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MOLECULAR VARIABILITY IN *STREPTOMYCES SPP.* A CAUSAL ORGANISM OF COMMON SCAB OF POTATO (*SOLANUM TUBEROSUM* L.)

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ABSTRACT
Common scab is an important disease of potato caused by *Streptomyces scabies* and other closely related species. Twenty seven cultures from different regions of Varanasi district were isolated and tested for their pathogenicity. Only Thirteen isolates were able to cause disease on potato tubers. Data on DNA fingerprinting was generated from genomic DNA extracted from all the 13 isolates of *Streptomyces*. Based on the genomic data generated by gel electrophoresis of BOX-PCR product, ERIC-PCR product and REP-PCR product and scored 0 and 1 either band present or not, dandograms of 13 isolates of *Streptomyces* are prepared by using NTSYS pc 2.02e. All isolates categorized into different clusters at based on 50 % similarity coefficient and sub clusters at based on 75 % similarity coefficient. A combined data of genetic diversity were presented including BOX, ERIC and REP-PCR by using UPGMA. It reveals that the all 13 isolates of *Streptomyces* divided into 10 clusters at 50% similarity coefficient. It indicates that genetic diversity of *Streptomyces* isolates are not affected by agro-climatic conditions and cultivars of potato crops. Perhaps the genetic diversity may obtain due to mutation in DNA sequence of individual isolate of bacteria.

INTRODUCTION

Potato suffers from a number of diseases of varied origins, which are responsible for decrease in yield and its quality. Among the bacterial disease of potato, Common scab of potatoes is caused by *Streptomyces scabies*, a very prevalent, soil-inhabiting bacterium. This serious disease can be found in all potato- growing areas throughout the world. The major loss from common scab is lower market quality because tubers are unsightly or disfigured and have poor customer appeal. Where scab is severe, yields also may be reduced. Due to its detrimental effect on appearance, grade and marketable yield, common scab of potato has been considered a disease of major economic importance (King *et al.*, 1992).

The taxonomy of potato-scab-causing *Streptomyces* spp. has been studied by many microbiologists on the bases of numerical analysis of phenotypic characteristics (Foucher *et al.*, 1995), fatty acid and protein profile analyses (Paradis *et al.*, 1994), DNA–DNA hybridization (Healy and Lambert, 1991; Bouchek-Mechiche *et al.*, 2000) and 16S rRNA gene sequence analysis (Takeuchi *et al.*, 1996; Kreuze *et al.*, 1999). Recently, the number, size and sequences of 16S-23S ITS regions were used for discrimination of *Streptomyces albidoflavus* strains (Hain *et al.*, 1997), genetic analysis of the genus *Nocardioides* (Yoon *et al.*, 1998) and clarification of the relationship between members of the family *Thermomonosporaceae* (Zhang *et al.*, 2001).

Repetitive elements (REP) polymerase chain reaction (PCR), which provides a more general representation of the inter and intra-species variability, is another useful approach for strain characterization and discrimination. By amplifying DNA found between repetitive elements dispersed throughout the genome, such as enterobacterial repetitive intergenic consensus (ERIC) and BOX elements, REP-PCR generates a strain-specific band profile. Careful and thorough characterization of pathogens and their pathogenicity and virulence-related genes is a prerequisite to the development of any successful control strategy.

So, the objective of this paper is to assess the genetic variability of *Streptomyces spp.* causing common scab of potato.

MATERIALS AND METHODS

The research work was conducted at the Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi and Molecular Variability was estimated at Laboratory of Division of Plant Pathology, IARI, New Delhi. Isolation of Pathogen was made by the techniques of Lawrence (1956) and Loria & Davis (1989). To test the pathogenicity in pots, the method described by Labruyere (1971) and Loria and Davis (1989) was followed. For Diagnosis of Genetic Variability DNA extraction was carried out by CTAB Method of Saghai-Maroof *et al.* (1984).

REP-PCR Fingerprinting

DNA extraction and REP-PCR amplification was done for all pathogenic isolates. For each strain REP-PCR is carried out by ERIC, REP and BOX primer. The primer sequence is given by Schaad *et al.*, 2001. PCR-based amplification of purified DNA

was carried out in a 25 μL reaction mixture. The reaction mixture contained 20ng DNA templates, Taq Polymerase, 25mM dNTPs, 25mM $MgCl_2$, 5X PCR buffer (Shah MM, Yen Y, Gill KS, 2000) and 2mM Primer (Himedia).

PCR condition

REP-PCR was performed in the thermo cycler (BIO_RAD, C1000™ Thermal cycler), under the following conditions as initial incubation of 95^{0}C for 7 min, followed by 30 cycles of 94^{0}C for 1 min and 53^{0}C for 1 min, 46^{0}C for 1 min, 48^{0}C for 1 min respectively for BOX-PCR, ERIC-PCR, and REP-PCR and 65^{0}C for 8 min. Lastly, final extension cycle at 65^{0}C for 15 min and a final waiting temperature of 8^{0}C (Pradiprao et al., 2015; Singh et al., 2013).

Gel Electrophoresis of PCR products

PCR amplified fragments were separated on agarose gel using 1X TAE (1X TAE prepared from a 50X TAE buffer stock solution that includes per litre 242g Tris Base, 57.1ml glacial acetic acid and 18.61g Na_2EDTA). PCR amplified product loaded into the well by combining with the 4μl of 6X Loading dye and 1 Kb ladder was also included. The Gel was run in 1X TAE for 6 h at a constant voltage of 100V at room temperature. After the running of gel, the gel was photographed using the gel documentation system (Singh *et al.,* 2013).

Analysis of the fingerprinting data

The digital image of each gel was analyzed. The normalized data generated from BOX, ERIC and REP fingerprinting profiles were used for generating similarity matrix by using SIMQUAL module for the NTSYS pc 2.02e. The similarity matrix thus generated was used for cluster analysis by unweighted pair group method of arithmetic average (UPGMA) using sequential, agglomerative, hierarchial, nested clustering module of NTSYS pc. The output data were graphically presented as a phylogenetic tree.

RESULTS AND DISCUSSION

Out of the 27 isolates, only thirteen (48%) were found to be pathogenic. This indicates that not only pathogenic ones but also saprophytic ones whose numbers may be more in the lesions of infected tubers. The same trend was also reported by Labruyere (1971) and Loria and Davis (1989).

Data on DNA fingerprinting was generated from genomic DNA extracted from all the 13 isolates of *Streptomyces*. DNA

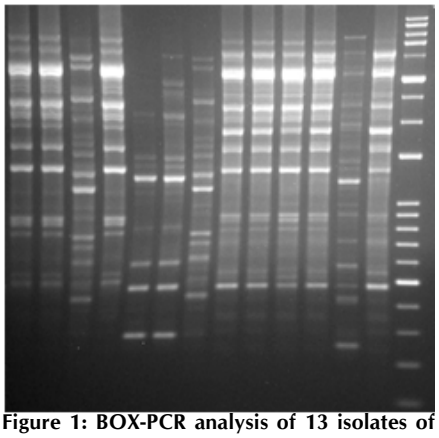

Figure 1: BOX-PCR analysis of 13 isolates of *Streptomyces*: Lane 1-13 (S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9, S_{10}, S_{11}, S_{12} and S_{13}), 1kb DNA Ladder

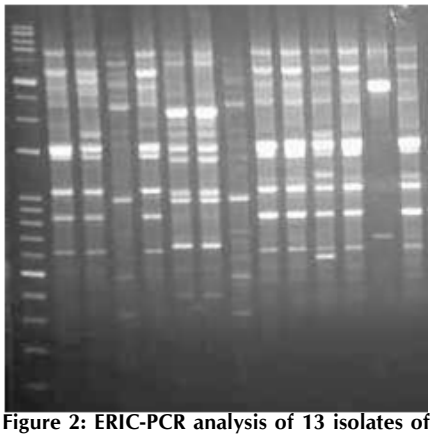

Figure 2: ERIC-PCR analysis of 13 isolates of *Streptomyces*: 1kb DNA Ladder, lane 1-13 (S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9, S_{10}, S_{11}, S_{12} and S_{13})

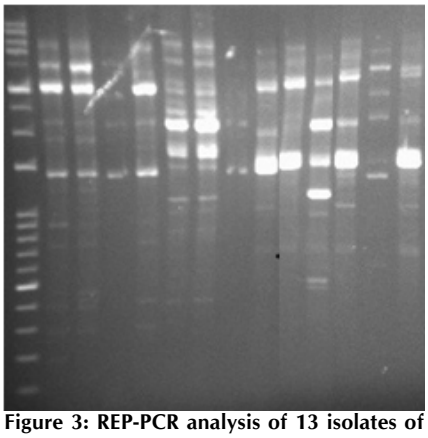

Figure 3: REP-PCR analysis of 13 isolates of *Streptomyces*: 1kb DNA Ladder, lane 1-13 (S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9, S_{10}, S_{11}, S_{12} and S_{13})

Table 1: Number of Bands obtained from *Streptomyces*

Isolates	No. of bands obtained from Streptomyces		
	BOX-PCR	ERIC-PCR	REP-PCR
S_1	9	7	8
S_2	9	7	8
S_3	9	6	4
S_4	9	7	7
S_5	5	7	8
S_6	8	8	8
S_7	7	4	2
S_8	9	8	6
S_9	9	8	5
S_{10}	9	9	8
S_{11}	9	8	7
S_{12}	12	3	5
S_{13}	7	8	5
Total number of amplicons in each method of fingerprinting	111	90	81
Range of amplicons produced in Streptomyces	5-12	3-9	2-8

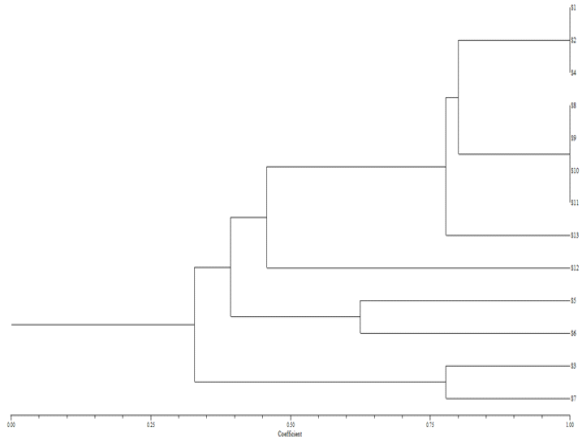

Figure 4: Cluster analysis of *Streptomyces* isolates for BOX-PCR: unweighted paired group mathematical average (UPGMA) dandograms were generated using Pearson's coefficient. The significant of each branch is indicated by the bootstrap percentage calculated for 1000 subsets

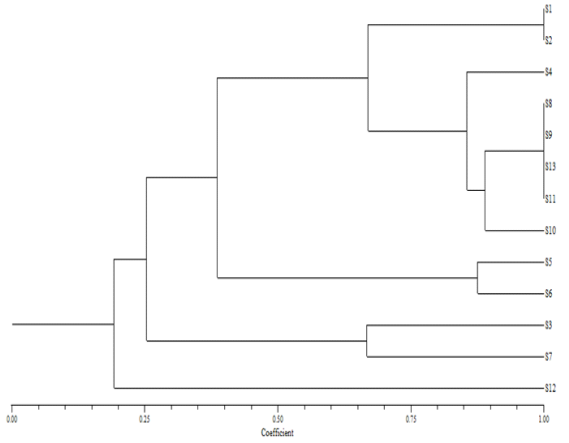

Figure 5: Cluster analysis of *Streptomyces* isolates for ERIC-PCR: unweighted paired group mathematical average (UPGMA) dandograms were generated using Pearson's coefficient. The significant of each branch is indicated by the bootstrap percentage calculated for 1000 subsets

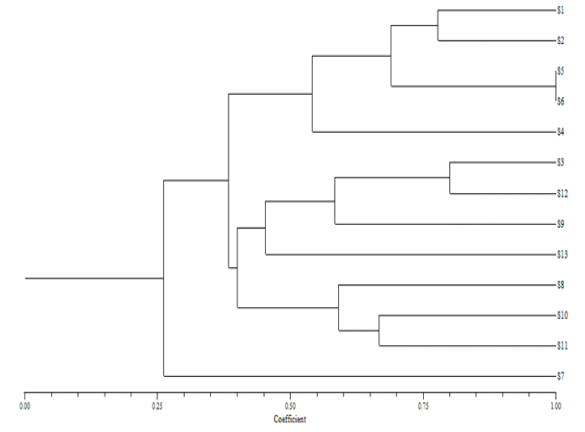

Figure 6: Cluster analysis of *Streptomyces* isolates for REP-PCR: unweighted paired group mathematical average (UPGMA) dandograms were generated using Pearson's coefficient. The significant of each branch is indicated by the bootstrap percentage calculated for 1000 subsets

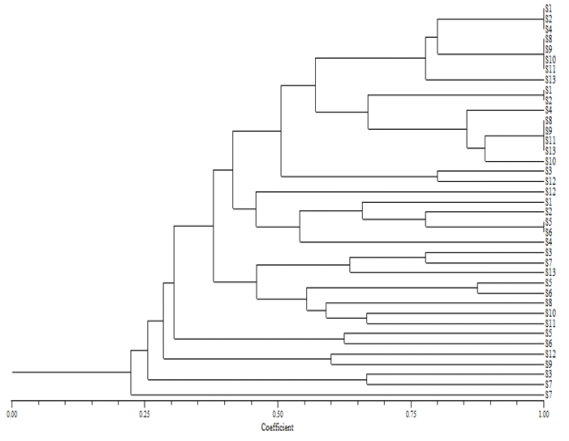

Figure 7: Combined cluster analysis of Streptomyces isolates for BOX, ERIC and REP-PCR: unweighted paired group mathematical average (UPGMA) dandograms were generated using Pearson's coefficient. The significant of each branch is indicated by the bootstrap percentage calculated for 1000 subsets

fragments of 300 base pair to 7kb were amplified in BOX-PCR, 350 base pair to 5kb in ERIC-PCR and 400bp to 8kb in REP-PCR and reveal a high level of genetic diversity among the isolates of *Streptomyces*. Maximum number of amplicons were found in BOX-PCR (111 amplicons) followed by ERIC-PCR (90 amplicons) and REP-PCR (81 amplicons) (Table-1). Amplification in each isolates of *Streptomyces* varied in all three method of PCR as amplicon 5-12 in BOX-PCR, 3-9 in ERIC-PCR and 2-8 in REP-PCR. In BOX-PCR maximum 12 fragments of amplicons were found in S_{12}. Based on the genomic data generated by gel electrophoresis of BOX-PCR product and scored 0 and 1 either band present or not, dandograms of 13 isolates of *Streptomyces* are prepared by using NTSYS pc 2.02e. All isolates categorized into 4 groups at based on 50 % similarity coefficient (Fig. 4). Further, the isolates were grouped into 5 sub clusters namely A, B, C, D and E at 75 % similarity coefficient. In sub cluster A 8 isolates of *Streptomyces* were in group and in sub cluster B 1 isolate, in sub cluster C 1 isolate, in sub cluster D 1 isolate and in sub cluster E 2 isolates were in group. In ERIC-PCR maximum 9 fragments of amplicons were found in S_{10} (Table-1). All isolates were categorized broadly into four clusters 1, 2, 3 and 4 at 50% similarity coefficient. At 75% similarity coefficient maximum isolates were group under cluster 1, which were further sub clustered into 2 sub group A and B. In REP-PCR maximum 8 fragments of amplicons were found in the isolates S_1, S_2, S_5, S_6 and S_{10}. All isolates were categorized into 4 groups at 50% similarity coefficient (Fig. 6). Further the isolates were grouped into 10 sub clusters namely A, B, C, D, E, F, G, H, I and J at 75% similarity coefficient. In sub cluster A, B and D 2 isolates were in group and in remaining sub clusters 1 isolate was in group. Maximum genetic diversity was recorded in

REP-PCR technique. A combined data of genetic diversity were presented in Fig. 7 including BOX, ERIC and REP-PCR by using UPGMA. It reveals that the all 13 isolates of *Streptomyces* divided into 10 clusters, cluster A, B, C, D, E, F, G, H, I and J at 50% similarity coefficient. Maximum number of isolates belong to cluster A followed by cluster D and F. it indicates that genetic diversity of *Streptomyces* isolates are not affected by agro-climatic conditions and cultivars of potato crops. Perhaps the genetic diversity may obtain due to mutation in DNA sequence of individual isolate of bacteria (Onge et al., 2008).

Genetic diversity of plant pathogenic bacteria has been studied by DNA based approaches to generate evidence of genome plasticity, ecological distribution and dispersal and evolution. The data obtain from genetic variability in this study confirms previous finding heterogeneity within *Streptomyces* strains (Wanner, L. A. 2006; Onge *et al.,* 2008; Sarwar *et al.*, 2016). For genomic variability REP-PCR finger printing using primer sets (REP, ERIC and BOX) are highly conserved repetitive sequences showed different banding patterns among *Streptomyces*. The analysis clearly reveals the potential of the REP-PCR to cluster the strains of *Streptomyces* to their pathogenic behavior. Fingerprinting of *Streptomyces* was done since it is faster cheaper and easily more discriminative than other techniques like EFG and AFLP. The unique fingerprinting profile generated by REP-PCR could be useful tool in diagnosis and differentiation of strain without a data base.

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