 (5.9%) in site 1, *Elytrosporangium sp.3*, showed high production of tyrosinase and the rest failed to show any activity (Figure 1E and F). Meanwhile, four isolates out of 18 (22.2%) of site 2, *Streptosporangium sp.2*, *Kitasatosporia*, *Nocardia sp.2*, and *Actinomadura sp.15* showed different tyrosinase activity. These isolates had a high potential for enzyme production except *Nocardia sp.2* which showed moderate activity.

For lipase production, all isolates from site 1 failed to show any activity. However, three isolates (16.7%) out of 18 isolates of site 2, *Nocardiopsis sp.1*, *Nocardiopsis sp.2* and *Kitasatosporia sp.*, were able to produce lipase in a weak to moderate manner of activity (Table 1).

Catalases are ubiquitous enzymes and have been isolated from a broad range of procaryotic and eukaryotic organisms (Zámocký *et al.*, 2012). Actinobacteria are aerobic bacteria and would be expected to have catalase activity; however, our actinomycete isolates recovered from both studied sites showed different ranges of positive activity (Figure 2). The catalase activity was observed at pH 7 of the growing medium for all isolates. Out of the isolated actinomycetes, *Kitasatosporia sp.* was the most active catalase producer, followed by 42.9% of the isolates have moderate catalase activity (Table 1). However, 18 isolates (51.4%) including different genera showed weak activity. The low expressed activity may be due to shortage of manganese ions or iron concentration in the growing medium, since catalases rely on iron or manganese for their activity (Mishra & Imlay, 2012).

Figure 2. Catalase activity of different isolates. **A** and **B** show the activity of isolates from site 1 and site 2 respectively. Evolved oxygen bubbles indicated a very strong activity and weak bubbles indicated a weak activity.

Further studies are needed to identify the factors that weaken the scavenger process.

Since phosphate always exists in unavailable form for plant growth, phosphatase activity is an important mechanism of solubilizing inorganic phosphate (Sharma *et al.*, 2013). In our study, all strains listed in Table 1, grown at room temperature (approximately $27\pm 2^\circ\text{C}$) on basal medium supplemented with CaHPO_4 (5 g/l) were tested for phosphatase activity at pH 7.0. Among the isolates, only *Streptosporangium* sp.1 and *Nocardiopsis* sp.2 showed phosphatase activity. The failure to detect of phosphatase activity by the remaining isolates may be due to the medium composition (Fredrikson *et al.*, 2002). In a recent study done by Ghorbani-Nasrabadi *et al.*, (2013), it was shown that substitution of nitrogen source in the growth medium by organic or inorganic nitrogen sources resulted in a significant reduction of phosphatase activity.

In conclusion, the need for low cost enzymes that can be applied in diverse biotechnological industries could be satisfied with the discovery of novel enzymes and metabolites. Moreover, the application of genetic engineering techniques in enzyme manufacturing is dramatically sparking the exploitation of new enzymes and the development of new enzyme properties. Actinobacteria have been proved a reservoir of important enzymes and metabolites due to their versatile genetic repertoire (Prakash *et al.*, 2013). Identification of new actinobacterial isolates in unique ecological environments could yield molecules that could become future players in green technology. Therefore our study contributes to explore new

ecological sites for actinobacterial identification. Actinobacterial isolates that inhabitant the northern part of Al-Aqsa Mosque, showed a diverse population in the two sites where the soil was collected. These genera have been identified in each site for the first time. However more suitable growth conditions should be tested to explore the metabolites and enzymatic activities of these organisms.

Author contributions

Mansour S., Abdelazeim A. and Deraz S. conceived the study. MS and AA designed the experiments. MS and DS carried out the research. AA and MS provided the expertise throughout the experiment. MS prepared the first draft of the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

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This manuscript describes the isolation and preliminary characterization of actinobacteria strains producing enzymes with potential biotechnological applications. However, the experimental design chosen by the authors, and the conclusions drawn from some results rise some concerns.

1. The major weakness of this work is that the assays used to investigate the enzymatic potential of the isolated actinobacteria are not fully convincing. Especially for what concerns cellulase activity, none of the strains isolated by the authors was positive to this test. The authors discuss that this unexpected result could be due to unsuitable culture conditions, such as pH. This is also the case for protease and catalase activities, that were found just in few isolates, and for which negative results could be biased by the experimental method. Authors should demonstrate that each enzymatic assay works in their hands, by using bacterial strains previously characterized for their ability and inability to produce the tested enzymes as positive and negative controls, respectively. Moreover, other experimental settings (e.g., cultures with different pH) should be considered to verify enzymatic activities in the isolates.
2. The authors state that enzymatic activity (EA) was evaluated as follow: "diameter of zone of tested substrate hydrolysis - diameter of colony in cm". Based on this calculation, the EA was estimated as excellent ($EA > 2$), good ($EA < 2$), or poor ($EA < 1$). Besides noticing that $EA < 2$ is not correct (it should be $1 < EA < 2$), this "EA" values are not mentioned anymore along the manuscript, where the detected activity is usually defined as "strong activity", "moderate activity" or "low activity". In addition, in Table 1 the enzymatic activity is represented with + or - symbols. Authors should choose one adjective/word to define a certain interval of activity and use always the same along the whole text.
3. The measurement of a diameter (see above) can be acceptable as a semi-quantitative method allowing the comparison of a certain enzymatic activity among different strains.

However, since catalase activity was detected by visualizing oxygen bubbles formation, it is not clear how the authors measured and compared this enzymatic activity.

4. Taxonomic allocation of the isolated strains should be confirmed by 16S rRNA sequencing.
5. Since the main aim of this article is to explore the enzymatic potential of newly isolated actinobacteria, the authors were expected to use protocols allowing maximal diversity of the isolated species. The authors discuss that in previous studies sample pre-treatment with specific agents has been successfully used to promote spore germination and to allow isolation of most actinobacteria inhabiting the soil, so it is not clear why they did not consider this possibility for the experimental plan.
6. Authors state that *"the differences observed among the genera of actinobacteria identified in both sites studied, may be due to the different human activities, including construction in such area. These differences may also indicate that the methods proposed and employed for actinobacteria isolation may not be suitable for site 1,..."*. In order to support this piece of discussion, authors should provide more information about the characteristics of the two isolation sites (e.g., anthropic impact, type of soil, type of vegetation). Were these sites similar or different with respect to one or more features? How these similarities/differences could be correlated to the results?

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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Sure it is of interest to evaluate the actinomycete populations of unique sites. However, the data should be sufficient and the goal has to be clearly defined and of interest.

The isolation procedure of the strains do not allow to cover all the actinomycetes population. One has to apply pre-treatment(s)¹ of the samples, use more media such as Humic Acid Vitamins Agar² and the agar-plates have to be incubated at least 3 weeks. With the procedure used one can isolate only the fast growing strains.

The identification of the strains to the genus level cannot be achieved only with the methods used.

It could be tentatively possible for some genera but not in any case for all. Actinomycetes taxonomy was previously based on morphology, which is inadequate in differentiating between various genera^{3,4}. Present approaches to the classification of prokaryotes are based on the combined use of genotypic and phenotypic data, that is, on polyphasic taxonomy^{5,6}. This approach is being driven increasingly by molecular biology, especially the impact of 16S rRNA gene sequence and DNA:DNA relatedness values on the delineation of taxa⁷.

"The differences observed among the genera of actinobacteria identified in both sites studied, may be due to the different human activities, including construction in such area." The authors should not forget that they isolated only a very small part of the actinomycetale populations, so the comparison is difficult. The only way would be to use culture-independent methods.

In addition according to Rule 37a, bacteriologists adhering to this proposal must change the name *Elytrosporangium* to *Streptomyces*.

The fact that the isolated strains showed various enzymatic activities is not surprising and such an observation can be made with all samples. However the description of the activities is well done, even if basic. There are many publications in this area. So the goal should be the detection of a rare enzymatic activity or the comparison of the enzymatic potential of the actinomycetes from this site to other ones or to characterize the enzymes somehow.

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Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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