Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 10 (11): 513-522, 2015
ISSN 1816-4935 / DOI: 10.3923/jm.2015.513.522

Taxonomic Diversity and Antimicrobial Activities of Actinomycetes from Manure Composts

A.L.M. Low, S.A.S. Mohamad and M.F.F. Abdullah
Faculty of Applied Sciences, Universiti Teknologi MARA, 40450, Shah Alam, Selangor Darul Ehsan, Malaysia

Corresponding Author: A.L.M. Low, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450, Shah Alam, Selangor Darul Ehsan, Malaysia

ABSTRACT

Manure compost is a unique microenvironment which harbours potential new microorganisms that is able to produce a variety of anti-infective agents. However, the exploration of this ecosystem for actinomycetes and its bioactive secondary metabolites remained understudied. Therefore, this study aims to study the diversity of manure compost actinomycetes and to investigate their antimicrobial potential using conventional disc diffusion method and a modified resazurin microtiter based approach. A collection of 191 actinomycete isolates were recovered from 5 types of manure composts collected around Selangor, Malaysia. Utilizing a combination of micromorphological characteristics and 16S rRNA sequence analysis, the isolated actinomycetes were grouped into 12 genera within 9 families. *Streptomyces* spp. dominated the culture collection (79.1%) while the rest belonged to the non-*Streptomyces* group (20.9%), including an unusual isolate from the genus *Verrucosispora*. The evaluation of antimicrobial activities demonstrated that 21.5% of the isolates exhibited antagonistic effect against at least one of the test microorganisms with strong inhibition observed against fungal strains compared to pathogenic bacteria. A modified resazurin microtiter based assay was also developed and displayed higher sensitivity (40.0%) compared to the disc diffusion assay (26.0%). All ten actinomycete isolates which displayed narrow and broad spectrum effects also produce pigmented extracts. The results demonstrated that manure compost actinomycetes could be a promising source of novel bioactive agents and that the resazurin microtiter based assay is a more sensitive approach in screening antimicrobial properties of large numbers of microbial extracts.

Key words: Compost actinomycetes, antimicrobial activity, resazurin

INTRODUCTION

The exploration of novel therapeutic drug has been intensively pursued especially in recent years due to the increasing rate of multi resistant pathogens, the rise of new rare diseases and as substitute to current toxic drugs. In fact, periodic replacement of the present drugs with the intention of halting pathogens from acquiring resistance is also significantly important thus making the discovery of novel effective drugs a priority (Ilic *et al*., 2005). For the last decade, advancement in technology has push forward the interest in synthesizing novel compounds in modern laboratory. Despite the development, there are still hitches in the synthesis method which slows down the yield of new compounds and based on the drug discovery documented in the past, most novel skeletons were and are still derived from natural products (Koehn and Carter, 2005; Genilloud *et al*., 2011). This is largely attributed to the structural diversity and complexity of the natural products which enable flexibility in binding to multiple target molecules (Ji *et al*., 2009;

Lahlou, 2013). Hence, more effort is put in improving the screening strategies and this has brought back the interest in natural product study as it is still seen as a valuable source of novel compounds. Recent studies to characterize specific pathways that contribute to pathogenicity e.g., biofilm formation and acid resistance (Abdul-Aziz *et al*., 2015) also uncovered a number of new proteins and molecules which may be used as novel target for drug screening.

Actinomycetes are ubiquitous and can be found in many niches, predominantly in soil. The genus *Streptomyces* in particular has built an impressive reputation in the pharmaceutical industry by contributing about 70% of the clinically useful medicines that is high of commercial value (Khanna *et al*., 2011). As a result of extensive screening programs, it is apparent that the rediscovery of known compounds from terrestrial actinomycetes is a growing problem in the research community. Venturing into unique and underexplored habitations seems to be rewarding as new actinomycete taxa with novel bioactivity are continuously being discovered every year (Elleuch *et al*., 2010; Xing *et al*., 2012).

Compost is a self-heated substrate formed when organic materials are broken down and recycled by successive groups of microorganisms in various composting stages (Ghazifard *et al.,* 2001). The compost microbial population is highly dominated by actinomycetes and they are known for their roles in degrading lignocellulosic materials efficiently under neutral conditions (Wang *et al*., 2014). Lacey (1997) reported the abundant distribution of *Thermomonospora* spp., *Saccharomonospora* spp., *Streptomyces* spp. and *Microtetraspora* spp. in mushroom, green waste and sewage compost which facilitate the degradation of complex molecule. With considerably high temperature and low nutrient condition, actinomycetes are able to thrive in the compost environment owing to the versatility of their metabolic pathways. In general, manure compost in particular contains higher pathogenic bacteria as compared to their mushroom or plant derived counterparts. Several studies have shown that the presence of actinomycetes can potentially serve as an indicator of compost maturity as they participate in suppressing pathogens in the curing stage (Steger *et al*., 2007; Tang *et al*., 2006). Although, many studies have been done especially on mushroom compost, the taxonomic diversity and bioactivity of actinomycetes particularly in manure compost is still understudied. With these promising potentials, it is relevant to venture into natural resources such as manure compost in the hope of attaining novel compounds.

Increasing the efficiency of natural product screening is of strategic importance as it encourages screening in large scale. When screening large amount of extracts, it is crucial to choose a fast pre-screening method in order to determine which extracts are worthy for further analysis. A blue redox dye called resazurin is an indicator solution which changes to pink due to the metabolic processes in cells (Hamid *et al*., 2004). It is sensitive, reliable and has been used previously with a series of modification to suit the screening needs. Unfortunately, most of the resazurin assay that was employed focus more on determining the minimum inhibitory concentration of the extract rather than just a simple antimicrobial screening. Thus, a simpler assay incorporating resazurin was developed to screen microbial extracts vastly. This study reports the diversity of isolated manure compost actinomycetes and its antimicrobial properties using a modified resazurin based assay.

MATERIALS AND METHODS

Collection of manure compost samples: Manure compost samples were collected from five animal farms around Selangor, Malaysia, from May 2010 to January 2013. Prior to sampling, the temperature of the manure compost was recorded. Each collection was taken in the middle part

of the compost heap using a sterile spatula and placed in a sterile ziplock bag. Samples were then crushed and used for the isolation of actinomycetes.

Isolation and maintenance of manure compost actinomycetes: Crushed manure compost samples were immediately processed to maintain the freshness and to minimize contaminants. One gram of crushed compost sample was serially diluted up to 10^{-6} dilution. Then 100 µL of 10^{-3}-10^{-6} diluted suspension was lawn onto Starch Casein Nitrate Agar (SCNA), Humic Acid Vitamin-B Agar (HVA), Starch Yeast Extract Agar (SYEA), Glucose Asparagine Agar (GAA), Oatmeal Agar (OMA) and Glycerol Asparagine Agar (GYAA) at pH 7.2 in duplicates. All plates were supplemented with 50 µg mL^{-1} of cycloheximide to suppressed fungal growth. Plates were then incubated at 30 and 40°C for 1-4 weeks. Actinomycetes formed were isolated and routinely sub-cultured onto fresh respective media and on ISP 2 (yeast extract malt extract agar). Pure colonies were stored as glycerol stock (25%) at -80°C and on plates at 4°C.

Macro and micromorphological characteristics of actinomycete isolates: Isolated actinomycetes were characterized after 7 days of incubation (at 30 or 40°C) on individual media and on ISP 2. The characterization was based on the color of aerial mycelium, reverse side pigmentation and soluble pigmentation. Direct microscopic examination was also conducted to observe the spore arrangement and mycelial structure using the cover slip method (Kawato and Shinobu, 1959) on ISP 2. The observed structures were compared to the Bergey and Holt (2000) and were then grouped or classified into their respective genus.

DNA extraction and PCR amplification: Morphologically distinct isolates were grown in 5 mL of Starch Casein Nitrate Broth (SCNB) with agitation for 7-14 days. Genomic DNA extractions were carried out using either DNeasy® blood and tissue kit (Qiagen, Germany). The 16S rRNA genes were amplified using primers PA: 5'-AGAGTTTGATCCTGGCTCAG-3' and PB: 5'-AAGGAGGTGAT CCAGCCGCA-3' (Qin *et al*., 2009). The PCR mixture contained 5 µL of 1X GoTaq® Buffer (Promega, Spain), 0.5 µL of 0.2 mM dNTPs, 1 µL of 0.5 mM $MgCl_2$, 1 µL of 0.5 µM each primer, 0.125 µL of 1.25 U DNA Polymerase (Promega) and 0.2 µL of template DNA. Each reaction was adjusted to a final volume of 25 µL with sterile double-distilled water and amplified in an automated thermal cycler (C1000 thermal cycler BIO-RAD laboratories). The following conditions were used for the PCR amplification: Initial denaturation at 94°C for 4 min, 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min and a final extension at 72°C for 10 min. The PCR products were electrophoresed on 1% agarose gel with 100 bp DNA ladder as the size reference.

Purified PCR amplicon were sequenced and used to interrogate the NCBI database via the BLAST web portal.

Antimicrobial screening: The antimicrobial activities were determined using direct broth disc diffusion method against a set of test bacteria including *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), fungi (*Candida albicans*) and a yeast (*Saccharomyces cerevisiae*). Test organisms were cultured overnight and adjusted to an absorbance of 0.03-0.05 at 600 nm (bacteria) and 630 nm (fungal) prior to use. Filter paper discs (6 mm) saturated with 10 µL of putative isolate broth (8 days old) were placed on Mueller-Hinton agar (for bacteria) and Sabouraud Dextrose agar (for fungal). Plates were then incubated for 24 and 48 h, respectively.

Fermentation and extraction of secondary metabolites: Pure cultures were grown in 30 mL of SCNB and incubated at 30°C in a rotary shaker (170 rpm) for 7 days. Secondary metabolites were recovered using the solvent extraction method where ethyl acetate (ratio 1:1, v/v) was added to the suspension and shaken for 2 h (170 rpm). The mixture was then left to stand at room temperature for an hour allowing the phases to be separated completely. The solvent phase containing the secondary metabolites was separated into pre-weighed vials, left to evaporate and weighed.

Antimicrobial screening using resazurin microtiter based assay: This assay was adopted from Sarker *et al*. (2007) with slight modifications. Overnight shaken test organisms were adjusted to an absorbance of 0.7-0.8 at 600 and 630 nm prior to use. Assay was conducted in 96 well flat bottom plates and labelled accordingly. A volume of 10 µL of test organisms was introduced to each well followed by 40 µL of test material (dissolved in 10% MeOH). After 18 h of incubation (37°C) of the plate, 20 µL of 0.5 mM resazurin 40 µL of extract was then pipetted into wells. After 18 h of incubation (37°C), 20 µL of 0.5 mM resazurin was added and re-incubated for 8 h. Nystatin, streptomycin and chloramphenicol were used as positive control whereas 10% MeOH as negative control. All tests were done in triplicates.

RESULTS AND DISCUSSION

A total of 191 actinomycetes were recovered from five different manure composts collected around Selangor, Malaysia (Table 1). Only mesophilic actinomycetes and certain thermo-tolerant strains such as *Saccharomonospora* sp. were recovered as the incubation temperatures chosen were

Table 1: Number of actinomycetes isolated from different manure composts

Sampling location	Type of compost	Actual compost temperature (°C)	Media	No. of strains isolated at 30°C	40°C	Total
Sg. Besar	Goat	41	SCNA	2		2
			HVA	4		4
Sg. Besar	Goat	45	SCNA	40	4	44
			HVA	11	11	22
Meru	Goat	36	SCNA	12		12
			HVA	2		2
			GAA	11		11
Meru	Goat	37	SCNA	14		14
			HVA	2		2
			GAA	3		3
Morib	Goat	38	SCNA	16	3	19
			HVA	12	3	15
			GAA	14	1	15
			SYEA	1	1	2
Shah Alam	Horse	33.2	HVA	1		1
			GAA	2		2
			OMA	3		2
			GYAA	3		3
Shah Alam	Deer	33.8	GAA	3		3
			OMA	1		1
			GYAA	4		4
Shah Alam	Porcupine	32.5	HVA	1		1
			GAA	1		1
			GYAA	5		5
Total				168	23	191

SCNA: Starch casein nitrate agar, HVA: Humic acid vitamin-B agar, GAA: Glucose asparagine agar, SYEA: Starch yeast extract agar, OMA: Oatmeal agar, GYAA: Glycerol asparagine agar

based on the measured temperature of the manure composts. In fact, incubation at 40°C gave comparatively lower counts as compared to 30°C (data not shown) and this actually displayed what appeared to be a typical characteristic of maturing compost. Steger *et al*. (2007) reported that even though actinomycetes can be found in all stages throughout composting, they mostly dominated during the maturing stage with estimation of up to 50% of the total microbial biomass. Using a combination of micromorphological characterization and 16S rRNA sequence analysis, these actinomycete isolates were tentatively classified into their respective genera. In decreasing order of abundance (Fig. 1), the isolates were *Streptomyces* spp., *Actinomadura* spp., *Pseudonocardia* spp., *Micromonospora* spp., *Saacharomonospora* spp., *Nocardiopsis* spp., *Verrucosispora* spp., *Glycomyces* spp., *Gordonia* spp., *Actinopolymorpha* spp., *Nonomuraea* spp. and *Actinoplanes* spp. which cover 9 families. The micromorphological characteristics of different genera are shown in Fig. 2. It is clear from the data that the non-*Streptomyces* only accounted for 20.9% of the overall isolates.

This is regularly reported in other compost related studies as *Streptomyces* grow faster under conventional culture conditions and special requirements are needed to grow the non-*Streptomyces* (Song *et al*., 2001; Jayasinghe and Parkinson, 2008). It was interesting that a motile actinomycete, *Actinoplanes* spp. was recovered from the cow manure compost. This strain has never been reported from manure compost before. However, it is usually found in compost leaves and normally requires special treatment such as pollen-baiting to acquire it (Hayakawa *et al*., 1991). Van Hop *et al*. (2011) reported the frequent isolation of *Actinoplanes* in composted leaves and highlighted their absence in fresh fallen leaves. Since it is a common practice to mix manure with straws or leaves during composting, this may explain the presence of *Actinoplanes* in the manure compost. Moreover, the hydrophilic motile spores that this genus possessed allow easy transmission through the wind and droplets of water during wet seasons. *Thermoactinomyces* sp. which is a common compost actinomycete was not observed in our sample and a similar situation was also reported by Hayakawa *et al*. (2010). Furthermore, both studies were consistent in reporting the presence of *Glycomyces* sp. in manure compost although past studies have shown that this genus was mostly found in soil samples and the inner part of plants (Qin *et al*., 2009). In a comprehensive research

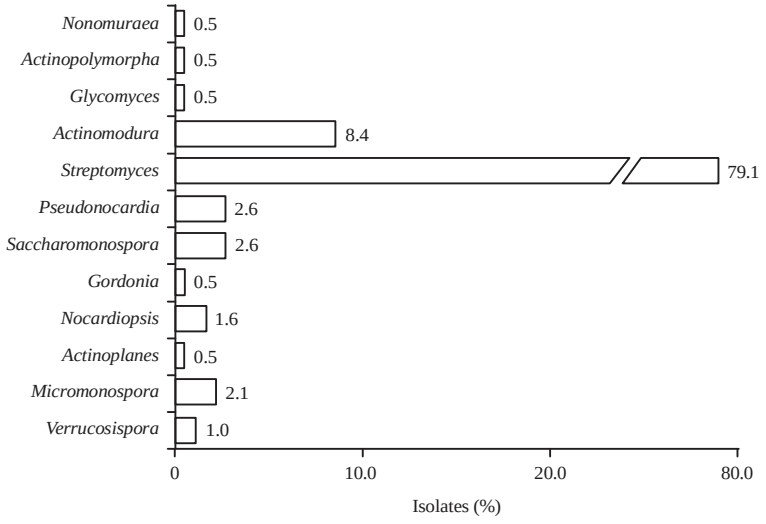

Fig. 1: Percentage of isolated actinomycete genera from manure composts

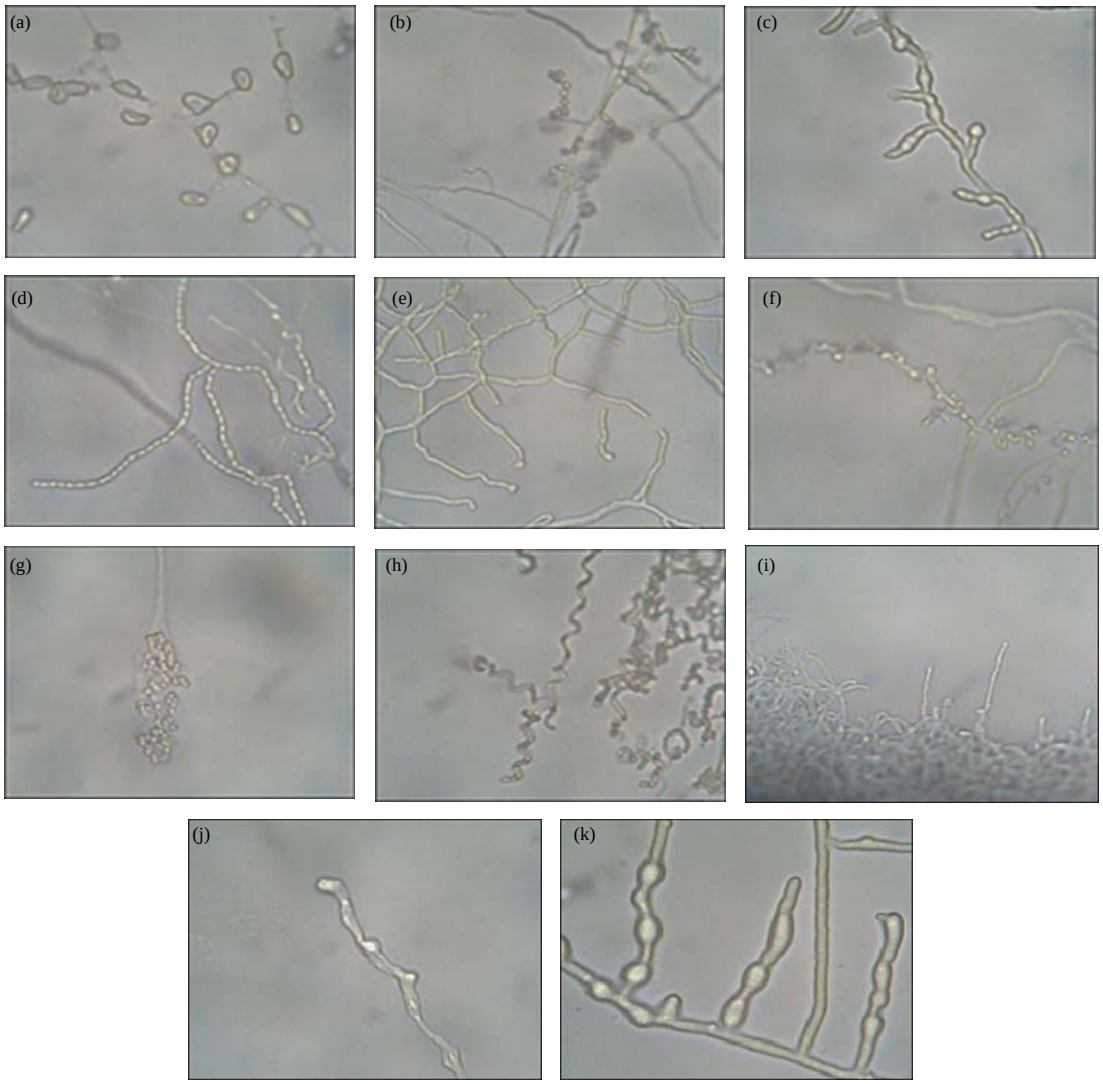

Fig. 2(a-k): Micromorphological characteristics of campost manure actinomycetes observed under light microscope (1000x), (a) *Actinoplanes* sp., (b) *Actinomadura cremea* (c) *Pseudonocardia* sp., (d) *Streptomyces* sp., (e) *Nocardiopsis chromatogenes* (f) *Saccharomonospora* sp., (g) *Verrucosispora* sp., (h) *Streptomyces* sp., (i) *Micromonospora echinofusca* (j) *Nonomuraea* sp. and (k) *Pseudonocardia* sp.

conducted by Yamada *et al*. (2008) using 16S rRNA gene-based clone library analysis, the authors revealed that the genus *Glycomyces* was among the major bacteria found in cow dung compost. These evidence implied that this strain is likely an inhabitant of specifically manure-based compost as there is still no study found to suggest otherwise.

In the present study also, one isolate (C2A1) recovered from cow manure compost showed closest similarity (99.1%) to *Verrucosispora maris* which was initially reported from deep-sea sediments (Goodfellow *et al*., 2012). It has been debated for many years without consensus whether marine actinomycetes are actually native marine population or simply a wash off terrestrial microorganisms (Jensen *et al*., 1991; Takizawa *et al*., 1993). According to Goodfellow *et al*. (2013), all six validly described *Verrucosispora* taxon members came from the marine environment

indicating that they are true autochthonous marine community and denying the theory of land runoff microorganisms. However, the present *Verrucosispora maris* strain was of terrestrial origin and able to grow without the presence of salt content which contradicts the fact of an autochthonous marine bacteria while suggesting the possibility of salinity adaptation. Although this finding is indeed an added value to the currently available information on the distribution of this taxon in natural habitats, further investigation is needed to finally conclude as to whether *Verrucosispora maris* in this study is a true terrestrial originated.

The antimicrobial properties of the manure compost actinomycetes were then investigated using direct broth disc diffusion method. From the data obtained, 41 isolates (21.5%) showed inhibitory activities against at least one of the test organisms. As expected, higher activities were seen in Gram positive rather than Gram negative bacteria (Oskay *et al.,* 2004; Basilio *et al.,* 2003). This is apparently attributed to the difference in composition of the cell walls between both cells which allow easier penetration of compounds into gram positive compared to gram negative bacteria. As depicted in Table 2, isolate G2A2 exhibited promising broad spectrum activities as it inhibited all test organisms except for *P. aeruginosa.* Isolate G1A6 showed the highest antifungal activities towards *C. albicans* and *S. cereviseae*. Stronger antifungal activity was also noticed compared to antibacterial ones but the percentage of antifungal (6.3%) was lower than antibacterial (16.2%). This is comparable with the work done by Kitouni *et al.* (2005) and Valan *et al.* (2012) where only a small percentage of actinomycetes showed antifungal properties. Generally, microorganisms' physiological activities and secondary metabolism are profoundly affected by the microenvironmental surroundings (Genilloud *et al.,* 2011). Since the later stages of composting is mostly dominated by actinomycetes and fungi (Steger *et al.,* 2007), it is believed that competition for nutrients and colonization causes actinomycetes to develop such strong antifungal properties. All the active isolates are from the genus *Streptomyces* sp.

To increase the sensitivity for detecting antimicrobial activities, a modified microtiter plate assay incorporating resazurin as cell growth indicator was used. Dried ethyl acetate extract of the actinomycete isolates were redissolved in 10% MeOH. Table 3 showed that 10 of the samples exhibited narrow and broad spectrum activities using the resazurin. Only isolate G2C32 exhibited broad spectrum effect which inhibitory was inclusive of *E. coli* and *C. albicans.* Comparing the results of both assays, there was a substantial elevation in the percentage of antimicrobial inhibitory (40%) using the resazurin microtiter method as compared to the disc diffusion method

Table 2: Antimicrobial activities of seven active actinomycete isolates

	Test microorganisms					
	Gram +ve		Gram -ve		Fungi	Yeast
Isolate No.	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Saccharomyces cereviseae*
G1A6	+	-	-	-	+	+
G1A7	+	+	+	-	-	-
G2A6	+	+	+	-	-	-
G2A31	+	+	-	-	+	+
G2C31	+	-	-	+	+	+
G1A20	+	+	+			
G2A2	+	+	+	-	+	+
+ve control	+	+	+	+	+	+
-ve control	-	-	-	-	-	-

Values are mean inhibition zone (mm) of two replicates, +ve control: Streptomycin: 400 μg mL^{-1}, Nystatin: 200 μg mL^{-1}, Chloramphenicol: 400 μg mL^{-1}, -ve control: Starch casein nitrate broth

Table 3: Antimicrobial activities of ten active extracts

	Test organisms						
	Gram+ve				Gram-ve	Fungi	
Isolate No.	*Staphylococcus aureus*	*Bacillus subtilis*	*S. cohnii*	*S. saprophyticus*	*Escherichia coli*	*Candida albicans*	Concentration of extract (mg mL^{-1})
G1A1	+	+	+	+	-	+	3.70
G2A24	+	+	+	+	-	+	0.55
C2A14	+	+	+	+	-	+	3.00
C2A16	+	+	+	+	-	+	0.80
C2C13	+	+	+	+	-	+	0.60
G1A30	+	+	+	+	+	-	0.72
D4Gy4	+	+	+	+	+	-	20.3
C2B6	+	+	-	+	-	+	0.35
G2B2	+	+	-	+	-	+	3.00
G2C32	+	+	-	-	+	+	1.30
+ve control	+	+	+	+	+	+	
-ve control	-	-	-	-	-	-	

Tests were conducted in triplicates, +: Positive inhibition, -: No inhibition

(26%). In fact, isolates that showed activities in disc diffusion assay displayed inhibitory against more strains in this assay. This implied that resazurin based assay is sensitive to small volume and efficient enough to give reproducible results. It is also conceivable that before extraction, the secondary metabolites were less concentrated; therefore, some isolates only exhibit antimicrobial activities with concentrated extracts. The present results also showed that all 10 actinomycete isolates exhibiting broad spectrum effects also produced diffusible pigments. This is in agreement with a study done by Selvameenal *et al*. (2009), where a yellow crude extracted from soil actinomycete displayed strong inhibitory against *Klebsiella* sp. and *E. coli* signifying that pigmented crude could be an indicator of an active extract. It is important to note that preliminary testing should always be taken prior to the actual assay as we discovered that resazurin was unsuitable to be tested against *P. aeruginosa* and *S. cerevisiae.* Since both strains possess efflux mechanisms, they tend to extrude external substrate which resulted in the unchanged color of resazurin (Webber and Piddock, 2003). Moreover, because resazurin assay relies mostly on the observation of color changes, we suggest that extracts with intense pigmentation should be diluted prior to the assay as it interferes with the physical observation of the dye.

CONCLUSION

Overall, manure composts indeed harboured considerable diversity of actinomycetes with the majority of isolates observed at 30°C and dominated by the genus *Streptomyces*. The ability of the present manure compost actinomycete isolates in inhibiting certain pathogens signified that there is potential in discovering interesting anti-infective activities and that these isolates are worthy for further analysis to finally identify the active compounds. Moreover, the successful development of resazurin microtiter based format is proven to be rapid, less laborious, sensitive enough and can be scaled up to HTS assays of large microbial natural product collection.

REFERENCES

Abdul-Aziz, A., M.F.F. Abdullah and N.H. Hussain, 2015. Inducible acid tolerance response in *Shigella sonnei* and *Shigella flexneri*. Res. J. Microbiol., 10: 320-328.

Basilio, A., I. Gonzalez, M.F. Vicente, J. Gorrochategui, A. Cabello, A. Gonzalez and O. Genilloud, 2003. Patterns of antimicrobial activities from soil actinomycetes isolated under different conditions of pH and salinity. J. Applied Microbiol., 95: 814-823.

Bergey, D.H. and J.G. Holt, 2000. Bergey's Manual of Determinative Bacteriology. 9th Edn., Lippincott Williams and Wilkins, Philadelphia, PA., USA.

Elleuch, L., M. Shaaban, S. Smaoui, L. Mellouli and I. Karray-Rebai *et al*., 2010. Bioactive secondary metabolites from a new terrestrial *Streptomyces* sp. TN262. Applied Biochem. Biotechnol., 162: 579-593.

Genilloud, O., I. Gonzalez, O. Salazar, J. Martin, J.R. Tormo and F. Vicente, 2011. Current approaches to exploit actinomycetes as a source of novel natural products. J. Ind. Microbiol. Biotechnol., 38: 375-389.

Ghazifard, A., R. Kasra-Kermanshahi and Z.E. Far, 2001. Identification of thermophilic and mesophilic bacteria and fungi in Esfahan (Iran) municipal solid waste compost. Waste Manage. Res., 19: 257-261.

Goodfellow, M., J.E.M. Stach, R. Brown and A.N.V. Bonda *et al*., 2012. *Verrucosispora maris* sp. nov., a novel deep-sea actinomycete isolated from a marine sediment which produces abyssomicins. Antonie van Leeuwenhoek, 101: 185-193.

Goodfellow, M., R. Brown, L. Ahmed, W. Pathom-Aree and A.T. Bull *et al*., 2013. *Verrucosispora fiedleri* sp. nov., an actinomycete isolated from a fjord sediment which synthesizes proximicins. Antonie van Leeuwenhoek, 103: 493-502.

Hamid, R., Y. Rotshteyn, L. Rabadi, R. Parikh and P. Bullock, 2004. Comparison of alamar blue and MTT assays for high through-put screening. Toxicol. *In vitro*, 18: 703-710.

Hayakawa, M., T. Sadakata, T. Kajiura and H. Nonomura, 1991. New methods for the highly selective isolation of *Micromonospora* and *Microbispora* from soil. J. Fermentat. Bioeng., 72: 320-326.

Hayakawa, M., H. Yamamura, Y. Nakagawa, Y. Kawa and Y. Hayashi *et al*., 2010. Taxonomic diversity of actinomycetes isolated from swine manure compost. Actinomycetologica, 24: 58-62.

Ilic, S.B., S.S. Konstantinovic and Z.B. Todorovic, 2005. UV/Vis analysis and antimicrobial activity of *Streptomyces* isolates. Med. Biol., 12: 44-46.

Jayasinghe, B.A.T.D. and D. Parkinson, 2008. Actinomycetes as antagonists of litter decomposer fungi. Applied Soil Ecol., 38: 109-118.

Jensen, P.R., R. Dwight and W. Fenical, 1991. Distribution of actinomycetes in near-shore tropical marine sediments. Applied Environ. Microbiol., 57: 1102-1108.

Ji, H.F., X.J. Li and H.Y. Zhang, 2009. Natural products and drug discovery. EMBO Rep., 10: 194-200.

Kawato, M. and R. Shinobu, 1959. Cover slip culture of *Streptomyces herbaricolour* nov. sp. supplement; A simple technique for the microscopical observation. Memoirs Osaka Univ. Liberal Arts Educ., 8: 114-119.

Khanna, M., R. Solanki and R. Lal, 2011. Selective isolation of rare actinomycetes producing novel antimicrobial compounds. Int. J. Adv. Biotechnol. Res., 2: 357-375.

Kitouni, M., A. Boudemagh, L. Oulmi, S. Reghioua and F. Boughachiche *et al*., 2005. Isolation of actinomycetes producing bioactive substances from water, soil and tree bark samples of the North-East of Algeria. J. Med. Mycol., 15: 45-51.

Koehn, F.E. and G.T. Carter, 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov., 4: 206-220.

Lacey, J., 1997. Actinomycetes in compost. Ann. Agric. Environ. Med., 4: 113-121.

Lahlou, M., 2013. The success of natural products in drug discovery. J. Pharmacol. Pharm., Vol. 4. 10.4236/pp.2013.43A003

Oskay, A.M., T. Usame and A. Cem, 2004. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. Afr. J. Biotechnol., 3: 441-446.

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

Sarker, S.D., L. Nahar and Y. Kumarasamy, 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth and its application in the *in vitro* antibacterial screening of phytochemicals. Methods, 42: 321-324.

Selvameenal, L., M. Radhakrishnan and R. Balagurunathan, 2009. Antibiotic pigment from desert soil actinomycetes; biological activity, purification and chemical screening. Indian J. Pharmaceut. Sci., 71: 499-504.

Song, J., H.Y. Weon, S.H. Yoon, D.S. Park, S.J. Go and T.W. Suh, 2001. Phylogenetic diversity of thermophilic actinomycetes and *Thermoactinomyces* spp. isolated from mushroom composts in Korea based on 16S rRNA gene sequence analysis. FEMS Microbiol. Lett., 202: 97-102.

Steger, K., A. Sjogren, A. Jarvis, J.K. Jansson and I. Sundh, 2007. Development of compost maturity and *Actinobacteria* populations during full-scale composting of organic household waste. J. Applied Microbiol., 103: 487-498.

Takizawa, M., R.R. Colwell and R.T. Hill, 1993. Isolation and diversity of actinomycetes in the Chesapeake Bay. Applied Environ. Microbiol., 59: 997-1002.

Tang, J.C., N. Maie, Y. Tada and A. Katayama, 2006. Characterization of the maturing process of cattle manure compost. Process Biochem., 41: 380-389.

Valan, A.M., S. Ignacimuthu and P. Agastian, 2012. Actinomycetes from western Ghats of Tamil Nadu with its antimicrobial properties. Asian Pacif. J. Trop. Biomed., 2: S830-S837.

Van Hop, D.V., Y. Sakiyama, C.T.T. Binh, M. Otoguro and D.T. Hang *et al*., 2011. Taxonomic and ecological studies of actinomycetes from Vietnam: Isolation and genus-level diversity. J. Antibiot., 64: 599-606.

Wang, C., X. Guo, H. Deng, D. Dong, Q. Tu and W. Wu, 2014. New insights into the structure and dynamics of actinomycetal community during manure composting. Applied Microbiol. Biotechnol., 98: 3327-3337.

Webber, M.A. and L.J.V. Piddock, 2003. The importance of efflux pumps in bacterial antibiotic resistance. J. Antimicrob. Chemother., 51: 9-11.

Xing, K., G.K. Bian, S. Qin, H.P. Klenk and B. Yuan *et al*., 2012. *Kibdelosporangium phytohabitans* sp. nov., a novel endophytic actinomycete isolated from oil-seed plant *Jatropha curcas* L. containing 1-aminocyclopropane-1-carboxylic acid deaminase. Antonie Leeuwenhoek, 101: 433-441.

Yamada, T., A. Suzuki, H. Ueda, Y. Ueda, K. Miyauchi and G. Endo, 2008. Successions of bacterial community in composting cow dung wastes with or without hyperthermophilic pre-treatment. Applied Microbiol. Biotechnol., 81: 771-781.