Comparative analysis of genetic diversity among fluorescent pseudomonads using RAPD and ISSR fingerprinting

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Abstract

The genetic diversity of fluorescent pseudomonads associated with the turmeric (Curcuma longa L.) rhizosphere of Tamil Nadu, India was analyzed. Selected isolates were identified by ITS sequence analysis. Based on 16S rRNA sequence similarity, the isolates were identified as Pseudomonas fluorescens, P. stutzeri and Pseudomonas sp. The genetic variability and relationship among 15 fluorescent pseudomonads were analysed using 15 Random Amplified Polymorphic DNA (RAPD) and 13 Inter-Simple Sequence Repeat (ISSR) markers.

Results revealed that ISSR markers were more efficient than the RAPD assay with regards to polymorphism detection. Also, total amplicons, the average number of polymorphic loci per primer, average polymorphic information content (PIC), marker index (MI) values and effective multiplex ratio (EMR) were more for ISSR than for RAPD. The similarity coefficients of fluorescent pseudomonads based on RAPD and ISSR markers ranged from 50.00 to 75.00 and 49.00 to 85.00 per cent respectively. Overall, we concluded that ISSR was found to be better than RAPD in assessing genetic diversity among fluorescent pseudomonads.

Keywords: Fluorescent pseudomonads, Genetic diversity, ISSR, RAPD, 16S rRNA sequence, Turmeric ecosystem.

Introduction

Beneficial free-living rhizosphere bacteria are generally referred as plant growth-promoting rhizobacteria (PGPR). Rhizosphere harbours a diverse array of microorganisms which show beneficial, neutral or detrimental effects on plant growth. Among the various PGPRs identified, fluorescent pseudomonads are of the most extensively studied rhizobacteria because of growth-promoting activity and antagonistic action against plant pathogens[1-5]. In addition to this, some fluorescent pseudomonads also produce antibiotics and are able to degrade or transform variouspesticides and soil organic pollutants. Because of the relative ease by which they can be cultured and their common association with plant root and leaf surfaces, there has been considerable interest in identifying the bacterial species and study their genetic diversity[6-10].

Variation in bacteria has been traditionally characterized on the basis of growth, cultural and biochemical characteristics. These methods are time-consuming, highly influenced by environment and thus are not very precise. To date, various molecular markers have been introduced to assess the genetic diversity among the isolates such as AFLP, DAMD, ISSR, ITS and RAPD. Among these, randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) are widely applicable because they are rapid, inexpensive, simple to perform, do not require prior knowledge of DNA sequence and require very little starting DNA template[11].

Thus, in order to assess the genetic diversity in fluorescent pseudomonads, PCR-based RAPD and ISSR-DNA fingerprinting are the best tools. RAPD primers are considered as random primers. ISSR primers are derived from an arbitrary nucleotide sequence of di and trinucleotide repeats with 5' or 3' anchoring sequence of few nucleotides to prevent strand-slippage. These nucleotide repeats are based on the presence of simple sequence repeats (SSRs) that are distributed throughout the genomes. ISSR markers have the potential to amplify the larger number of polymorphic fragments per primer than RAPD. In this investigation, objectives of the present study were: 1) to detect the genetic diversity and polymorphism among fluorescent pseudomonads isolates and 2) to estimate the relative efficiencies of both RAPD and ISSR markers.

Material and Methods

Fluorescent pseudomonads: The fluorescent pseudomonads were isolated from turmeric rhizosphere soil samples of Tamil Nadu, India. Kings' B (KB) medium was used for the isolation. The isolates were confirmed according to the description given in Bergey's manual of systematic bacteriology[12]. *P. fluorescens* strains FP7 and Pf1 were obtained from the Culture Collection Section, Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. These isolates were maintained at -80 ^{0}C with 50% glycerol.

DNA Extraction: *Pseudomonas* cultures were grown in 5 ml KB broth with 10% glycerol (v/v) for 72 h at 27°C. Eppendorf tube of 1.5 ml was used to centrifuge the cells at 13,000 rpm for 5 min and the pellet was suspended in 200 µl Tris 0.1 mol L^{-1} and added with 200 µl of lysis solution (NaOH 0.2N and 1% SDS), mixed and deproteinized with 700 µl of phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v). It was then homogenized and centrifuged for 10 min at 13,000 rpm. Top

layer containing bacterial DNA was taken without disturbing the bottom layer and it was transferred to 1.5 ml microcentrifuge tube. To this, 700 µl of ice-cold 95% ethanol was added to precipitate the DNA and spun. Final washing was given with 70% ethanol and centrifuged at 8000 rpm for 5 min. Precipitated DNA was dried at room temperature and suspended in 100 µl of water. The samples were electrophoresed on 0.8% agarose gels stained with ethidium bromide and photographed under UV light.

ITS – PCR: To confirm isolates as *Pseudomonas* sp. 16S-23S rRNA intervening sequence ITS1F (5'-AAGTCGTAACAA GGTAG-3'); ITS2R (5'GACCATATATAACCCCAAG- 3') primers were used to get an amplicon size of 560 bp[13]. PCR reactions were carried out in 20 µl reaction mixture containing 10X buffer (with 2.5 mM $MgCl_2$), 2 µl; 2 mM dNTP mixture, 2 µl; 2 M primer, 5 µl; *Taq* DNA polymerase, 3 U; H_2O, 8 µl and 50 ng of template. DNA samples were amplified on DNA thermal cycler (Eppendorf Master Cycler Gradient, Westbury, New York) using the PCR conditions 92 ^{0}C for 4 min, 55 ^{0}C for 1 min and 72 ^{0}C for 2 min. The total number of cycles was 40 with the final extension time of 10 min. The PCR products were resolved on 2% agarose at 50 V stained with ethidium bromide (0.5 µg ml^{-1}) and photographed and analysed using gel documentation system (Alpha Innotech Corporation, San Leandro, California).

RAPD and ISSR: Out of 35 RAPD primers, 15 primers were selected to detect polymorphic RAPD bands among the fifteen isolates of fluorescent pseudomonads. Thirteen random primers were selected for ISSR Analysis. The PCR was carried out in a Mastercycler gradient. PCR for RAPD and ISSR analysis was carried out in 0.2 ml PCR tubes containing 50-80 ng genomic DNA, 10 µM of primer, 0.25 mM dNTP, 1.5 mM $MgCl_2$, 2 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India) and 1X PCR reaction buffer. For RAPD, DNA amplification was performed using the following parameters: 40 cycles of 94^0 C for 1 min, 36^0 C for 1 min and 72^0 C for 2 min[14].

For ISSR, Amplification conditions were: 94^0 C for 5 min; 40 cycles of 94^0 C for 30 s, 46-52^0 C for 1 min and 72^0 C for 1 min; followed by a final extension for 10 min at 72^0 C[15]. The integrity and yield of RAPD and ISSR products were analyzed by a 1.5 % (w/w) agarose gel. The PCR reactions were repeated at least three times to confirm the reproducibility of each PCR band.

Data Analysis: The banding patterns were scored for RAPD and ISSR primers in each fluorescent pseudomonads isolate starting from the small size fragment to large sized one. Polymorphic DNA bands were documented as presence (1) and absence (0). The genetic similarity coefficients between each isolate were calculated by the Numerical Taxonomy Multivariate Analysis System (NTSYS-PC), version 2.10 (Exeter Software, Setauket, New York) software package[16]. A dendrogram was constructed based on Jaccard's similarity coefficient using the marker data from the fluorescent pseudomonads with unweighted pair group method (UPGMA)[17].

For each primer, Polymorphism information content (PIC), Marker index (MI) and Assay efficiency index (AEI) was calculated. The PIC was calculated using the formula described by Anderson et al[18]:

$$PIC_j = 1 - \sum_{l=1 to L} P^2_{lj}$$

where P_{lj} is the relative frequency if the l^{th} allele for the locus j and is summed across all the alleles (L) over all lines.

PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed but also the relative frequencies of those alleles. PIC values may range from 0 (monomorphic) to 1 (very highly discriminative), with many alleles in equal frequencies. Genotypic gene diversity was calculated as described by Mariette et al[19]:

$$Hg = 1 - (p_i^2 - q_i^2)$$

where $p_i^2 - q_i^2$ are the frequencies of the dominant and null alleles, respectively. Here, allele frequencies were calculated based on the frequency of the null allele (i.e. the number of individuals without the band) where q_i represents the frequency of the null allele and p_i represents the frequency of the dominant allele,

$$q_i = \left\{ \frac{\text{No. of individuals for which the band was not present}}{\text{Total no. of individuals surveyed}} \right\}^{1/2}$$

$$p_i = 1 - q_i$$

Marker Index (MI) was determined as the product of PIC and the number of polymorphic bands per assay unit and EMR (E) is the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay[20].

$$EMR\ (E) = n_p\ (n_p / n)$$

where `n_p' is the number of polymorphic loci and n is the total number of loci.

Results

Isolates: A total of sixty fluorescent pseudomonads were isolated from turmeric growing areas of Tamil Nadu, India (Supplementary material). All the isolates were screened against *P. aphanidermatum*, the causal agent of rhizome rot pathogen of turmeric crop. Based on the screening results (data not shown), fifteen effective isolates were taken for genetic diversity studies (Table 1).

ITS region: The *Pseudomonas* genus specific primers amplified a fragment of approximately 560 bp corresponding to the region of the 16S-23S rRNA

intervening sequence for *Pseudomonas* sp. All the isolates showed amplification of 560 bp. Isolates were sequenced and deposited in NCBI, gene bank (Table 1).

RAPD analysis: In RAPD analysis, polymorphic fragments were generated in fluorescent pseudomonads by 15 oligonucleotides decamers. The size of the amplified products varied from 100 to 2500 bp. Of the 15 primers used, seven primers *viz.* OPA-01, OPA11, OPE-04, OPF-10, OPF-12, OPF-14 and R3 were found to show 100 per cent polymorphism. Of the 94 total alleles observed, 82 alleles were polymorphic and maximum numbers of 10 alleles were obtained with primer OPA-01 followed by primers OPA-11, OPF-10 and OPG-19 with 9 alleles. The higher PIC, MI and EMR were observed in OPA01 followed by OPF10 (Table 2).

The coefficient of genetic similarity ranged from 50 – 75 percent. The maximum similarity of 75 per cent was noticed between TPF 17 and TPF 18. The UPGMA clustering divided the isolates into two main groups I and II. The isolates TPF12, TPF13 and TPF14 were placed in one group with approximately 58 per cent similarity coefficient between them and TPF17, TPF18, TPF20, TPF33, TPF34, TPF35, TPF40, TPF41, TPF53, TPF54, FPF and Pf1 were grouped in another group which showed 50 percent similarity. In group II, there were two clusters namely A and B.

Cluster A was divided into sub-cluster C and D with approximately 52 percent similarity. In the case of cluster C, two sub-clusters namely E and F were noticed with 56 percent similarity. Sub-cluster F has two isolates TPF35 and TPF40 whereas, sub-cluster E had further cluster namely G and H. Sub cluster G had the isolates of TPF17, TPF18, TPF20 and TPF33. Sub-cluster H had the isolates of TPF34, TPF41, TPF54 and FP7 with 65 percent similarity (Fig. 1).

ISSR analysis: Totally 142 alleles were generated by 13 ISSR markers. Among that 133 were polymorphic bands and 9 were monomorphic bands. Clearly detectable amplified ISSR fragments ranged from 100 to 3500 bp in size. PIC was an average of 0.88 with a range of 0.82 by $(GA)_8T$ to 0.93 by $(ACTG)_4$ and $(AG)_8$ C. The maximum MI and EMR were observed in $(AG)_8$ C (Table 3). Jaccard's similarity coefficients among the fifteen isolates showed 49 to 85 % genetic similarity.

Maximum percentage of similarity (85.00 %) was observed among the strains of FP7 and Pf1 followed by TPF12 and TPF13 which showed 79.00 percent similarity. UPGMA cluster analysis showed two main distinct clusters, designated as I and II exhibiting overall genetic relationship among the isolates. Cluster I was subdivided into IA and IB. Cluster IA consisted of TPF12, TPF13 and TPF14. Cluster IB was further divided into sub-cluster IC and ID. Isolate TPF40 belongs to the sub-cluster ID. Sub-cluster IC consisted of TPF17, TPF18, TPF20, TPF33, TPF34 and TPF35. Cluster II was divided into IIA and IIB. Cluster IIA has further sub-cluster IIC and IID. Isolate TPF41 belongs to sub-cluster IIC and isolates TPF53 and TPF54 come under the sub-cluster IID. Cluster IIB had the strains of FP7 and Pf1 with 85 percent similarity (Fig. 2).

Table 1
Effective isolates of fluorescent pseudomonads from Tamil Nadu, India

S. N.	Strains	Place of isolation /Source	District	Species	Accession Number
1	TPF12	Chellapapalayam	Erode	*P. fluorescens*	KP887810
2	TPF13	Sathyamangalum	Erode	*P. fluorescens*	KP887811
3	TPF14	Sathyamangalum	Erode	*P. fluorescens*	KP887812
4	TPF17	Thondamuthur	Coimbatore	*P. fluorescens*	KP706448
5	TPF18	Thondamuthur	Coimbatore	*Pseudomonas* sp.	KP887816
6	TPF20	Chengam	Dharmapuri	*P. fluorescens*	KP887814
7	TPF33	Athur	Salem	*P. fluorescens*	KP887813
8	TPF34	Echangatore	Salem	*P. fluorescens*	KP714262
9	TPF35	Pichanoor	Salem	*Pseudomonas* sp.	KP887815
10	TPF40	Anangi	Theni	*P. stutzeri*	KP699579
11	TPF41	Anangi	Theni	*P. stutzeri*	KP887817
12	TPF53	Gobi	Erode	*P. fluorescens*	KR818037
13	TPF54	Nallur	Namakkal	*P. fluorescens*	KP714263
14	FP7	TNAU, Culture Collection Centre		*P. fluorescens*	GU797088
15	Pf1	TNAU, Culture Collection Centre		*P. fluorescens*	AY818674

Table 2
Polymorphism detected by RAPD markers

S. N.	Primers	Total allele	MB	PB	% MM	% PM	Allele range	Total amplicons	PIC	Genotypic gene diversity	MI	EMR	AEI
1.	OPA-01	10	0	10	0.00	100.00	250-2500	92	0.89	0.84	8.90	10.00	5.46
2.	OPA-11	9	0	9	0.00	100.00	250-2500	61	0.85	0.99	7.65	9.00	
3.	OPE-02	7	3	4	42.86	57.14	100-2000	55	0.80	0.97	3.20	2.29	
4.	OPE-04	8	0	8	0.00	100.00	100-1500	65	0.85	0.94	6.80	8.00	
5.	OPF-06	5	2	3	40.00	60.00	100-1500	42	0.77	0.91	2.31	1.80	
6.	OPF 7	4	1	3	25.00	75.00	100-1500	28	0.68	1.05	2.04	2.25	
7.	OPF 8	8	1	7	12.50	87.50	250-1500	64	0.81	0.95	5.67	6.13	
8.	OPF10	9	0	9	0.00	100.00	100-2500	75	0.86	0.92	7.74	9.00	
9.	OPF11	4	1	3	25.00	75.00	250-1000	28	0.67	1.09	2.01	2.25	
10.	OPF12	7	0	7	0.00	100.00	250-1500	49	0.83	0.83	5.81	7.00	
11.	OPF14	4	0	4	0.00	100.00	250-1000	27	0.73	0.69	2.92	4.00	
12.	OPG-19	9	2	7	22.22	77.78	100-2000	68	0.84	0.77	5.88	5.44	
13.	OPH-19	5	1	4	20.00	80.00	250-1500	50	0.78	0.92	3.12	3.20	
14.	R3	3	0	3	0.00	100.00	100-1000	24	0.62	0.95	1.86	3.00	
15.	Pgs2	2	1	1	50.00	50.00	250-1000	13	0.60	0.95	0.60	0.50	
Total		94	12	82	237.58	1262.42		741	11.58	13.77	66.51	73.85	
Mean		6.26	0.80	5.46	15.83	84.16		49.40	0.772	0.918	4.43	4.92	

Table 3
Polymorphism detected by ISSR markers

S. N.	Primers	Total allele	MB	PB	% MM	% PM	Allele range	Total amplicons	PIC	Genotypic gene diversity	MI	EMR	AEI
1.	$(CAG)_5$	13	0	13	0.00	100.00	250-2500	105	0.89	0.78	11.57	13.00	10.23
2.	$(GTG)_5$	13	0	13	0.00	100.00	100-3000	111	0.92	0.87	11.96	13.00	
3.	$(AGG)_5$	9	1	8	11.11	88.89	100-1000	45	0.87	0.79	6.96	7.11	
4.	$(GACA)_4$	12	2	10	16.67	83.33	250-2500	85	0.91	0.80	9.1	8.33	
5.	$(ACTG)_4$	14	0	14	0.00	100.00	100-2000	93	0.93	0.72	13.02	14.00	
6.	$(GACAC)_3$	11	0	11	0.00	100.00	250-2500	94	0.90	0.70	9.9	11.00	
7.	$(TGTC)_4$	10	2	8	20.00	80.00	250-2000	70	0.88	0.86	7.04	6.40	
8.	$(TCC)_5$	7	1	6	14.29	85.71	250-1000	61	0.83	0.82	4.98	5.14	
9.	$(CAG)_3$	10	0	10	0.00	100.00	250-2000	83	0.89	0.70	8.9	10.00	
10.	$(CAC)_5$	13	2	11	15.38	84.62	250-2500	87	0.90	0.69	9.9	9.31	
11.	$(AG)_8T$	8	0	8	0.00	100.00	100-1000	55	0.87	0.78	6.96	8.00	
12.	$(AG)_8C$	16	1	15	6.25	93.75	250-3500	110	0.93	0.79	13.95	14.06	
13.	$(GA)_8T$	6	0	6	0.00	100.00	250-1000	46	0.82	0.76	4.92	6.00	
Total		142	9	133	83.69	1216.30		1045	11.54	10.06	119.16	125.35	
Mean		10.92	0.69	10.23	6.43	93.56		80.38	0.88	0.77	9.16	9.64	

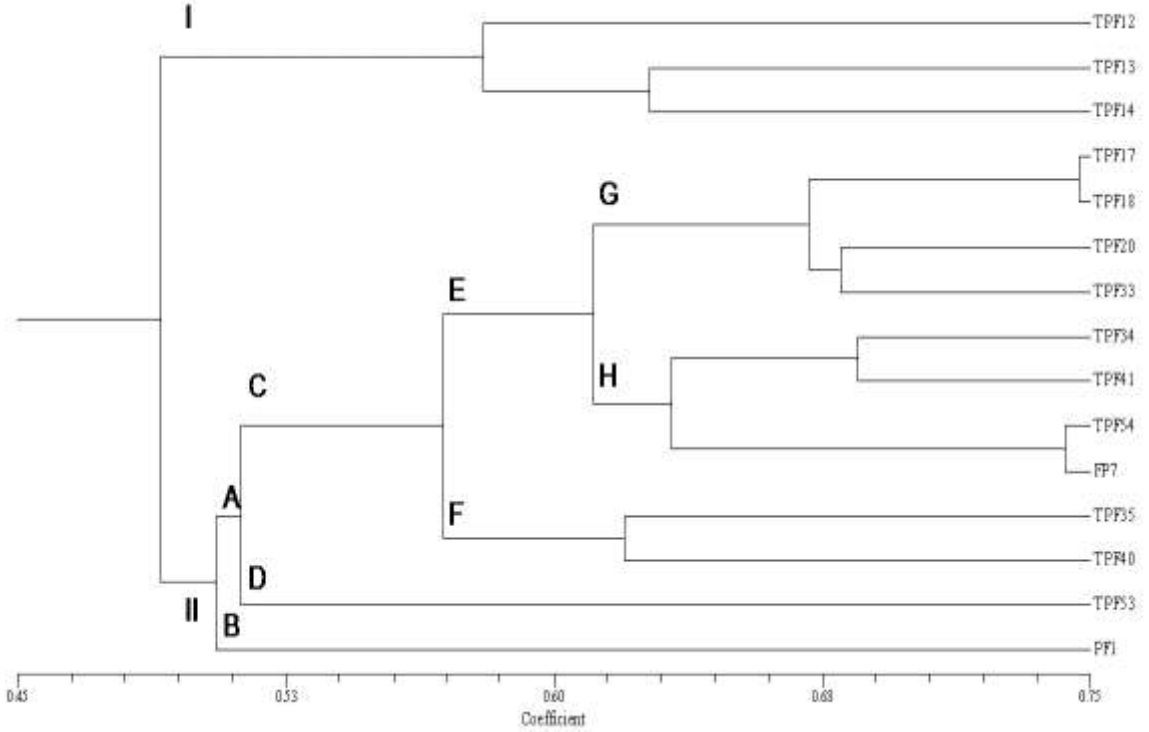

Fig. 1: UPGMA cluster analysis of fluorescent pseudomonads based on RAPD primers

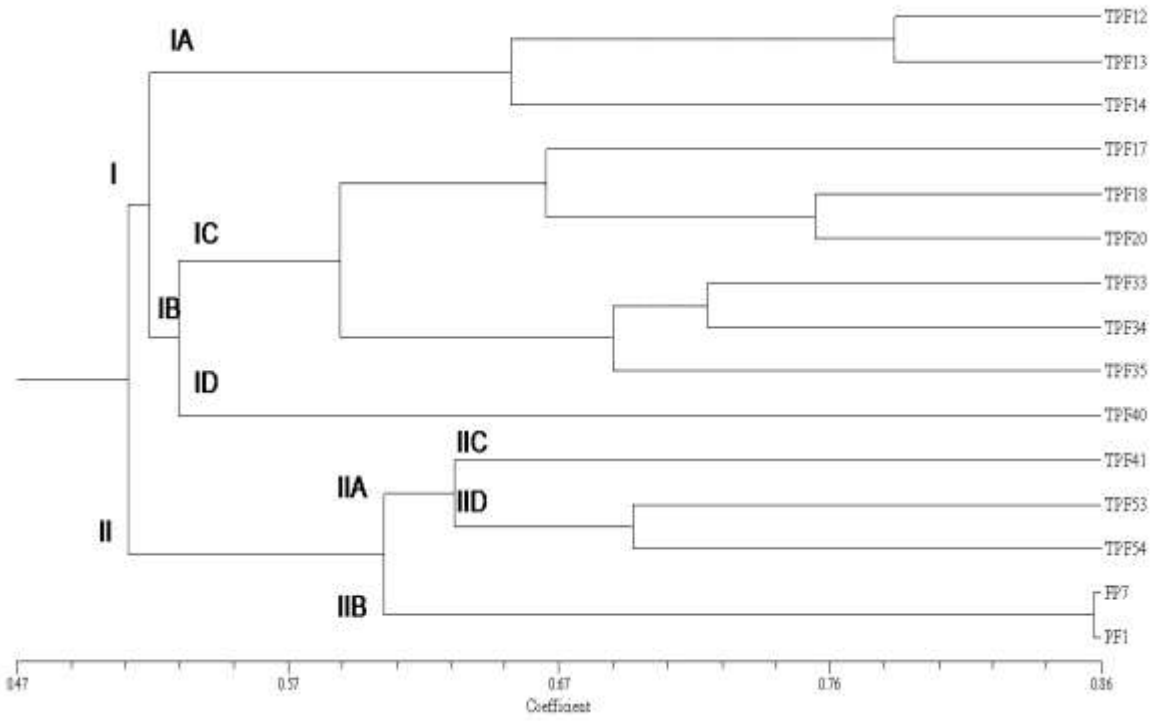

Fig. 2: UPGMA cluster analysis of fluorescent pseudomonads based on ISSR primers

Comparison of RAPD and ISSR marker systems: Various parameters were recorded as criteria to differentiate the RAPD and ISSR markers. ISSR primers yielded better results than RAPD in different criteria. The mean number of alleles per assay unit and number of polymorphic per assay unit in ISSR analysis was 10.92 and 10.23 respectively which was significantly superior over RAPD primers accounting 6.26 and 5.46 alleles. But a number of monomorphic per assay unit in ISSR markers (0.69) was less when compared to RAPD markers (0.80). The ISSR marker index (9.16) indicative of marker utility and mean polymorphic information content per assay (0.88) was superior to RAPD. It is due to ISSR's

higher effective multiplex ratio (9.64). The mean genotypic gene diversity was 0.91 for RAPD analysis, while for ISSR it was 0.77, despite the higher multiplex ratio and marker index. Further, the higher percentage of polymorphic bands was obtained from ISSR analysis (93.56%) as compared to 84.16% for RAPD (Table 4).

Table 4
Comparative analysis of banding patterns generated by RAPD and ISSR

S.N.	Components	RAPD	ISSR
1.	Number of assay units	15.00	13.00
2.	Total amplicons	741	1045
3.	Total number of alleles	94	142
4.	Mean number of alleles per assay unit	6.26	10.92
5.	Number of polymorphic bands per assay unit	5.46	10.23
6.	Mean (%) polymorphism per assay	84.16	93.56
7.	Number of monomorphic bands per assay unit	0.80	0.69
8.	Mean PIC per assay	0.77	0.88
9.	Mean genotypic gene diversity	0.91	0.77
10.	Mean marker Index (MI)	4.43	9.16
11.	Effective multiplex ratio	4.92	9.64
12.	Assay Efficiency Index	5.46	10.23

Supplementary material
List of fluorescent pseudomonads from turmeric ecosystem of Tamil Nadu, India

S. N.	Isolates*	Place	District	S. N.	Isolates*	Place	District
1	TPF 1	Puliyampatti	Erode	31	TPF 31	Rasipuram	Namakkal
2	TPF 2	Thandukaranpalayam	Erode	32	TPF 32	Athur	Salem
3	TPF 3	Thandukaranpalayam	Erode	33	TPF 33	Athur	Salem
4	TPF 4	Seyur	Erode	34	TPF 34	Echangatore	Salem
5	TPF 5	Kumarapalayam	Namakkal	35	TPF 35	Pichanoor	Salem
6	TPF 6	Seyur	Coimbatore	36	TPF 36	Thindal	Salem
7	TPF 7	Annur	Coimbatore	37	TPF 37	Mottalore	Salem
8	TPF 8	Annur	Coimbatore	38	TPF 38	Pichanoor	Salem
9	TPF 9	Nambiyur	Erode	39	TPF 39	Vellaikottai	Salem
10	TPF 10	Kavizhipalayam	Erode	40	TPF 40	Anangi	Theni
11	TPF 11	Karapatti	Erode	41	TPF 41	Anangi	Theni
12	TPF 12	Chellapapalayam	Erode	42	TPF 42	Bhavani	Erode
13	TPF 13	Sathyamangalum	Erode	43	TPF 43	Bhavani	Erode
14	TPF 14	Sathyamangalum	Erode	44	TPF 44	Perumanallur	Coimbatore
15	TPF 15	Madukarai	Coimbatore	45	TPF 45	Pongalur	Erode
16	TPF 16	Devarayapuram	Coimbatore	46	TPF 46	Pallipalayam	Namakkal
17	TPF 17	Thondamuthur	Coimbatore	47	TPF 47	Vadugapatti	Theni
18	TPF 18	Thondamuthur	Coimbatore	48	TPF 48	Bhavani	Erode
19	TPF 19	Pandiyampalayam	Coimbatore	49	TPF 49	Pallipalayam	Namakkal
20	TPF 20	Chengam	Dharmapuri	50	TPF 50	Gobi	Erode
21	TPF 21	Chengam	Dharmapuri	51	TPF 51	Sivagiri	Coimbatore
22	TPF 22	Azhangkattur	Erode	52	TPF 52	Seyur	Coimbatore
23	TPF 23	Vellankovil	Erode	53	TPF 53	Gobi	Erode
24	TPF 24	Kullumpalayam	Erode	54	TPF 54	Nallur	Namakkal
25	TPF 25	Kodumudi	Erode	55	TPF 55	Nallur	Namakkal
26	TPF 26	Bhavanisagar	Erode	56	TPF 56	Kovilpalayam	Coimbatore
27	TPF 27	Perundurai	Erode	57	TPF 57	TNAU orchard	Coimbatore
28	TPF 28	Narasipuram	Namakkal	58	TPF 58	Gobi	Erode
29	TPF 29	Gangaikovil	Erode	59	TPF 59	TNAU orchard	Coimbatore
30	TPF 30	Oonjalur	Erode	60	TPF 60	Bhavani	Erode

Isolates* - All the isolates were isolated from turmeric rhizosphere

Discussion

The present study has confirmed the fifteen isolates of *Pseudomonas* sp. using 16S-23S rRNA intervening sequences. ITS sequences helped in distinguishing the species in comparison of the sequences deposited in NCBI gene bank. These results were in agreement with Adhipathi[21] who characterized and confirmed the twenty strains of *Pseudomonas* based on the 16S-23S rRNA ITS region. Susilowati et al[22] distinguished the *Pseudomonas* sp. into seven groups based on 16S sequences. The differentiation of isolates belonging to fluorescent pseudomonads was achieved through several genomic DNA fingerprinting techniques. Among them, RAPD and ISSR are the most extensively used techniques to detect genetic variation among plants and micro-organisms[23,24].

Erdogan et al[25] reported the phylogenetic grouping of fluorescent pseudomonads in Turkey based on RAPD data and showed the similarity coefficient of 40 to 87 percent. Dibakar Pal et al[26] used nine ISSR primers to study the genetic diversity among 45 isolates of *Pseudomonas* sp. In this study, the potential ability of two molecular markers was tested to evaluate the genetic diversity among isolates. Various parameters have taken as criteria to distinguish the two marker systems. Results showed that the total amplicons, total alleles, percent polymorphism, primer information content (PIC), marker index (MI) and effective multiplex ratio (EMR) values were higher in ISSR as compared to RAPD marker.

Reports from the previous studies showed that RAPD produced less reliable bands than ISSR. The ISSR produce more complex marker patterns than RAPD method, which is more advantageous when differentiating closely related species[27,28]. Nagoaka and Ogihara[29] reported that ISSR primers are more informative than RAPD because of the higher percentage of polymorphic bands. Recently, Rayer et al[30] found that ISSR produced slightly better results in primer information content, marker index; percent polymorphism and average expected gene diversity (Hi) values than RAPD approach.

Cluster analysis of RAPD and ISSR marker profiles broadly grouped the fifteen isolates into two clusters. However, the formation of sub-clusters within the main cluster varied between RAPD and ISSR. These differences may be attributed to marker sampling errors and or the level of polymorphism detected, reinforcing again the importance of the number loci and their coverage in the genome of the isolates[30].The similarity coefficients of fluorescent pseudomonads based on fifteen RAPD markers and thirteen ISSR markers ranged from 50.00 to 75.00 and 49.00 to 85.00 per cent respectively. A possible explanation for the difference in resolution of RAPDs and ISSRs is that the two-marker techniques target different portions of the genome.

The ability to resolve genetic variation among different genotype may be more directly related to the number of polymorphisms detected with each marker technique rather than a function of which technique is employed[31]. Comparative studies in different species using various marker systems were successfully conducted by other researchers and it was concluded that ISSR would be a better tool than RAPD for phylogenetic studies[32]. It may be due to highly polymorphic, abundant nature of the microsatellites due to slippage in DNA replication[33]. But the combination of these two techniques will be better for comprehensive genetic analysis of fluorescent pseudomonads strains.

Conclusion

The present study has shown the usefulness of RAPD and ISSR for diversity analysis of fluorescent pseudomonads. The results from a comparison of two markers indicated that ISSR markers are better than RAPD in terms of revealing the genetic diversity. This information will improve the basic understanding of the genetic variability among fluorescent pseudomonads strains.

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