



A Consolidated Method for Selective Isolation of Actinomycetes Based on Choice of Substrate

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ABSTRACT

Actinomycetes are known as filamentous, Gram positive bacteria. They form the majority of the microbial load in various niches; soils, composts, etc. The study aimed to evaluate the method of isolating slow growing actinomycetes from four different sources: garden soil, cow dung manure compost, floral waste compost, and floral waste vermicompost. In this study, an integrated method consisting of physical and chemical pretreatment of the sample and the use of selective media was used to isolate actinomycetes. Physical treatment includes air drying, sun drying, dry heating in an oven, and moist heat treatment, whereas chemical treatment includes enrichment of the sample with CaCO₃ followed by plating on actinomycetes-specific media with the incorporation of antibacterial and antifungal antibiotics. The actinomycetes count on the plate was reported in CFU/gm of dry wt. Morphological and microscopic characteristics of purified isolates were noted. The results were compared, and it was found that the pretreatment method of a particular sample depends on the choice of substrate. Overall, physical treatment followed by chemical enrichment showed relatively higher counts on the plate and better results. Here we also found the dominance of fluorescent *Pseudomonas* sp. in case samples from vermicompost. The study can be of great importance in isolating novel and rare genera of actinomycetes. These methods can help speed up the isolation and screening of novel actinomycetes which will ultimately be important for the discovery of antibiotics and other industrially vital bioactive compounds.

Keywords: Actinomycetes, Pre-treatment, Enrichment.

1 Introduction

Actinomycetes are aerobic gram-positive bacteria, filamentous in nature with a fungal type of morphology. They are one of the major groups of soil population, widely distributed in nature and inhabit various niches other than terrestrial soil such as marine sediments, mangrove environment, composts, vermicomposts etc (Yami *et al.*, 2003; Baniya and Vaidya, 2011; Mohseni *et al.*, 2013; Gopalkrishnan *et al.*, 2014; Mohanta, 2014). They are saprophytic in nature and play an important role in decomposition of waste and recycling of organic matter (Goodfellow and Williams, 1983). They have DNA rich in GC content, 57-75% (Lo *et al.*, 2002). They have the ability to produce antibiotics of agriculture and medicinal use. About 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora* (Pandey *et al.*, 2004). They have also been recognized as prolific producers of enzymes such as amylase, lipase, protease (Lekshmi *et al.*, 2014), cellulase (Mohanta, 2014), glucose isomerase (Sheetal and Modi, 2013) etc. of commercial interest, plant hormones (Gopalkrishnan *et al.*, 2014) and other secondary metabolites. Thus, they have allured considerable research interest. They are the part of microbial flora of most natural substrates (El-Nakeeb and Lechevalier, 1962). Numerous methods have been suggested to facilitate the isolation of actinomycetes and to separate them from other non-actinomycetes bacteria and fungi. Isolation of actinomycetes itself is a challenging task (Williams and Cross, 1971). They need to be cultivated than to be cultured. These methods include pretreatment of samples and use of selective media



for increasing the slow growing actinomycetes flora from substrate before plating out. Pretreatment of sample can be physical such as air drying at room temperature (Salim *et al.*, 2017), sun drying (Sheetal and Modi, 2013), dry heat in oven (Abidin *et al.*, 2016), moist heat treatment (Mohseni *et al.*, 2013), centrifugation etc (El-Nakeeb and Lechevalier, 1962) or can be chemical such enriching the sample with 1% CaCO₃ (Tsao *et al.*, 1960) or chitin etc. Lechevalier (1963) suggested that CaCO₃ treatment gave highest colony counts. Physical treatment together with chemical treatment favors growth of actinomycetes and reduction in the number of other microbes. Selective media supplemented with antibiotics have been used by many researchers to separate actinomycetes from other bacteria and fungi. These antibiotics do not affect the growth of actinomycetes and thus can be successfully used (El-Nakeeb and Lechevalier, 1962). The purpose of our present study is to compare various pretreatment methods and find out the method that can give relatively higher counts of actinobacteria on plate.

2 Materials and Methods

2.1 Sample Collection

Soil samples were collected from garden nearby village school campus. For isolation of actinomycetes soil was to be taken 6-12 inches at depth (Iyer *et al.*, 2014). Four different samples were taken in this study 1) Soil samples 2) 20 days old cow dung left to degrade, turns black 3) Sample from compost prepared from floral waste and cow dung in ratio 1:1 (2kg each) matured in 60 days (approx.). Sample from vermicompost prepared from floral waste and cow dung in ratio 1:1 kg each (Shouche *et al.*, 2011) matured in 46-50 days (approx.). 400 gm of mature compost and vermicompost sample kept for further processing was used in this study. All the samples were collected by maintaining aseptic conditions.

2.2 Pretreatment of Sample

All the four samples were pretreated before plating out on selective media (Table 1). Samples without pretreatment were kept as control. The selective isolation was done using following combinations mentioned in table below.

Table 1: Selective isolation of actinomycetes

Pretreatment of sample	Selective media	Antibacterial and antifungal supplements
No treatment (control)	SCA	-
No treatment (control)	AIA	-
No treatment (control)	SCA	CHL and ICZ
No treatment (control)	AIA	CHL and ICZ
Dry treatment		
Air drying at RT	SCA	-
Air drying at RT	AIA	-
Air drying at RT	SCA	CHL and ICZ
Air drying at RT	AIA	CHL and ICZ
Sun drying	SCA	-
Sun drying	AIA	-
Sun drying	SCA	CHL and ICZ
Sun drying	AIA	CHL and ICZ
Wet treatment		
Air drying at RT + heating in water bath at 60°C	SCA	CHL and ICZ
Air drying at RT + heating in water bath at 60°C	AIA	CHL and ICZ
Sun drying + heating in water bath at 60°C	SCA	CHL and ICZ
Sun drying + heating in water bath at 60°C	AIA	CHL and ICZ
Chemical enrichment		
Sample enriched with CaCO ₃ for 7 days and air dried	SCA	CHL and ICZ
Sample enriched with CaCO ₃ for 7 days and air dried	AIA	CHL and ICZ

Note: RT= Room Temperature, SCA = Starch Casein Agar, AIA = Actinomycete Isolation Agar, CHL = Chloramphenicol, ICZ = Itraconazole.

The Starch Casein Agar (SCA) and Actinomycete Isolation Agar (AIA) used here were procured from HiMedia, Mumbai, India. For chemical enrichment, the particular sample was mixed with for 1% of CaCO₃ (Tsao *et al.*, 1960). It was kept on filter paper moistened with sterile water in a petridish for 7 days and then air dried for further use. All the pretreated samples were crushed into powder and sieved and were subjected to serial dilution up to 10⁻⁶ before plating on agar medium.

2.3 Isolation of Actinomycetes from Pretreated Samples

For the isolation of actinomycetes 10gm of dried pretreated sample was added to 90ml of sterile water in conical flask and shaken in vortex mixer vigorously for detachment of spore chains (Workie and Abate, 2016). It is then allowed to settle for 10 minutes. From the suspension 1ml was taken and added to 9 ml of sterile water followed by serial dilution up to 10⁻⁶ of which 0.1 ml was spread using sterile L - shaped glass rod on SCA (Kuster and Williams, 1964) and AIA (Abidin *et al.*, 2016) supplemented with or without antibiotics. The antibiotics used in the medium were Chloramphenicol - 50µg/ml as an antibacterial drug (Williams and Davies, 1965) and Itraconazole - 75µg/ml as an antifungal drug (Peela and Porana, 2017). After plating the sample plates were kept inverted at 30°C for 7-14 days (Kuster and Williams, 1964; Jeffrey, 2008; Anusuya and Geetha, 2012). In case of moist heat pretreatment, the process is same as mentioned above except serially diluted soil suspensions before plating were kept in water bath for 50°C for 10min (Arifuzzaman *et al.*, 2010; Mohseni *et al.*, 2013) Colonies with tough or powdery texture, dry or folded appearance and branching filaments, substrate or aerial mycelium were sub cultured on SCA for further studies.

2.4 Microscopic Characterization

Microscopy was performed with simple stain technique using methylene blue was used (Kahasabuli and Kibera, 2014). A mass of pure colony of isolate was picked or scooped using a sterile wire loop, spread on clean glass slide, air dried and fixed by passing over flame gently. The slide was the flooded with methylene blue stain and after few seconds excess stain washed off in running tap water. The slide was dried using blotting paper and observed using a light microscope under 10X, 40X and also under 100X using immersion oil. Nature of hyphae and spore chain was observed and identified according to Shirling and Gottlieb, 1966 and photograph was taken.

3 Results

From the four different samples collected actinomycetes isolated were represented as CFU/g dry wt. (Table 2, 3, 4, 5). Soil Samples without treatment showed no growth of actinomycetes in any of the media SCA or AIA (Table 2, Fig. 1).



Figure 1: Plate with sample pretreatment and without Ab – no actinomycetes visible



Figure 2: Plate with sample pretreatment and without Ab

Samples without treatment but with use of antibiotics also showed no growth of actinomycetes (Table 2 Fig. 2). Similar is the case with other cowdung compost, floral compost and floral vermicompost. Samples with physical treatment such as air dry and sun drying along with the use of antibiotics showed some actinomycetes isolates and fungal inhibition (Fig 3). Results showed that highest no. of actinomycetes count was achieved when subjected to both dry and wet heat treatment followed by plating on SCA and AIA agar supplemented with antibiotics (Fig 1-7). However, in case of cow dung compost actinomycetes count was better with air dried sample at room temperature on agar medium with antibiotics. The bacterial counts were higher when the sample was subjected to CaCO₃ enrichment (Fig 4 & 5). Hence chemical enrichment showed better results in this case. On the other hand, in case of floral waste compost good results were obtained only when sample enriched with CaCO₃ (Table 3).

Table 2: Selective isolation of actinomycetes from soil near plant area:

Pretreatment of sample	Selective Media	Growth of actinomycetes	Growth of other microbes	CFU/gm of dried sample
No treatment	SCA	No growth	Bacterial and fungal contamination	-
No treatment	AIA	No growth	Bacterial and fungal contamination	-
No treatment	SCA + CHL+ICZ	No distinct actinomycetes appear	No bacteria; fungal contamination of <i>Aspergillus niger</i>	-
No treatment	AIA + CHL +ICZ	No distinct actinomycetes	No bacteria; fungal contamination of <i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i> , green mold	-
Dry treatment				
Air dried at RT	SCA	No growth	Bacterial and fungal contamination	-
Air dried at RT	AIA	No growth	Bacterial and fungal contamination	-
Air dried at RT	SCA + CHL+ICZ	Poor growth	<i>Aspergillus niger</i> contamination	2x10 ⁻⁵
Air dried at RT	AIA +CHL +ICZ	Poor growth	<i>Aspergillus niger</i> contamination; But other fungi is inhibited	1x10 ⁻⁵
Sundried	SCA	No growth	Bacterial and fungal contamination	-
Sundried	AIA	No growth	Bacterial and fungal contamination	-
Sundried	SCA + CHL+ICZ	Poor growth	<i>Aspergillus niger</i> contamination; but other fungi is inhibited	2x10 ⁻⁵
Sundried	AIA +CHL +ICZ	Poor growth	<i>Aspergillus niger</i> contamination; but other fungi is inhibited	2x10 ⁻⁵
Wet treatment				
Air dried at RT + heated in water bath for 60°C	SCA + CHL+ICZ	Poor growth	<i>Aspergillus niger</i> contamination; but other fungi is inhibited	2x10 ⁻⁵
Air dried at RT+ heated in water bath for 60°C	AIA +CHL +ICZ	Poor growth	<i>Aspergillus niger</i> contamination; but other fungi is inhibited	2x10 ⁻⁵
Sundried + heated in water bath for 60°C	SCA + CHL+ICZ	Good	<i>Aspergillus</i> and fungal contamination is restricted to corner of plate	6x10 ⁻⁵
Sundried+ heated in water bath for 60°C	AIA +CHL +ICZ	Good	<i>Aspergillus</i> and fungal contamination is restricted to corner of plate	5x10 ⁻⁵

Chemical enrichment				
Sample enriched with CaCO ₃ for 7 days and air dried	SCA + CHL+ICZ	Better	Less contamination of other microbes	7x10 ⁻⁵
Sample enriched with CaCO ₃ for 7 days and air dried	AIA+ CHL+ICZ	Better	Less contamination of other microbes	6x10 ⁻⁵

Note: RT= Room Temperature, SCA = Starch Casein Agar, AIA = Actinomycete Isolation Agar, CHL = Chloramphenicol, ICZ = Itraconazole.

Table 3: Selective isolation of actinomycetes from cow dung compost

Pretreatment of sample	Selective Media	Growth of actinomycetes	Growth of other microbes	CFU/gm of dried sample
No treatment	SCA	No growth	Bacterial and fungal contamination	-
No treatment	AIA	No growth	Bacterial and fungal contamination	-
No treatment	SCA + CHL+ICZ	No distinct actinomycetes appear	No bacteria; fungal contamination	-
No treatment	AIA + CHL +ICZ	No distinct actinomycetes appear	No bacteria; fungal contamination	-
Dry treatment				
Air dried at RT	SCA	No growth	Bacterial and fungal contamination	-
Air dried at RT	AIA	No growth	Bacterial and fungal contamination	-
Air dried at RT	SCA + CHL+ICZ	Good	Fungal contamination of <i>Aspergillus niger</i>	4x10 ⁻⁵
Air dried at RT	AIA +CHL +ICZ	Good	Fungal contamination of <i>Aspergillus niger</i> and <i>Aspergillus</i>	5x10 ⁻⁵
Sundried	SCA	No growth	Bacterial and fungal contamination	-
Sundried	AIA	No growth	Bacterial and fungal contamination	-
Sundried	SCA + CHL+ICZ	Poor growth	<i>Aspergillus niger</i> contamination restricted to corner of plates, but other fungi is inhibited	2x10 ⁻⁵
Sundried	AIA +CHL +ICZ	Poor growth	<i>Aspergillus niger</i> contamination; but other fungi is inhibited	2x10 ⁻⁵
Wet treatment				
Air dried at RT + heated in water bath for 60°C	SCA + CHL+ICZ	Good	<i>Aspergillus niger</i> contamination; but other fungi is inhibited	4x10 ⁻⁵
Air dried at RT+ heated in water bath for 60°C	AIA +CHL +ICZ	Good	<i>Aspergillus niger</i> contamination; but other fungi is inhibited	4x10 ⁻⁵
Sundried + heated in water bath for 60°C	SCA + CHL+ICZ	Poor	Less fungal contamination still growth is poor	2x10 ⁻⁵
Sundried+ heated in water bath for 60°C	AIA +CHL +ICZ	Poor	Less fungal contamination still growth is poor	2x10 ⁻⁵

Chemical enrichment				
Sample enriched with CaCO ₃ for 7 days and air dried	SCA + CHL+ICZ	Better	Less contamination of other microbes	6x10 ⁻⁵
Sample enriched with CaCO ₃ for 7 days and air dried	AIA+ CHL+ICZ	Better	Less contamination of other microbes	5x10 ⁻⁵

Note: RT= Room Temperature, SCA = Starch Casein Agar, AIA = Actinomycete Isolation Agar, CHL = Chloramphenicol, ICZ = Itraconazole.

Table 4: *Selective isolation of actinomycetes from floral compost*

Pretreatment of sample	Selective Media	Growth of actinomycetes	Growth of other microbes	CFU/gm of dried sample
No treatment	SCA	No growth	Bacterial and fungal contamination	-
No treatment	AIA	No growth	Bacterial and fungal contamination	-
No treatment	SCA + CHL+ICZ	No distinct actinomycetes appear	No bacteria; fungal contamination	-
No treatment	AIA + CHL +ICZ	No distinct actinomycetes appear	No bacteria; fungal contamination	-
Dry treatment				
Air dried at RT	SCA	No growth	Bacterial and fungal contamination	-
Air dried at RT	AIA	No growth	Bacterial and fungal contamination	-
Air dried at RT	SCA + CHL+ICZ	Poor	Fungal contamination	1x10 ⁻⁵
Air dried at RT	AIA +CHL +ICZ	Poor	Fungal contamination	1x10 ⁻⁵
Sundried	SCA	Poor	Bacterial and fungal contamination	-
Sundried	AIA	Poor	Bacterial and fungal contamination	-
Sundried	SCA + CHL+ICZ	Poor	Aspergillus niger contamination restricted to corner of plates, but other fungi is inhibited	2x10 ⁻⁵
Sundried	AIA +CHL +ICZ	Poor	Aspergillus niger contamination; but other fungi is inhibited	2x10 ⁻⁵
Wet treatment				
Air dried at RT + heated in water bath for 60°C	SCA + CHL+ICZ	Poor	Aspergillus niger contamination; but other fungi is inhibited	3x10 ⁻⁵
Air dried at RT+ heated in water bath for 60°C	AIA +CHL +ICZ	Poor	Aspergillus niger contamination; but other fungi is inhibited	2x10 ⁻⁵
Sundried + heated in water bath for 60°C	SCA + CHL+ICZ	Poor	Less fungal contamination still growth is poor	2x10 ⁻⁵
Sundried+ heated in water bath for 60°C	AIA +CHL +ICZ	Poor	Less fungal contamination still growth is poor	2x10 ⁻⁵

Chemical enrichment				
Sample enriched with CaCO ₃ for 7 days and air dried	SCA + CHL+ICZ	Better	Less contamination of other microbes	8x10 ⁻⁵
Sample enriched with CaCO ₃ for 7 days and air dried	AIA+ CHL+ICZ	Better	Less contamination of other microbes	5x10 ⁻⁵

Note: RT= Room Temperature, SCA = Starch Casein Agar, AIA = Actinomycete Isolation Agar, CHL = Chloramphenicol, ICZ = Itraconazole.

Table 5: Selective isolation of actinomycetes from floral vermicompost

Pretreatment of sample	Selective Media	Growth of actinomycetes	Growth of other microbes	CFU/gm of dried sample
No treatment	SCA	No growth	Fluorescence <i>Pseudomonas</i> sp. and other bacteria, no fungi	-
No treatment	AIA	No growth	Fluorescence <i>Pseudomonas</i> sp. and other bacteria, no fungi	-
No treatment	SCA + CHL+ICZ	No distinct actinomycetes appear	Fluorescence <i>Pseudomonas</i> sp., no other bacteria and fungi	-
No treatment	AIA + CHL +ICZ	No distinct actinomycetes appear	Fluorescence <i>Pseudomonas</i> sp., no other bacteria and fungi	-
Dry treatment				
Air dried at RT	SCA	No growth	Fluorescence <i>Pseudomonas</i> sp.growth inhibiting fungi. No other bacterial contamination	-
Air dried at RT	AIA	No growth	Fluorescence <i>Pseudomonas</i> sp.growth inhibiting fungi. No other bacterial contamination	-
Air dried at RT	SCA + CHL+ICZ	No growth	Fluorescence <i>Pseudomonas</i> sp. growth inhibiting <i>Aspergillus spp.</i> No other bacteria and fungi	-
Air dried at RT	AIA +CHL +ICZ	No growth	Fluorescence <i>Pseudomonas</i> sp.growth inhibiting <i>Aspergillus spp.</i> No other bacteria and fungi	-
Sundried	SCA	No growth	Fluorescence <i>Pseudomonas</i> sp. growth inhibiting fungi. No other bacterial contamination	-
Sundried	AIA	No growth	Fluorescence <i>Pseudomonas</i> sp. growth inhibiting fungi. No other bacterial contamination	-
Sundried	SCA + CHL+ICZ	No growth	Fluorescence <i>Pseudomonas</i> sp.	-
Sundried	AIA +CHL +ICZ	No growth	Fluorescence <i>Pseudomonas</i> sp.	-

Wet treatment				
Air dried at RT + heated in water bath for 60°C	SCA + CHL+ICZ	No growth	Fluorescence <i>Pseudomonas</i> sp.	-
Air dried at RT+ heated in water bath for 60°C	AIA +CHL +ICZ	No growth	Fluorescence <i>Pseudomonas</i> sp.	-
Sundried + heated in water bath for 60°C	SCA + CHL+ICZ	No growth	Fluorescence <i>Pseudomonas</i> sp.	-
Sundried+ heated in water bath for 60°C	AIA +CHL +ICZ	No growth	Fluorescence <i>Pseudomonas</i> sp.	-
Chemical enrichment				
Sample enriched with CaCO ₃ for 7 days and air dried	SCA + CHL+ICZ	Better	Less contamination of other microbes	8x10 ⁻⁵
Sample enriched with CaCO ₃ for 7 days and air dried	AIA+ CHL+ICZ	Better	Less contamination of other microbes	7x10 ⁻⁵

Note: RT= Room Temperature, SCA = Starch Casein Agar, AIA = Actinomycete Isolation Agar, CHL = Chloramphenicol, ICZ = Itraconazole.

In case of vermicompost of floral waste however, growth of actinomycetes was only achieved with enrichment of sample with CaCO₃ (Table 4). Colony color of actinomycetes strains isolated was categorized into puffy white, grayish brown, brownish, brownish black, grey centered white (Fig. 8). Actinomycetes colonies were identified through microscopical techniques showing filamentous hyphae and spores morphology. All the isolates were showing spore chain morphology and were identified as *Streptomyces* sp (Fig.9). In case of floral vermicompost, the colonies of actinomycetes appeared only after CaCO₃ enrichment (Table 5). In other pretreatment methods there was dominance of *Pseudomonas* sp. with fluorescence appearance (Fig 6). The presence of *Pseudomonas* was confirmed when purified on nutrient agar and SCA. Both the plates turned green within 24 hrs incubation and oxidase test was found to be positive as per Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). It was found that these colonies have secreted some volatile substances when plates were under incubation for 7-8 days and have killed the *Aspergillus* sp. and other fungi like colonies (Fig 7). A kind of pungent smell was detected on these agar plates. Only in case of soil sample 10⁻⁶ dilution was preferable to get countable colonies. Rest in other cases dilution up to 10⁻³ gave expected results.



Figure 3: Pretreated sample (Physical) – actinomycetes grown on plate



Figure 4: Pretreated sample (CaCO₃ enrichment)



Figure 5: Pretreated sample (CaCO_3 enrichment showing better growth of actinomycetes and more in number

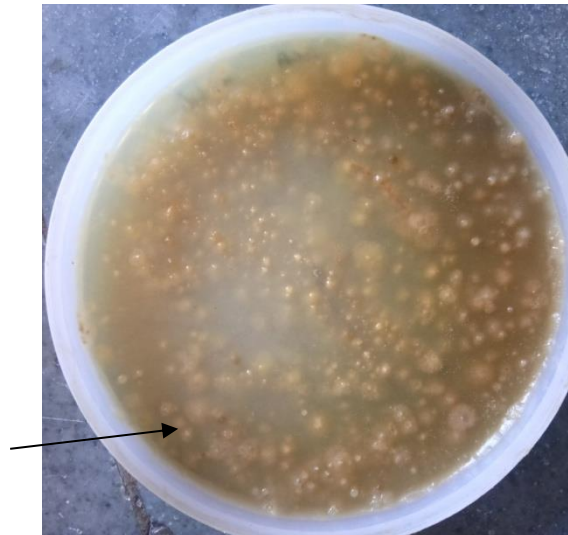


Figure 6: Greenish colony of fluorescent *Pseudomonas* sp. covers the whole plate.



Figure 7: Fluorescent *Pseudomonas* killed fungus after 7 days

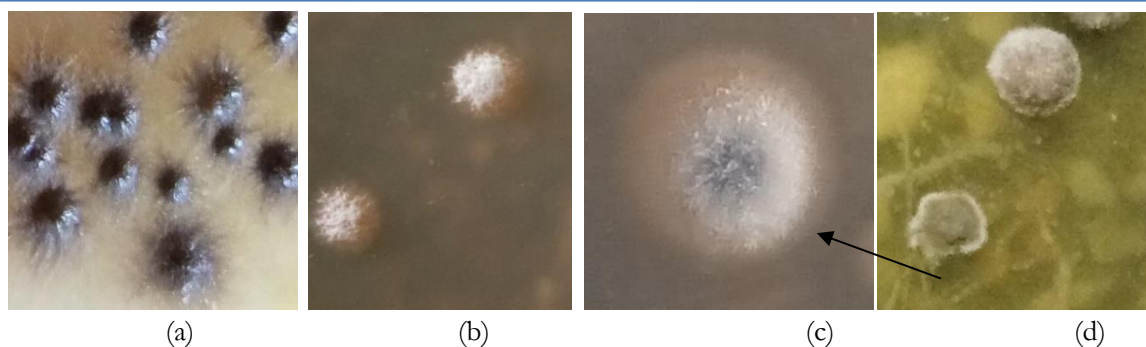


Figure 8: Color of some of the purified colonies a) Blackish brown b) white puffy c) grayish centered white d) chalky brownish



Figure 9: Microscopic view of some of the actinomycetes isolates showing spore chain morphology

In the entire above cases SCA agar and AIA agar gave almost same results. However, *Aspergillus* sp. contamination was more in AIA agar than SCA. Total 12 isolates were obtained with different colour colony. All the 12 presumed actinomycetes isolated were coded as ACT 1-12 and tested under microscope under 10X and 100X showed filaments and spore chains characteristics.

4 Discussions

From the above results it is clear that pretreatment of sample and growth of actinomycetes depends on choice of substrate or source from which sample was collected (Khama *et al.*, 2011; Chavan *et al.*, 2013). In case of soil since it constitutes mixed flora in large quantity both wet and moist treatment required to achieve isolation of actinomycetes. In case of cow dung compost a different type of black colored colonies was observed of filamentous nature. It is better achieved in air dried sample than in sun dried one. This may be due to sun drying the mesophilic actinomycetes growth has been restricted since compost was maintained in moist condition and room temperature around 30-32 C. So, this may have provided good favorable niche for mesophilic actinomycetes to grow. In case of floral waste, the growth was achieved well only when enriched with CaCO₃. This may be due to rare actinomycetes genera inhabiting the niche in the maturing phase of composting and thus need to be enriched for their isolation from mixed population of microbes (Hayakawa, 2008). Vermicompost of floral waste on the other hand showed the presence of a type of *Pseudomonas* sp. Colonies appears fluorescent and transparent same as color of media in 24 hours and turns to green in next 24 hrs with pungent smell. The similar results were observed from vermicompost reported by Sequeria and Chandrashekhar, 2015. Involvement of HCN produced by fluorescent *Pseudomonas* in disease suppression by induction of plant resistance has been reported by Voisard *et al.*, 1981. HCN production by *Pseudomonas fluorescens* is reported to suppress black root rot of tobacco (Hass *et al.*, 1991). The suppression of *Aspergillus* and other fungi in our case by fluorescent *Pseudomonas* sp. may be

due production of HCN. However, the presence of such species only in vermicompost samples may be due to the presence of such species in digestive tract of earthworms (Vega and Victoria, 2009) which later have come out of earthworms along with the castings and might have multiplied rapidly as compared to slow growing actinomycetes and thus dominated the other flora and same results were appeared on agar medium too. On chemical enrichment the production of volatile substance by *Pseudomonas* sp. might have inhibited due to change in pH and thus it might have favored the isolation of actinomycetes. Talking about dilution factor only in case of soil sample 10^{-6} dilution was preferable to get countable colonies. Rest in other cases dilution up to 10^{-3} gave expected results. This may be due abundance of micro flora in soil in vegetative or spore forms constituting gram negative, gram positive bacteria, fungi, actinomycetes hence to selectively isolate actinomycetes from soil 10^{-6} dilution is preferable to get countable colonies else it will give massive growth on less dilution factor. However, in case of composts the dominance of actinomycetes mainly occurs at later stages since there is succession of microbial colonies; bacteria, fungi, actinomycetes (Rebollido *et al.*, 2008), hence dilution factor up to 10^{-3} can give expected results. Higher dilution factor in this case did not give good results. During the whole experiment except in case of vermicompost samples which shows fluorescent *Pseudomonas* colonies rest all samples though on pretreatment and enrichment showed actinomycetes colonies on media supplemented with antibiotics still they were not able to inhibit the growth of fungi completely. There is mainly contamination of *Aspergillus* sp. This may be due to resistance of some of the fungal species to these antifungal drugs. Growth of non-actinomycetes bacteria was however completely inhibited. Hence use of antibiotics can reduce the number of fungi but cannot completely suppress them.

5 Conclusion

From the present study we can conclude that selective isolation of actinomycetes can be done effectively by combining pretreatment either physical or chemical or both along with suitable media supplemented with specific antibiotics. The range of pretreatment methods can be applied depending on the substrate chosen in study. Though 80% of the actinomycetes comprise *Streptomyces* and can be easily isolated and pretreatment is mainly required to isolate rare genera of actinomycetes as mentioned by many researchers. But from our study we conclude that a consolidated approach is needed for better results. One should remember that our study was quantitative in nature therefore, there is a possibility of different ways of pretreatment methods might have favored the repeated isolation of same types of actinomycetes. This can be judged simply by morphological characteristics and microscopy. The isolates with distinct morphology after purification and microscopic characteristics can be selected for further studies. The study also demonstrated that not only soils but other niches such as manure composts, floral waste composts, vermicomposts etc. can be utilized for isolation of actinomycetes as an addition source for the screening of novel bioactive compounds.

6 Declarations

6.1 Acknowledgement

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6.2 Competing Interests

The authors have no conflicts of interest to declare.

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