IJPSR (2018), Volume 9, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 April, 2017; received in revised form, 30 July, 2017; accepted, 02 August, 2017; published 01 January, 2018

PHENOTYPICALLY AND GENETICALLY VARIATION OF *Rif r* MUTANTS STRAINS OF *PSEUDOMONAS FLUORESCENCE* pf2T2 AND pf2m1

V. Priyadarshini, Sonamilli, S. Shruthipooja and N. Hariram*

Department of Biotechnology, Kalasalingam University, Krishnankoil - 626126, Tamil Nadu, India.

Keywords:

Rif r, pf2T2, Rhizosphere, NaCl, pH

Correspondence to Author:
N. Hariram

Department of Biotechnology, Kalasalingam University, Krishnankoil - 626126, Tamil Nadu, India.

E-mail: n.hariram@klu.ac.in

ABSTRACT: The present study was Rifampicin-resistant (*Rif r*) mutants of *Pseudomonas fluorescens* pf2T2 and pf2m1 were isolated from different localities of onion plant rhizosphere soil in an around the Tamil Nadu. However, the mutants of *P. fluorescens* were of two general categories. Group 1 showed fast growing in terms of growth rate, competitive fitness, and membrane protein composition. Group 2 showed a slower growth rate in both minimal and enriched media and an altered membrane protein profile. These mutants also demonstrated decreased competitive fitness compared with the wild-type strain based on the study of colony morphology, soil characters, different temperature, pH, NaCl (salt tolerant), and gram staining has been done. Although, the Rifampicin - resistant (*Rif r*) mutants of *Pseudomonas fluorescens* pf2T2 and pf2m1 were distinctly in the media.

INTRODUCTION: The beneficial microorganisms of onion plant rhizosphere soil have been proposed for biological control of soil borne crop. Bio-control of pathogenesis organism relies on this very fact in nature. In the biological management, different groups of organisms from viruses, bacteria, fungi and insects, depending on the target organisms are put into service for management of diseases. In agriculture, bacteria belonging to the genera of *Bacillus* and *Pseudomonas* have shown effectiveness in the bio-management of different crops. Among the bio-control bacteria, fluorescent *Pseudomonas* has become the bacterium of the choice for its versatility and ability to contain a large number of plant pathogens in diverse target environments.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(1).188-96

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(1).188-96

The management of soil borne pathogens has become one of the major concerns in onion plant field. Fluorescent pseudomonads are ubiquitous soil microorganisms and common inhabitants of onion plant rhizosphere. Certain strains suppress plant diseases by protecting roots from infection of soil-borne pathogens. In recent years, considerable attention has been paid to plant growth promoting rhizobacteria (PGPR) primarily fluorescent pseudomonads, which are aggressive root colonizers. They play a major role in biological control of plant pathogens. Not all the fluorescent pseudomonads dominate in the rhizosphere and possess several properties that have made them as biocontrol agent of choice [6].

Antagonistic activity of *Pseudomonas fluorescens* and *P. putida* in the rhizosphere has been recognized as major factor in the suppression of many phytopathogens. Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites.

However, mostly beneficial soil microbes to deliberate release metabolic like antibacterial and antifungal compounds into the environment is being practiced and considered as a means to improve agriculture and environmental quality (Halvorson *et al*., 1985). This approach requires a means of monitoring the organisms after their release. To be enumerated, a released organism must have a selective characteristic which does not interfere with its inherent ability to survive or colonize the environment (Turco *et al*., 1986). Spontaneous antibiotic resistance has provided a potentially simple and effective method to genetically mark bacterial strains for monitoring after introduction into complex ecosystems. Such mutants are being used to study root nodulation and competition among symbiotic nitrogen-fixing strains of rhizobia (Bromfield and Gareth Jones, 1979; Pankhurst CE 1977), the survival of fecal indicator bacteria in waters (Pettibone *et al.*,1987) and soil (Temple *et al*., 1980), and the persistence of organisms of potential use in genetic engineering (Liang *et al.,* 1982.).

However, spontaneous antibiotic resistance mutations may not be innocuous genetic lesions (Bromfield, *et al.,* 1985). Among *Rhizobium* spp., decreased symbiotic effectiveness was associated with streptomycin resistance (Gareth Jones and Bromfield, 1978; Onderdonk *et al.,* 1981), and rifampin-resistant (*Rif* ^{r}) strains failed to compete in nodulation studies with wild-type strains (Lewis *et al*., 1987). Mutants of *Rhizobium* spp. resistant to both streptomycin and rifampicin had significantly slower growth rates *in vitro* than wild-type strains had (Turco *et al*., 1986). In addition to physiological insult, antibiotic-resistant mutants may be viable in the environment of presentation but not easily culturable on media containing sufficiently high concentrations of selective agents (Rozak, and Colwell, 1987).

Resistance to rifampicin, generally mediated by a mutation in the, subunit of RNA polymerase (Sippel and Hartmann, 1968), is unusual among soil bacteria. The chromosomal nature of the mutation affords greater stability than occurs with plasmid-borne markers and is also advantageous since the mutation is not easily transferable. The objectives of these studies were to evaluate the physiological and ecological fitness of Rifampicin resistant mutants derived from *Pseudomonas fluorescens* pf2T2 and pf2m1. And to study the behaviour of these genetically marked strains when reintroduced into the live soil from which they were isolated. Fitness of the mutant strains was confirmed by their growth rates *in vitro*, membrane protein composition, and ability to successfully compete with wild-type parents in sterile-soil assays. The fate of the *Rif* ^{r} mutant organisms was then monitored after their introduction into live soil. The present study was Rifampicin-resistant (*Rif* ^{r}) mutants of *Pseudomonas fluorescens* pf2T2 and pf2m1 were isolated from different localities of black gram rhizosphere soil in an around the Tamil Nadu. However, the mutants of *P. fluorescens* were of two general categories. Group 1 showed fast growing in terms of growth rate, competitive fitness, and membrane protein composition. Group 2 showed a slower growth rate in both minimal and enriched media and an altered membrane protein profile. These mutants also demonstrated decreased competitive fitness compared with the wild-type strain based on the study of different temperature, pH, NaCl (salt tolerant), colony morphology, and gram staining has been done. Although, the Rifampin-resistant (*Rif* ^{r}) mutants of *Pseudomonas fluorescens* pf2T2 and pf2m1 were distinctly in the media.

MATERIALS AND METHODS:

Soil Samples Collection: Onion plant rhizosphere soil samples were collected from all over the Tamil Nadu plastic in bags. The isolation of bacterial isolate was carried out by serially diluting the volatile samples in sterile water and subsequently plating on the King's B agar base medium containing with 2.5% Glycerol with 0.001% Tween 20 by using the pour plate method.

Soil Character Analysis: Onion plant rhizosphere soil samples were the nutrient and mineral contents of the organic soil were determined and physical character was tested in Tamil Nadu Agricultural University, Madurai. However most of the 65% soil samples were in sandy loam and, 35% black soil. The macro nutrients Nitrogen was 49.0 to 66.2 mg/kg; P_2O_5 10.2 to 28.0; Potash 18 to 387mg/kg soil and micronutrients was Zn, Mg, Mn and Fe was tested. The physical characters Temperature, EC = dSm^{-1} and pH was analyzed (**Table 1**).

Effect of Initial pH: The effect of initial pH was accessed in the range of 5.5, 7.0 and 8.0 was carried out different types of fast and slow growing mutants *vs.* control strains using shake flask cultures. The medium containing 50ug/ml of Rifamycin was maintaining the culture **(Fig.1)**.

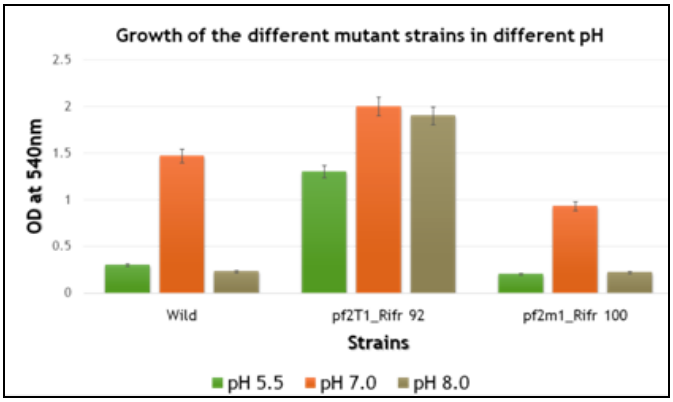


FIG. 1: GROWTH IN DIFFERENT pH

Effect of Temperature: The effect of different types of temperature were incubated in King's broth supplement with 50ug/l was tested in 37 ºC, 55 ºC and 65 ºC while keeping the pH of fermentation media was tested in both fast and slow growing mutant stains *Pseudomonas fluorescence* **Fig. 2**.

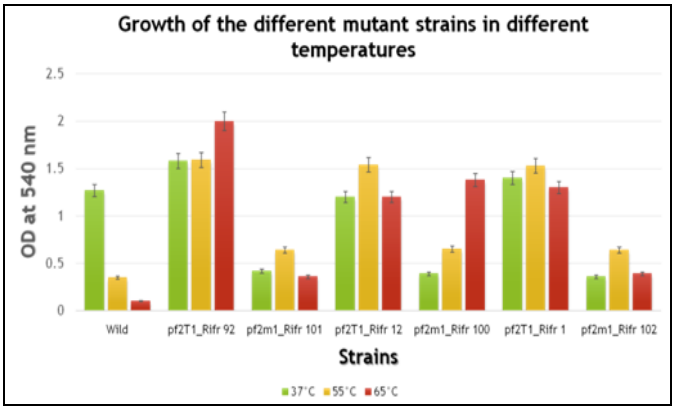


FIG. 2: GROWTH IN DIFFERENT TEMPERATURE

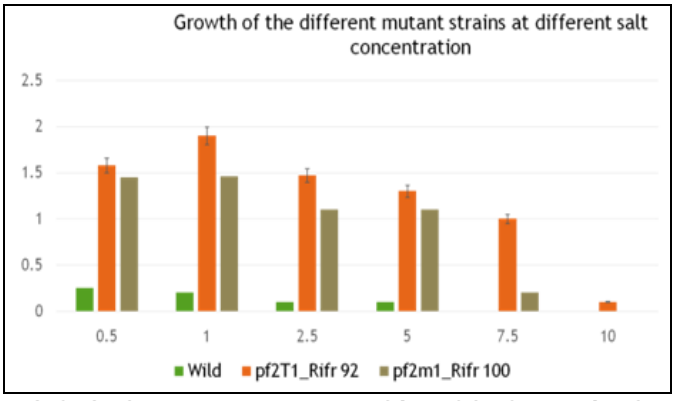


FIG. 3: GROWTH IN DIFFERENT SALT CONCENTRATION

Different Percentage of Salt: The effect of different percentage of salt (NaCl - 0.5, 1.0, 1.5, 2.5, 5.0, 7.5, 10.0%) were supplemented in King's broth containing with 50ug/l Rifamycin. The fast and slow growing was tested in 37 ºC while keeping the pH of fermentation media were growing mutant stains of *Pseudomonas fluorescence* **Fig. 3**.

Mutation Study: Selection for rifampin resistance colonies was selected based spontaneous *Rif* ^{r} strains of *Pseudomonas fluorescens* pf2T2 and pf2m1 were isolated from King's B medium containing 50µg of rifampin per ml at a frequency of 5 - 8 and 8 - 10. The *Rif*' mutation was stable without reversion to wild type after more than 15 passages in minimal and nutrient broths and several passages in sterile soil for each strain tested. The mutants were maintained on King's B medium containing 50µg of rifampin per ml **Fig. 4**.

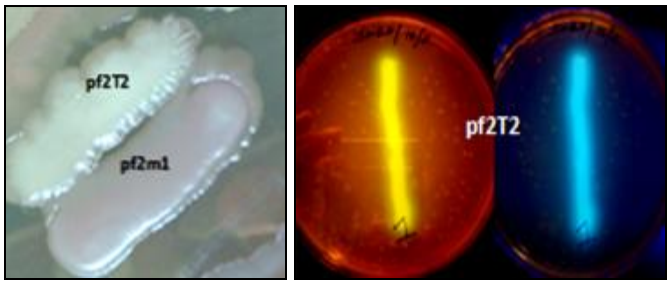


FIG. 4: SHOWS WILD STRAINS OF PSEUDOMONAS FLUORESCENCE pf2T2 AND pf2m1

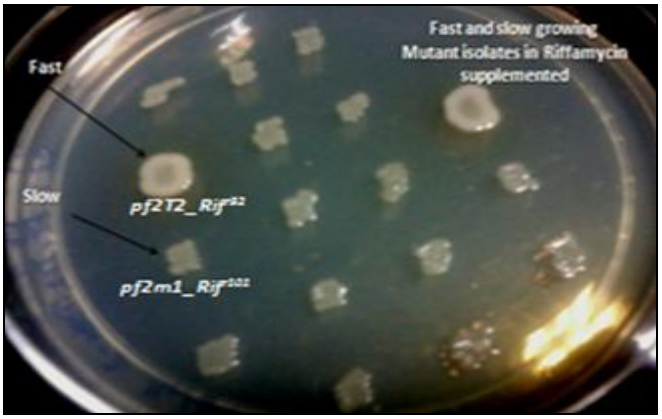
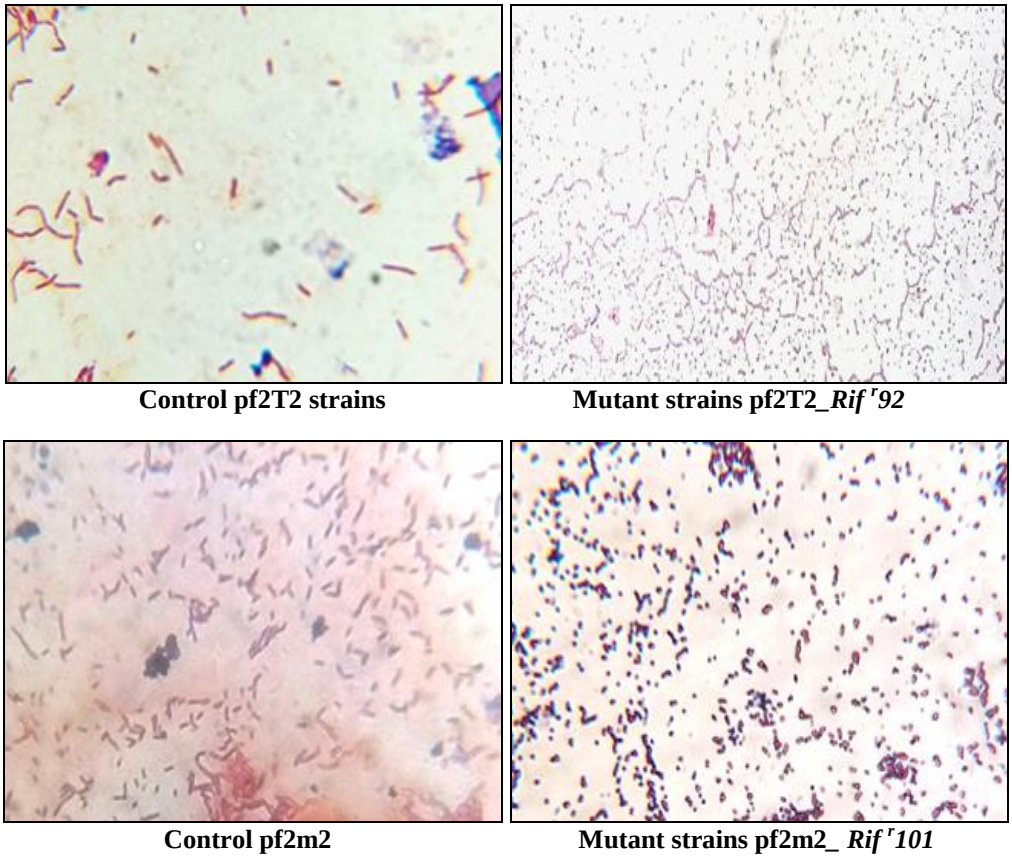


FIG. 5: SHOWS MUTANT STRAINS OF *PSEUDOMONAS FLUORESCENCE* pf2T2_*Rif* ^{r}*92* AND pf2m1_*Rif* ^{r}*101*

Grams Staining: A "smear" of bacteria is made on a microscope slide, fixed, stained, dried and, without using a cover slip, examined with the aid of a microscope. Aseptic technique must be observed when taking samples of a culture for making a smear.

A culture on agar medium is much preferable to a liquid culture for making a smear. A smear that is thin and even enables the shape and arrangement of cells to be clearly seen and ensures that the staining procedure is applied uniformly **Fig. 5**.

Antibacterial Study: Antibacterial study against pf2T2 non mutant *vs*. pf2T2 *Rif ʳ92* mutant was performed well under the King's B media with and without supplemented of Rifamycin 50ug/l **Fig. 6.**



Control pf2T2 strains **Mutant strains pf2T2_*Rif ʳ92***

Control pf2m2 **Mutant strains pf2m2_ *Rif ʳ101***

FIG. 6: GRAMS STAINING OF MUTANT STRAIN *PSEUDOMONAS FLOURESCENCE* pf2T2_*Rif ʳ*92

RESULTS AND DISCUSSION: The diversity of microbes associated with Onion plant plant roots is enormous, in the order of tens of thousands of species. This complex plant-associated microbial community, also referred to as the second genome of the plant, is crucial for plant health. Recent advances in plant - microbe interactions research revealed that plants are able to shape their rhizosphere microbiome, as evidenced by the fact that different plant species host specific microbial communities when grown on the same soil.

In this study, we discuss evidence that upon pathogen or insect attack, plants are able to recruit protective microorganisms, and enhance microbial activity to suppress pathogens in the rhizosphere. A comprehensive understanding of the mechanisms that govern selection and activity of microbial communities by plant roots will provide new opportunities to increase crop production.

The fluorescent pseudomonad was isolate and identified from the cultivated Onion plant rhizosphere soil sample was collected in plastic bags from all over the Tamil Nadu, India. The isolation of rhizosphere fluorescent pseudomonades for specifically bacterial isolate was carried out by serially diluting the agricultural soil samples in sterile water and subsequently plating on the 1.5% Kings *et al.*, 1959 agar base medium containing glucose by using the pour plate method**.** The plates were incubated at 37 °C for 48-72 hrs and observe in zone formation. A greenish blue or yellowish florescent zone pf2T2 and pf2m1 around the colonies indicates the florescent pseudomonas (Kings *et al*, 1959) and was studied in grams staining.

Soil Samples and *Pseudomonas fluorescence*: Fluorescent pseudomonas was screened from different localities of Onion plant rhizosphere soil

samples in Tamil Nadu **Table 1**. The present investigation on biological and molecular characterization of indigenous wild type of fluorescent Pseudomonas isolates for biocontrol activity of balance wheel of nature. Soil samples were collected from different localities of all over the Tamil Nadu. The rhizosphere soil of black gram field of Perambalur, Madurai, Trichy, Tirunelveli, Nagarkovil, Salem, Coimbatore, Chennai North, Rameshwaram, Kadalur, Naively, and Krishnagiri. These samples were analysis of Tamil Nadu Agricultural University at Madurai for macronutrients such as Nitrogen, Phosphorus and potassium and micronutrients of Zn, Mg, Mn, Fe of availability and physical and chemical character has been done (**Table 2**).

TABLE 1: SHOWING DIFFERENT LOCATION OF ONION PLANT RHIZOSPHERE SOIL SAMPLES ANALYSIS OF FLUORESCENT PSEUDOMONADS BACTERIAL COLONIES WERE ISOLATION BASED ON ANTI-BACTERIAL AND ANTIFUNGAL ACTIVITY

	Sources of sample			Number colonies / plate			Percentage of Fluorescent bacterial colony	
S. No	Name of the Place	Isolate Code number	Dilution Factor 10^{-7}	Fluorescent colonies	Other colonies	CFU/ml^3	Total No. colonies	% of Antibiotic and antifungal activity
1	Perambalur	Pf-2	10^{-7}	14±001	33±001	$3.5x10^7$	47	29.7
2	Madurai	M-1	10^{-7}	19±001	19±001	$3.5x10^7$	38	50.0
3	Trichy	T-1	10^{-7}	15±003	22±005	$3.5x10^7$	37	40.5
4	Tirunelveli	TRE-1	10^{-7}	10±002	29±002	$3.5x10^7$	39	25.6
5	Nagarkovil	NGK-1	10^{-7}	29±003	18±001	$3.5x10^7$	47	61.7
6	Salem	SLM-1	10^{-7}	19±001	12±002	$3.5x10^7$	31	61.2
7	Coimbatore	COag-1	10^{-7}	18±004	19±005	$3.5x10^7$	37	48.6
8	Chennai North	MDN-1	10^{-7}	21±001	24±003	$3.5x10^7$	45	46.6
9	Rameshwaram	Ram-1	10^{-7}	20±002	19±002	$3.5x10^7$	39	51.2
10	Kadalur	KAD-1	10^{-7}	26±003	19±002	$3.5x10^7$	45	57.7
11	Naively	NVL-1	10^{-7}	33±002	33±002	$3.5x10^7$	66	50.0
12	Krishnagiri	KRG-1	10^{-7}	27±001	20±001	$3.5x10^7$	47	57.5

TABLE 2: SOIL ANALYSIS OF ONION PLANT FIELD OF AGRICULTURAL LAND IN ALL OVER THE TAMIL NADU

S. No	Soil samples/Places	Parameters									
		Macronutrients in mg/kg of soil			Micronutrients in mg/kg of soil				Physical Character		
		N	P_2O_5	K_2O	Zn	Mg	Mn	Fe	Temperature	EC dSm^{-1}	pH
1	Perambalur	66.2	22.2	221	0.0017	0.9	0.002	0.003	31	0.4	6.9
2	Madurai	57.0	23.0	199	0.0022	0.01	0.0071	0.06	28	0.5	6.9
3	Trichy	51.9	26.2	267	0.0033	0.001	0.0013	0.0015	30	0.3	7.0
4	Tirunelveli	60.9	20.1	300	0.0021	0.0012	0.0013	0.81	32	0.4	7.1
5	Nagarkovil	58.8	19.9	198	0.0018	0.1	0.0016	0.1	25	0.4	6.7
6	Salem	49.0	18.2	209	0.0011	0.011	0.01	0.91	34	0.4	7.7
7	Coimbatore	64.7	28.0	234	0.0018	0.0015	0.001	0.01	26	0.5	6.8
8	Chennai North	64.0	20.7	256	0.0010	0.1	0.1	0.001	30	0.3	7.2
9	Rameshwaram	39.0	10.2	387	0.0013	0.0001	0.01	0.9	31	0.2	7.9
10	Kadalur	56.7	16.6	331	0.0015	0.0061	0.009	0.055	32	0.4	7.7
11	Naively	60.0	25.0	270	0.0015	0.0019	0.091	0.001	38	0.4	7.0
12	Krishnagiri	55.9	13.3	211	0.0015	0.001	0.1	0.001	29	0.4	7.0

Introduction to Mutation: Past 40 years rediscovery of Mendel's work and the rebirth of genetics, they were considered too simple to have genes, undergo mutation, or reproduce sexually. Today bacteria are an important tool in the study of genetics and biotechnology in the field of mutagenesis. The repair capacity of organisms not only determines their survival after the exposure to agents which damage their DNA, but also influences their response to the mutagenic effects of these agents. The management of soil application with *P. fluorescens* strain pf2T2 were isolated from black gram soil its genetically modified derivatives of fast growing pf2T2_*Rif^{r}92* and slow growing pf2m1_*Rif^{r}101* was apply onion field for significantly ($p < 0.05$) reduced Onion root pink disease compared to the control while the wild type strain pf2T2 had no significant impact in this respect (**Fig. 7**).

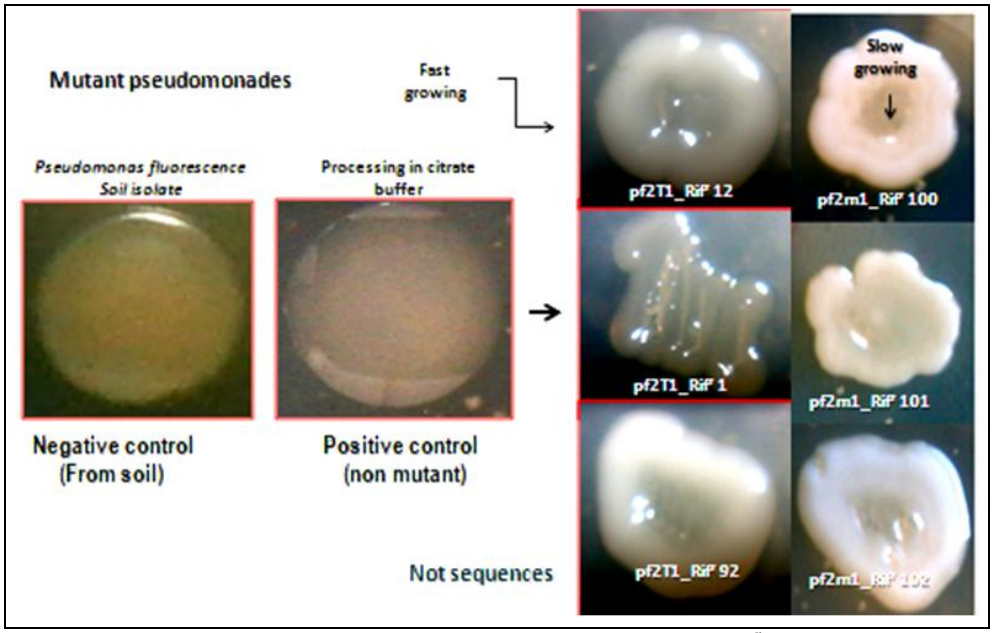


FIG. 7: SHOWS FAST AND SLOW GROWING MUTANT STRAINS OF pf2T2_*Rif*^{r}*1, 12, 92* and pf2m1_*Rif*^{r}*100, 101, 102*

Studies on Different pH, Temperature, Salt and Grams Staining: Gram staining is done to distinguish the two large groups of bacteria based on their cell wall constituents. Gram staining procedure was done and the resulting image showed pink coloured rod shaped strains which indicate that *Pseudomonas fluorescens* is gram negative. This shows that the bacteria have a thin layer of peptidoglycan and high lipid content **Fig. 5**. The culture was inoculated in Kings B broth and the OD was taken at 540nm for every hour. Graph was plotted for both the wild and mutant strains and finally their growth was compared (**Fig. 8, 8a-c**).

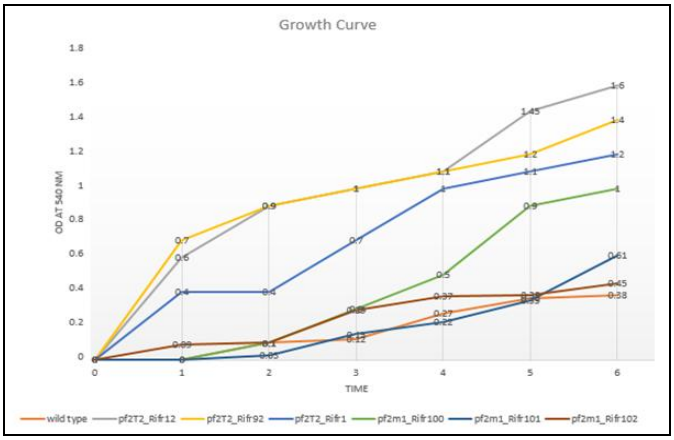


FIG. 8: BACTERIA HAVE A THIN LAYER OF PEPTIDOGLYCAN AND HIGH LIPID CONTENT

When the strains were grown in three different temperatures, the strain pf2T2_*Rif*^{r}92 showed good growth in all the three temperatures. And the slow growing strain pf2T2_*Rif*^{r}*100* grew very well even at 65 °C (**Fig. 2**). The X-axis shows the name of the strains used and the Y-axis shows the OD at 540nm (**Fig. 6**). When the strains were grown in 3 different pH, the strain pf2T2_*Rif*^{r} *92* grew very well in all the three pH (**Fig. 1**).

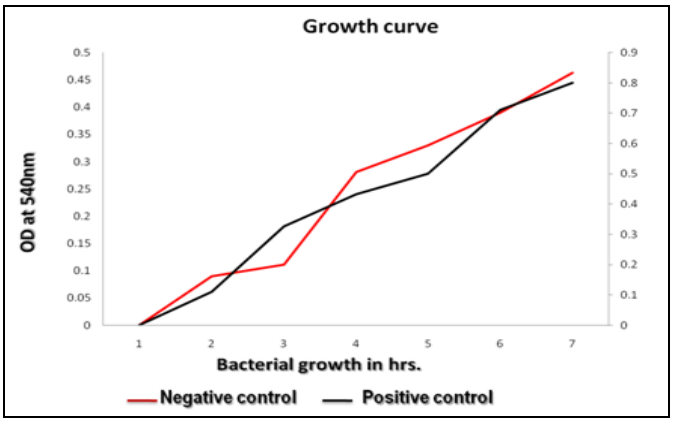
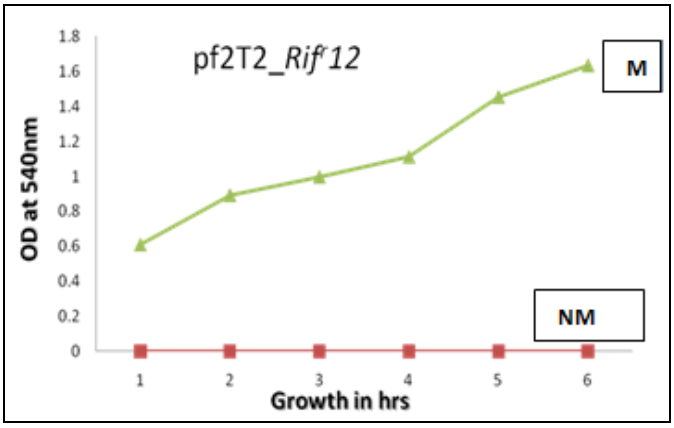
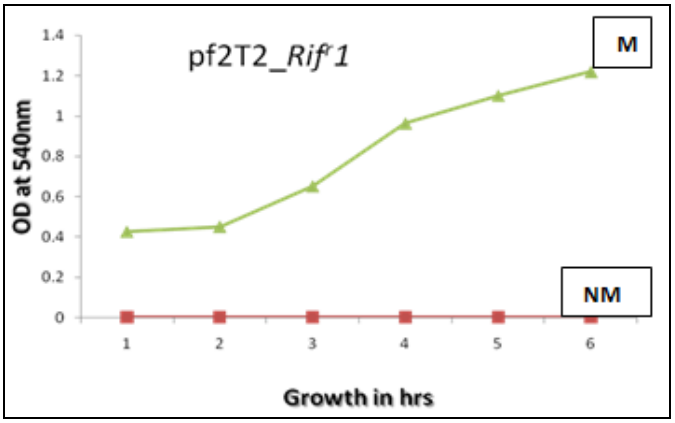


FIG. 8a: SHOWS IN POSITIVE AND NEGATIVE CONTROL *PSEUDOMONAS* STRAINS pf2T2 AND ON GROWTH CURVE STUDIES IN KING'S B BROTH





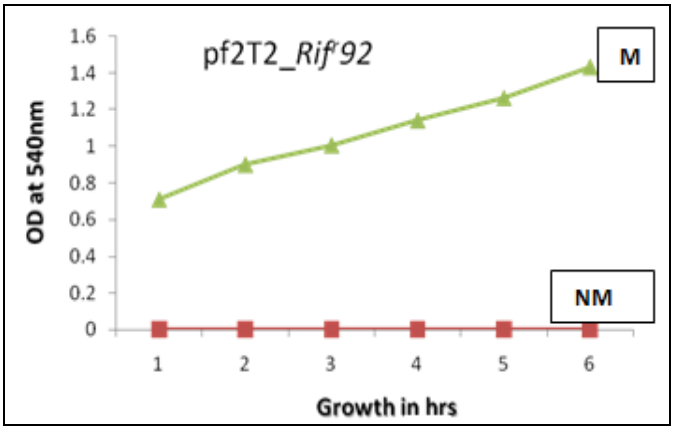
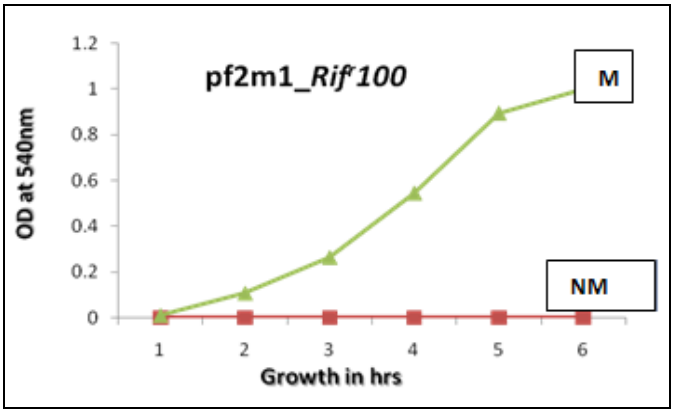
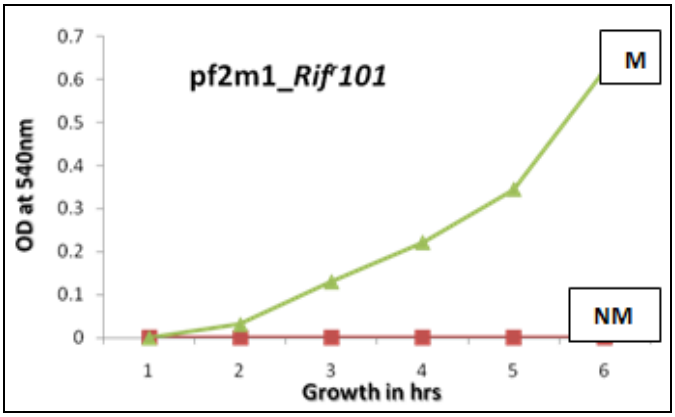


FIG. 8B: 1 - 3 SHOWS DIFFERENT FAST GROWING *PSEUDOMONAS* MUTANT STRAINS pf2T2_*Rif*^{r1}, pf2T2_*Rif*^{r}*12* AND pf2T2_*Rif*^{r}*92* GROWING IN KING'S B BROTH





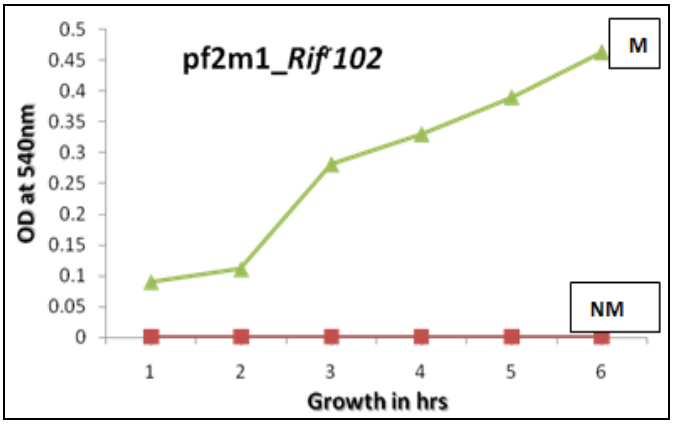


FIG. 8c: 1 - 3 SHOWS DIFFERENT SLOW GROWING *PSEUDOMONAS* MUTANT STAINS pf2m1_ *Rif* ^{r}*100*, pf2m1_ *Rif* ^{r}*101* and pf2m1_ *Rif* ^{r}*102* GROWING IN KING'S B BROTH

Mutation Study: A mutation rate is an estimation of the probability of a mutation occurring per cell division and corresponds to the probability of a mutation occurring in the lifetime of a bacterial cell. A mutation frequency is simply the proportion of mutant bacteria present in a culture. These terms are often used interchangeably, causing confusion.

The relationship between mutation frequency and the rate at which mutations occur is uncertain. If a mutation arises early in the culture period, then a large number of mutant progeny occur and this would be represented by a high frequency. Mutation is an essential cellular process that contributes to the genetic variability and acts as the driving force of evolution. A mutation is a permanent alteration in the sequence of nitrogenous bases of a DNA molecule [11]. The similar result was found in mutation is generally a change in the end-product specified by that gene.

In some cases, a mutation can be beneficial if a new metabolic activity arises in a microorganism, or it can be detrimental if a metabolic activity is lost. Mutations can be spontaneous for Rifampicin based on fast and slow growing mutants were analysed, or induced by a mutagen in the environment. The present study of the mutant results was indicated that fast and slow growing *Pseudomonas fluorescence* was analyzed. The fast growing mutant was pf2T2_*Rif* ^{r}*1, 12, 92* and slow growing mutants pf2m1_*Rif* ^{r} *100, 101,102* (**Fig. 2** and **3**).

Growth rate of mutants pf2T2_*Rif* ^{r}*1, 12, 92* and pf2m1_*Rif* ^{r}*100, 101,102.*

Growth characteristics of *Rif* ^{r} Pseudomonas spp. Seven different *Rif* ^{r} derivatives of *P. putida* and 10 derivatives of *P. fluorescens* were selected by growth on NA supplemented with 100ug of rifampin per ml. The generation time at 30 °C of the *P. putida* mutant strains tested did not differ from that of the wild-type strain Ppl-1, *i.e.*, a doubling time of 100 min in minimal medium and 48 min in nutrient medium. The *Rif* ^{r} mutant strains of *P. fluorescens* Pfl-1 were found to comprise two types, one type with growth rates identical to that of the wild type and a second type with slower growth rates in both media. A member of each group was chosen for further study. *P. fluorescens* Pfl-1 and the Rif' derivative Pfl-2 had similar generation times in minimal medium (120 min) and in nutrient medium (63 min). *P. fluorescens* Pfl-8 represented the group of *Rif* ^{r} mutants that had longer growth rates. The generation times of this strain were 153 min in minimal medium and 81 min in nutrient medium [14].

Recent work has shown that many bacterial populations harbor a proportion of cells with a mutator phenotype. These cells have a mutation rate that is increased from 10 to 50 up to 10,000 times [11], generally as a consequence of a defective methyl-directed mismatch repair system. Because such highly mutable bacteria can rapidly emerge in a previously homogeneous population, the overall mutation rate will increase. The mutator phenotype allows bacteria to develop a large variability of alleles that can evade (by mutation) stressful environments during the infective process or antibiotic treatment [12, 13]. Interestingly mutants *vs*. Non mutant strains were grow in Kings B broth supplemented with Rifampicin. The non-mutants positive and negative control of field isolates pf2T2, pf2m1and mutants of fast and slow growing strains of pf2T2_*Rif* ^{r}*1, 12, 92* and pf2m1_*Rif* ^{r}*100, 101,102* was calculated by different hour's intervals (**Fig. 4, 5** and **6**). The monomer composition of polyhydroxyalkanoates produced pf2T2_*Rif* ^{r}*1, 12, 92* and pf2m1_Rif ^{r}*100, 101,102* grown on glucose or dextrose as sole carbon source (**Fig. 4**) was similar to that by the wild type reported previously [9, 10].

Antibacterial Study: Biocontrol of soil borne pathogens has become one of the major concerns in agriculture. Fluorescent pseudomonads are ubiquitous soil microorganisms and common inhabitants of rhizosphere. Certain strains suppress plant diseases by protecting roots from infection of soil-borne pathogens. In recent years, considerable attention has been paid to plant growth promoting rhizobacteria (PGPR) primarily fluorescent pseudomonads, which are aggressive root colonizers. They play a major role in biological control of plant pathogens.

Fluorescent pseudomonads dominate in the rhizosphere and possess several properties that have made them as biocontrol agent of choice [1, 4]. The similarity was found in our mutants strains of two (pf2T2_*Rif* ^{r}*92* and pf2m1_*Rif* ^{r}*101*) potential mutant strains isolates of *Pseudomonas fluorescens* was evaluated against a Onion root pink disease and correlated with antagonistic activity of *Pseudomonas fluorescens* and *P. putida* in the rhizosphere has been recognized as major factor in the suppression of many phytopathogens [6].

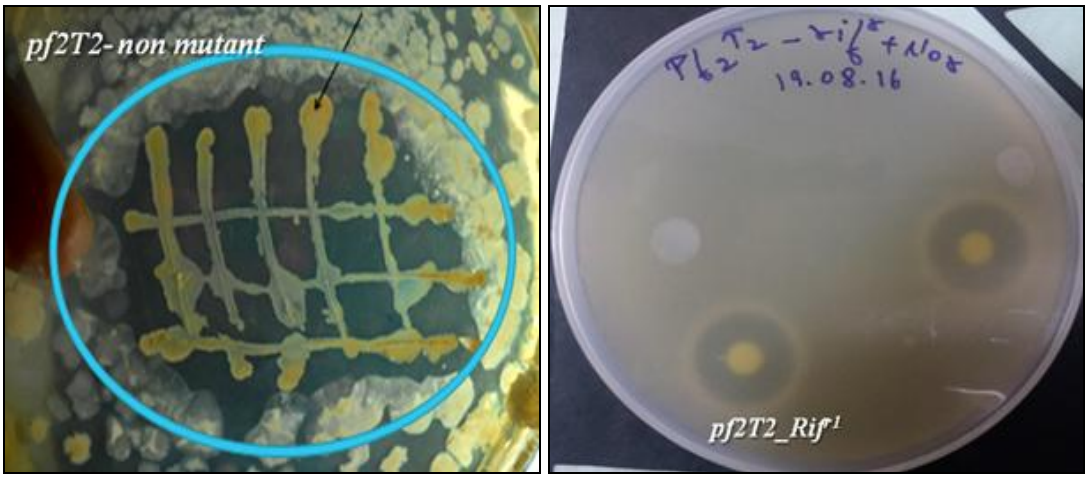


FIG. 9: ANTIBACTERIAL STUDY

CONCLUSION: We have stated that the "mutation rate" is not a simple characteristic of specific bacterial understanding molecular mechanisms of mutational processes requires test systems for monitoring mutation rates and analyzing mutation spectra. Several mutation detection systems have been developed for bacteria, however the majority are well suited for use in *E. coli*. In recent years, studies have been focused on mutational mechanisms in other bacteria such as the diverse and ecologically significant *Pseudomonas* genus Kivisaar M (2010). There are a limited number of test systems for analyzing mutations in *Pseudomonas* spp.

ACKNOWLEDGEMENT: We are sincerely thankful to Director, Chancellor and Vice Chancellor, Kalasalingam University, Krishnankoil, Viruthunagar District, Srivilliputhur - 626126 Tamil Nadu, India.

REFERENCES:

1. Anand M, Naik MK, Ramegowda G and Devika Rani GS: Biocontrol and plant growth promotion activity of indigenous *Pseudomonas fluorescens* isolates J. Mycopathol. Res. 48(1): 45-50.
2. Siddiqui LA, Ehteshamul-Haque S and Shaukat SS: Use of rhizobacteria in the control of root rot-root knot disease complex of mungbean. Journal of Phytopathology, 2001; 149: 337-346.
3. Weller DM: Biological control of soil-borne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology 1988; 26: 379-407.
4. Johri BN, Rao CVS and Goel R: Fluorescent pseudomonads in plant Disease management. In Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production. Ed. Dadarwal R, Jodhpur Science Publishers. Jodhpur 1997; 193-122.
5. King EO, Ward MK and Raney DE: J. Lab and Clin. Med. 1954; 44: 301-307.
6. Manjunatha H, Naik MK, Patil MB, Lokesha R and Vasudevan SN: Isolation and characterization of native fluorescent pseudomonads and antagonistic activity against major plant pathogens, Karnataka J. Agric. Sci., 2012; 25(3): 346-349.
7. Maki H: Origins of spontaneous mutations: specificity and directionality of base-substitution, frame shift, and sequence-substitution mutageneses. Annu Rev Genet 2002; 36: 279–303.
8. Kivisaar M: Mechanisms of stationary-phase mutagenesis in bacteria: mutational processes in pseudomonads. FEMS Microbiol Lett 2010; 312: 1–14.
9. Lee HJ, Rho JK and Yoon SC: Growth temperature dependent conversion of de novo-synthesized unsaturated fatty acids into polyhydroxyalkanoic acid and membrane cyclopropane fatty acids in the psychrotrophic bacterium *Pseudomonas fluorescens* BM07. J Microbiol Biotechn 2004b; 14: 1217–1226.
10. Lee HJ, Choi MH, Kim TU and Yoon SC: Accumulation of polyhydroxyalkanoic acid containing large amounts of unsaturated monomers in *Pseudomonas fluorescens* BM07 utilizing saccharides and its inhibition by 2-bromooctanoic acid. Appl Environ Microb 2001; 67: 4963–4974.
11. Miller JH: Spontaneous mutators in bacteria: insights into pathways of mutagenesis and repair. Annu. Rev. Microbiol. 1996; 50: 625–643.
12. Matic I, Radman M, Taddei F, Picard B, Doit C, Bingen E, Denamur E and Elion J: Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. Science 1997; 277: 1833–1834.
13. Le Clerc JE, Li B, Payne WL and Cebula TA: High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. Science 1996; 274: 1208–1211.
14. Geoffrey C, Al-Achi BJ, Evangelia P and Stuart BL: Survival of Rifampin-Resistant Mutants of *Pseudomonas fluorescens* and *Pseudomonas putida* in Soil Systems. Applied and Environmental Microbiology 1988; 54(10): 2432-2438.

How to cite this article:
Priyadarshini V, Sonamilli, Shruthipooja S and Hariram N: phenotypically and genetically variation of *Rif r* mutants strains of *Pseudomonas fluorescence* pf2T2 and pf2m1. Int J Pharm Sci Res 2018; 9(1): 188-96.doi: 10.13040/IJPSR.0975-8232.9(1).188-96.