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# Genetic Diversity of Cellulolytic *Streptomyces* spp. Isolated from Decomposing Waste

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**ABSTRACT: *Streptomyces* have been reported to exhibit significant genomic divergence and unique metabolic gene content. Present study was designed to explore the genetic diversity in cellulolytic *Streptomyces* from same natural ecological niche. Nineteen *Streptomyces* spp. cultures were isolated from decomposing rural and urban wastes collected from different parts of Western Maharashtra. Random amplified polymorphic DNA (RAPD) tool was used for assessment of genetic diversity between cellulolytic *Streptomyces* isolates. Genomic DNA from 19 isolates and one reference strain were amplified using 16 random primers. The PCR amplification products of the isolates with different primers produced 507 bands out of which 323 were polymorphic, while 127 were unique. Electrophoretic and cluster analysis of the amplification products revealed incidence of polymorphism among the isolates and none of them was identical to the reference strains although there were some common amplification bands. The average polymorphism observed was 98.22%. The genetic similarity index based on the pooled data of RAPD profiles ranged from 0.19 to 0.70. The dendrogram constructed from the pooled data of *Streptomyces* spp. had two major groups showing wide genetic diversity between isolates. The results highlights RAPD as an efficient method for discriminating and studying genetic diversity among *Streptomyces* isolates. The protocol was found useful to detect similarities and differences in different isolates but it is not possible to gain further information on the biochemical parameters which are correlated with diversity amongst each other. Further genomic research will help in understanding the actual differences in biochemical parameters, enzyme production levels and functional potential within closely related population.**

**Keywords:** *Streptomyces*, genetic diversity, RAPD-PCR, decomposing waste.

## INTRODUCTION

The decomposition process is carried out by various microorganisms including bacteria, fungi and actinomycetes. Different communities of microorganisms predominate during the various composting phases. Initial decomposition is carried by mesophilic microorganisms primarily which include bacteria, which rapidly biodegrade the soluble and easily degradable compounds. As temperature increases on oxidation of carbon compounds, thermophiles take over. During active phase of composting temperature in a compost typically follows a pattern of rapid increase for several weeks, in which easily degradable compounds and oxygen are consumed. As the active composting phase subsides, temperature gradually declines to around 38°C and mesophilic microorganisms including fungi and actinomycetes take over the other types of microorganisms and the curing phase begins (Fourti *et al*., 2008). Apart from several bacteria and fungi, actinomycetes plays a active role in decomposition process. Some *Streptomyces* species participate in the degradation of biopolymers such as cellulose, hemicelluloses or lignocellulose (Amore *et al*., 2012; Li *et al*., 2012).

*Streptomyces* have been reported to play an important role in the carbon cycle and plant biomass degradation in soils (Book *et al.*, 2016). Genomes of *Streptomyces* have revealed several carbohydrate-active enzymes (CAZy), which include cellulases, hemicellulases, and lyticpolysaccharide monooxygenases (Pinheiro *et al.*, 2016). In a mesocosm, cellulose and lignin inputs increased *Streptomyces* density more than glucose (Schlatter *et al*., 2009). In addition to carbon cycling, Constant *et al.* (2010) showed that, in *Streptomyces* the presence of a high affinity hydrogenase is involved in

dihydrogen uptake. Apart from that, the role of *Streptomyces* is well known in agriculture. They are effective colonizers of rhizosphere and rhizoplane tissues with the potential to enhance plant growth resulting in yield boost (Dias *et al*., 2017). *Streptomyces* spp. promote plant growth through the production of phytohormones like auxins, cytokinins, and gibberellins, additionally they produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase whose activity is important in the suppression of plant stress (Sadeghi *et al*., 2012; Verma *et al*., 2011). They also play active role in phosphate solubilization (Jog *et al*., 2014) and siderophore production (Verma *et al*., 2011) as well.

*Streptomyces* are Gram-positive filamentous bacteria of the actinomycetal order with more than 600 described species ubiquitous in soils, sediments and seawater (Labeda *et al*., 2012). These organisms are one of the most abundant genera in soils and recently have been identified living in symbiotic relationships with a wide variety of eukaryotic hosts (Aylward *et al*., 2014). They grow as a filamentous hyphal mass, often in low nutrient environments. While *Streptomyces* have been extensively investigated for their ability to produce antibiotic compounds, relatively little is known about the distribution and evolution of plant biomass degtradation across this, or any, genus of bacteria (Bibb, 2005; Hopwood *et al*., 1995). *Streptomyces* have been reported to exhibit significant genomic divergence and unique metabolic gene content (Chevrette *et al*., 2019). Reports suggest that within *Streptomyces* lineages, diversification of metabolism can overlap with divergence which leads to variation in metabolic capacity even within identical species (Antony-Babu *et al*., 2017; Choudoir *et al*., 2018). Many *Streptomyces* isolates have been described as having functions that influence their environment, e.g. biogeochemical cycles or in the community structure of other microbes. However, studies generally focus on a single or few isolates considered the most efficient after a single function screening (Da Vinha *et al*., 2011). Thus, less information is available regarding function variation between *Streptomyces* isolates of the same natural population.

The work presented here aimed specifically to isolate the cellulolytic *Streptomyces* from decomposing rural and urban wastes and to analyze the similarities and differences of these isolates according to their RAPD-PCR fingerprints, and to compare RAPD clustering with the conventional phenotypic grouping. It is well documented in the literature that changing in the ecological niche or geographical region there is a definite genetic diversity at genus level, species level, etc. But, it is interesting to study genetic diversity at genus / species level from the same ecological niche. Hence, present study was designed to explore the diversity in cellulolytic *Streptomyces* isolated from the rural and urban wastes with the help of molecular tools.

## MATERIAL AND METHODS

Cellulolytic actinomycetes were isolated on starch casein agar medium from 34 samples of decomposing rural and urban wastes collected from different parts of Western Maharashtra. Cultures were screened for cellulase activity and the efficient 19 cellulolytic actinomycete isolates were selected for further studies. The selected isolates were identified as *Streptomyces* species on the basis of gram reaction, acid fast staining, mycelium colour and spore chain morphology, as per description given by Waksman (1961); Lechevalier (1989); Anonymous (1957); Cappuccino and Sherman (1992).

The genetic diversity among ninteen cellulolytic *Streptomyces* isolates along with the reference strain (BNF/AS-41) was analyzed by employing random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. Initially, the pure culture of *Streptomyces* spp. were grown in LB broth for late exponential phase and centrifuged to collect cell pellets. Genomic DNA was isolated by following the methodology of Stahl and Flesher (1987) with few modifications. The DNA pellet was dried and suspended in 100 µl of TE buffer. Quantification of DNA was done by gel electrophoresis and NanoDrop ND-1000 USA, UV visible spectrophotometer. The purified genomic DNA samples from the individual isolates were diluted to working concentration of 20 ng/µl and used as template DNA. The DNA sequences of sixteen RAPD primers used for DNA amplification are given in Table 1.

One primer at a time was used to study polymorphism within each group of selected *Streptomyces* isolates with genomic DNA extracts from all the strains as template DNA. The PCR amplification was done as per following profiles: predenaturation at a temperature of 94°C for 5 min, followed by 40 cycles of denaturation stages at a temperature of 94°C for 1 min, annealing at a temperature of 36°C for 1 min, elongation at 72°C for 2 minutes, and finalizing at a temperature of 72°C for 10 minutes. Twenty µl of the amplified products from each tube along with 4 µl of loading dye were separated on 1.2 per cent agarose gel using 1x TBE buffer of pH 8.0 along with l00 bp DNA ladder as DNA molecular weight marker. Electrophoresis was performed for 3 hrs at 80 volts in a submarine electrophoresis apparatus. The gel was stained and photographed using gel documentation system.

The amplification profiles for all the primers were compared with each other and the bands of DNA fragments were scored as present (1) or absent (0) generating the binary matrices. The binary data was analyzed under the SIMQUAL module of NTSYS PC-2.0 software programme (Rohlf, 1998) using DICE coefficient (Nei and Li, 1979). A dendrogram based on the UPGMA clustering method (SAHN) (Sneath and Sokal 1973) was used to generate a tree. Principle coordinate analysis was performed to estimate the genetic distance between each group of the isolates by using NTSYS software. Then analyzing the product matrix by EIGEN to get

Eigenvectors and Eigenvalues, and finally getting its 2D scatter plot graph.

## RESULTS AND DISCUSSION

The genomic DNA of 20 strains of *Streptomyces* spp. was subjected to PCR amplification with 16 random primers. A total of 507 bands were produced with different primers, out of which 323 were polymorphic bands, 175 were unique while, nine were monomorphic (Table 1). The number of bands produced per primer varied from 24 to 43 with an average of 31.69 bands per primer. Average polymorphism observed was 98.22%. Maximum bands were produced by primers RBA-1 (43) while, least bands were observed with primer RBA-2 and RBA-7. Most of the primers produced polymorphic bands varied from 11 to 30. The amplified DNA fragment ranged from 173 to 2295 bp. The number of polymorphic loci amplified varied with different primers and isolates. The fingerprints of 16 random primers were converted into a binary matrix based on the presence of band marked as "1" and absence of band marked as "0". Genetic variation among different strains can be documented by using different molecular markers (Sabir *et al*., 2013). Genetic analysis using RAPD is attractive as no prior knowledge of the DNA sequence is available and primers are designed randomly with the sole constraint being GC contents. It permits simultaneous investigation of multiple loci in a single PCR reaction (Bhattacharya and Ranade 2001).

Though some of the isolates share common bands most of the isolates differed in their banding pattern. None of the *Streptomyces* sp. isolate share all the bands and were therefore genetically variable. Genetic similarity index was computed based on pooled data of RAPD profiles for *Streptomyces* as DICE coefficient (Dice, 1945; Nei and Li, 1979) using the similarity routine of NTSYS PC 2.0 software (Table 2). The genetic similarity index based on the pooled data of RAPD profiles from all the 16 primers ranged from 0.19 to 0.70. The highest genetic similarity index of 0.70 was between the isolates A-47 and A-42. The minimum genetic similarity index was in between isolates A-41 and BNF/AS-41 (0.19). From the genetic similarity coefficient, the results indicate the distinct diversity among *Streptomyces* isolates. Andam *et al*. (2016) reported that ancestral inter-species homologous recombination in *Streptomyces* contributes towards genetic diversity which is further influenced by phylogeography. High degree of variability of average nucleotide identity (ANI) and ortholog distribution have been observed within *Streptomyces*, even in strains found identical on the basis of 16S rRNA gene homology (Chevrette *et al*., 2019). The results of the present study also corroborate these findings explaining the genetic diversity of *Streptomyces*. The findings of the study by Kumar *et al. (*2023) have also shown that there is a high level of diversity within *Streptomyces* spp. present in the agriculture fields of Tamil Nadu i.e. from same ecological niche.

The dendrogram representing the genetic relationship among isolates based on RAPD analysis was developed and presented in figure 1. On the basis of RAPD analysis, *Streptomyces* isolates were broadly classified into five groups, while the dendrogram constructed from pooled data has two major groups. The group I consisted of 6 subgroups. The subgroup Ia consisted of A-4 while, subgroup Ib consisted of isolate A-18 and BNF/AS-41 strain having similarity coefficient 0.56. The subgroup Ic consisted of isolates A-24, A-38, A-40 and A-35, subgroup Id consisted of isolates A-42, A-47, A-52, A-46 and A-62. The subgroup Ie consisted of single isolate A-22, while the subgroup If consisted of two isolates A-17 and A-48 with genetic similarity coefficient 0.36. The group II consisted 3 subgroups. Subgroup IIa consisted of isolates A-15 and A-41 with genetic similarity coefficient 0.35, subgroup IIb consisted of two isolates A-20 and A-63 with similarity coefficient 0.48 while subgroup IIc consisted single isolate A-29. Similar grouping was also observed in 2D scatter plot (Fig. 2). The isolates A-18, A-24, A-38, A-40, A-35, A-42, A-47, A-52, A-46, A-62 and BNF/AS-41 were genetically closely related in dendrogram and also represented in 2D scatter plot graph. The isolates A-63 and A-20 were also graphically closely represented in 2D scatter plot graph. While the remaining isolates were distinctly placed on the graph. From the genetic similarity coefficient, it is observed that the isolates showed significant genetic diversity among each other.

Similar results were reported by Malkawi *et al*. (1999) who used 10 primers to identify and assess genetic diversity among *Streptomyces* isolates and two reference strains. Cluster analysis revealed incidence of polymorphism among the isolates and none of them was identical to reference strain although there were some common amplification bands. Boroujeni *et al*. (2012) screened the actinomycetes isolates with 20 different primers. The dendrogram showed that the tested isolates fell into one super cluster which consisted of three clusters and one out group. Bontemps *et al*., (2013) also showed that a large diversity of *Streptomyces* exists in the investigated forest soil and that it constitutes an interesting reservoir for describing new species. Singh *et al*. (2022) reported that high genetic diversity is responsible for multifarious bioactive compound production by the *Streptomyces* isolates and tremendous genetic diversity of the *Streptomyces* isolates from Meghalaya forms the underlying base of extraordinarily metabolic diversity.

**Table 1: RAPD analysis of *streptomyces* isolates.**

| Primer | Primer sequence | Total No. of bands | No. of monomorphic bands | No. of unique bands | No. of polymorphic bands | % poly - morphism |
|---|---|---|---|---|---|---|
| RBA 1 | 5’ AAA ACC GGG C 3’ | 43 | 0 | 15 | 28 | 100.00 |
| RBA 2 | 5’ ACA GGG CTC T 3’ | 24 | 0 | 10 | 14 | 100.00 |
| RBA 3 | 5’ ACA GGG GTG T 3’ | 33 | 0 | 13 | 20 | 100.00 |
| RBA 4 | 5’ ACC GGG TTT C 3’ | 30 | 0 | 7 | 23 | 100.00 |
| RBA 5 | 5’ AGG GGC GGC A 3’ | 34 | 1 | 3 | 30 | 97.05 |
| RBA 6 | 5’ ATC CTG CCT G 3’ | 33 | 0 | 13 | 20 | 100.00 |
| RBA 7 | 5’ ATC GGG TCC T 3’ | 24 | 2 | 9 | 13 | 91.67 |
| RBA 8 | 5’ ATC GGG TCG A 3’ | 32 | 0 | 10 | 22 | 100.00 |
| RBA 9 | 5’ ATC TGC GAG C 3’ | 34 | 0 | 12 | 22 | 100.00 |
| RBA 10 | 5’ CCC GCC TTC C 3’ | 38 | 1 | 11 | 26 | 97.37 |
| RBA 11 | 5’ CCG GCC CCA A 3’ | 29 | 0 | 12 | 17 | 100.00 |
| RBA12 | 5’ CCG GCC TTA A 3’ | 28 | 1 | 12 | 15 | 96.43 |
| RBA 13 | 5’ CCG GCC ATA C 3’ | 29 | 1 | 12 | 16 | 96.55 |
| RBA 14 | 5’ CCG GCC TTC C 3’ | 30 | 2 | 5 | 23 | 93.33 |
| RBA 15 | 5’ CCG GCT GGA A 3’ | 37 | 1 | 13 | 23 | 97.30 |
| RBA 16 | 5’ CCG GGG AAA C 3’ | 29 | 0 | 18 | 11 | 100.00 |
| **Total** | | **507** | **9** | **175** | **323** | **98.22** |

**Table 2: Genetic similarity as DICE coefficient based on pooled data of RAPD profiles for *Streptomyces* spp.**

| | A-4 | A-15 | A-17 | A-18 | A-20 | A-22 | A-24 | A-29 | A-35 | A-38 | A-40 | A-41 | A-42 | A-46 | A-47 | A-48 | A-52 | A-62 | A-63 | BNF/AS-41 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A-4** | 1.00 | | | | | | | | | | | | | | | | | | | |
| **A-15** | 0.30 | 1.00 | | | | | | | | | | | | | | | | | | |
| **A-17** | 0.35 | 0.34 | 1.00 | | | | | | | | | | | | | | | | | |
| **A-18** | 0.46 | 0.27 | 0.31 | 1.00 | | | | | | | | | | | | | | | | |
| **A-20** | 0.21 | 0.31 | 0.26 | 0.29 | 1.00 | | | | | | | | | | | | | | | |
| **A-22** | 0.37 | 0.24 | 0.27 | 0.39 | 0.31 | 1.00 | | | | | | | | | | | | | | |
| **A-24** | 0.31 | 0.25 | 0.24 | 0.43 | 0.24 | 0.42 | 1.00 | | | | | | | | | | | | | |
| **A-29** | 0.26 | 0.30 | 0.30 | 0.32 | 0.35 | 0.33 | 0.27 | 1.00 | | | | | | | | | | | | |
| **A-35** | 0.42 | 0.29 | 0.29 | 0.56 | 0.27 | 0.39 | 0.52 | 0.25 | 1.00 | | | | | | | | | | | |
| **A-38** | 0.41 | 0.27 | 0.31 | 0.58 | 0.26 | 0.45 | 0.64 | 0.24 | 0.62 | 1.00 | | | | | | | | | | |
| **A-40** | 0.40 | 0.27 | 0.31 | 0.54 | 0.26 | 0.43 | 0.63 | 0.27 | 0.61 | 0.62 | 1.00 | | | | | | | | | |
| **A-41** | 0.24 | 0.35 | 0.33 | 0.25 | 0.31 | 0.25 | 0.27 | 0.27 | 0.27 | 0.29 | 0.29 | 1.00 | | | | | | | | |
| **A-42** | 0.42 | 0.25 | 0.31 | 0.54 | 0.28 | 0.40 | 0.57 | 0.23 | 0.58 | 0.61 | 0.56 | 0.29 | 1.00 | | | | | | | |
| **A-46** | 0.37 | 0.30 | 0.32 | 0.48 | 0.26 | 0.38 | 0.47 | 0.26 | 0.52 | 0.58 | 0.64 | 0.28 | 0.64 | 1.00 | | | | | | |
| **A-47** | 0.36 | 0.24 | 0.29 | 0.51 | 0.23 | 0.37 | 0.53 | 0.22 | 0.62 | 0.64 | 0.61 | 0.24 | 0.70 | 0.68 | 1.00 | | | | | |
| **A-48** | 0.26 | 0.27 | 0.36 | 0.42 | 0.29 | 0.29 | 0.33 | 0.29 | 0.37 | 0.39 | 0.39 | 0.31 | 0.38 | 0.36 | 0.38 | 1.00 | | | | |
| **A-52** | 0.44 | 0.27 | 0.36 | 0.48 | 0.25 | 0.42 | 0.50 | 0.28 | 0.51 | 0.55 | 0.55 | 0.29 | 0.66 | 0.65 | 0.67 | 0.32 | 1.00 | | | |
| **A-62** | 0.39 | 0.25 | 0.30 | 0.45 | 0.24 | 0.35 | 0.48 | 0.22 | 0.42 | 0.48 | 0.45 | 0.29 | 0.63 | 0.60 | 0.56 | 0.32 | 0.63 | 1.00 | | |
| **A-63** | 0.22 | 0.30 | 0.28 | 0.22 | 0.48 | 0.31 | 0.26 | 0.33 | 0.23 | 0.26 | 0.26 | 0.34 | 0.30 | 0.29 | 0.28 | 0.30 | 0.30 | 0.25 | 1.00 | |
| **BNF/AS-41** | 0.36 | 0.25 | 0.27 | 0.56 | 0.23 | 0.39 | 0.51 | 0.25 | 0.50 | 0.58 | 0.49 | 0.19 | 0.57 | 0.53 | 0.56 | 0.35 | 0.54 | 0.57 | 0.23 | 1.00 |

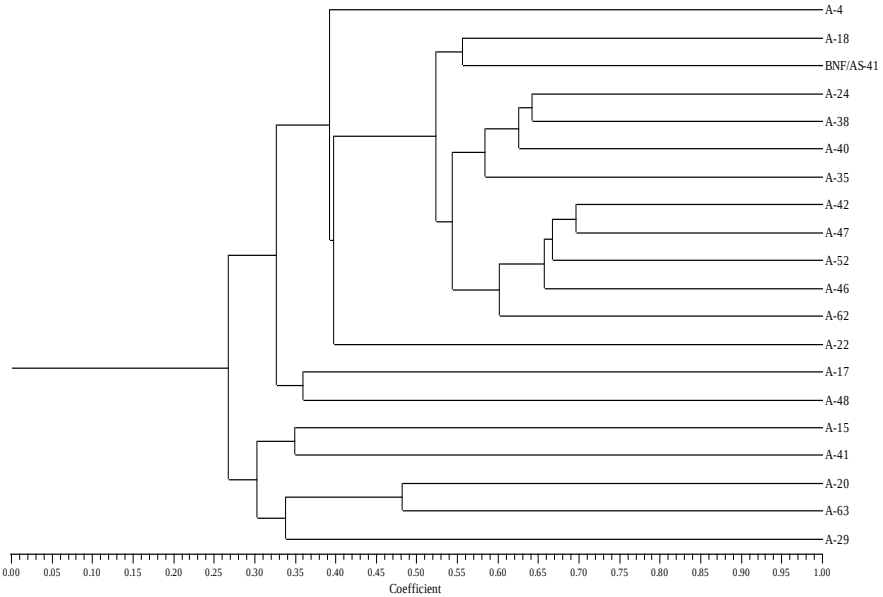


**Fig. 1.** Dendrogram representing the clustering among different isolates of *Streptomyces* spp.

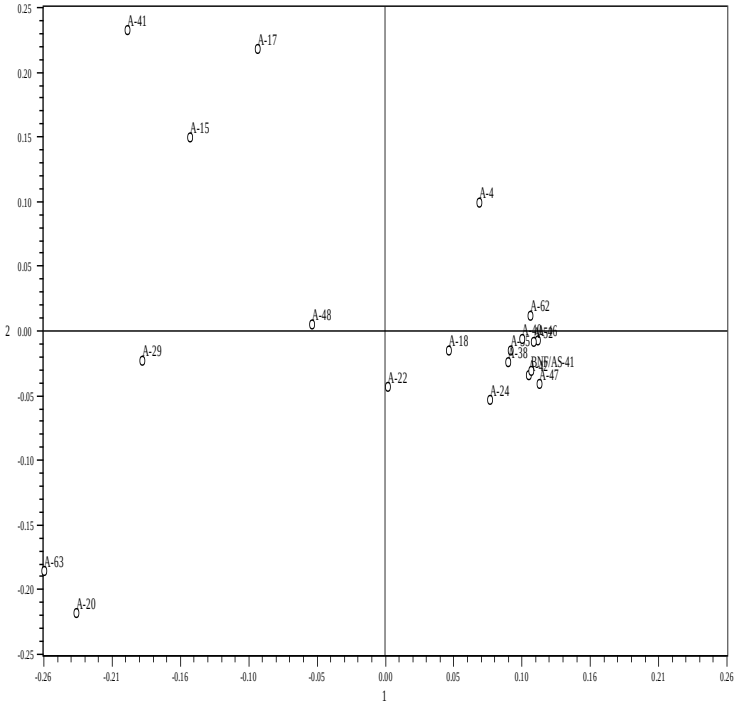


**Fig. 2.** RAPD 2D PCO scatter plot representing the phylogenetic relationship among different isolates of *Streptomyces* spp.

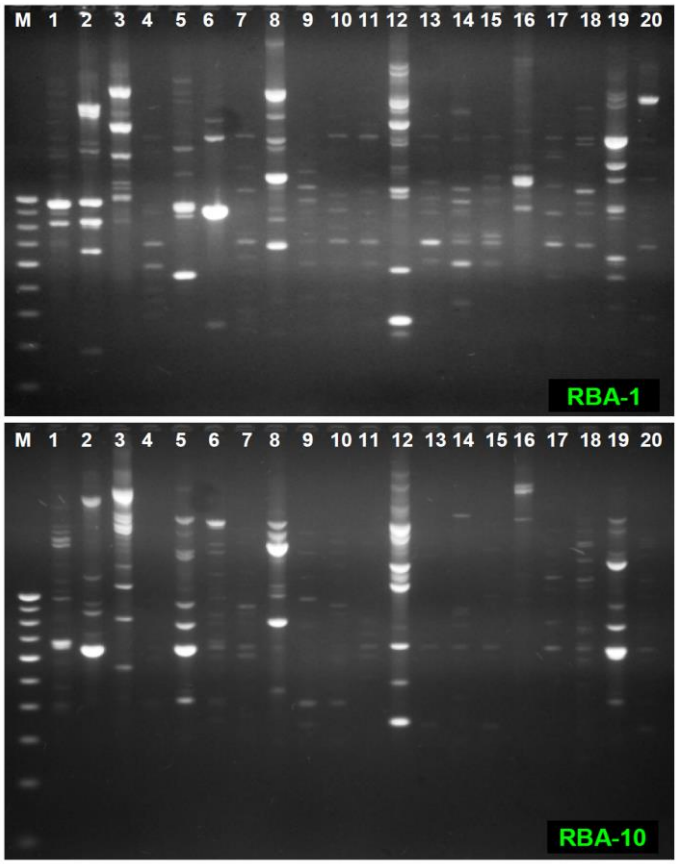


**Plate 1. Amplification profiles of different isolates of *Streptomyces* spp. with RBA-1 and RBA-10 primer**

**Lane M : Marker 100 bp (StepUp Low Range DNA Ladder)**
***Streptomyces* spp. isolates:1. A-4 2. A-15 3. A-17 4. A-18 5. A-20**
**6. A-22 7. A-24 8. A-29 9. A-35 10. A-38 11. A-40 12. A-41 13. A-42**
**14. A-46 15. A-47 16. A-48 17. A-52 18. A-62 19. A-63. 20. BNF/AS-41**

## CONCLUSIONS

There was a significant genetic diversity among *Streptomyces* isolates at genus level though they were from same ecological niche, may be due to the genetic differences in the strains due to differences at species level, differential expression of genes or diversification of metabolism. RAPD PCR protocol was found useful to detect similarities and differences in different

isolates. Data generated by RAPD might help in further genomic research on differences in enzyme production levels within closely related population. Further studies can be correlated with the functional potential and genomic diversity within same group of closely related microorganisms.

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**Conflict of Interest.** None.

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