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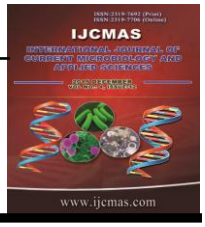


**Original Research Article**

# Screening of Pathogenic Strains of *Pseudomonas aeruginosa* from Clinical Samples using *Lactuca sativa* as a Plant Model

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**Keywords**

*Pseudomonas aeruginosa*; *Lactuca sativa*; Plant model; Lettuce leaves; Lesion; Pathogen

**A B S T R A C T**

The present study suggested the use of plant as a model for screening the pathogenic isolates of *Pseudomonas aeruginosa* from clinical samples in which the pathogenicity level varies from strain to strain. Totally 10 clinical samples were collected, 4 isolates were identified as *Pseudomonas aeruginosa.* Pathogenicity of the 4 isolates sputum (A), pus (B), urine (C and D) has been evaluated in plant model (*Lactuca sativa*) by injecting 10 µl of bacteria suspension ($0.8 \times 10^9$ CFU/ml) into separate smaller (6 cm) and larger (10 cm) lettuce (*Lactuca sativa*) leaves. The result suggested that isolate (A) seems to be more pathogenic in which the extent of lesion is 86% in large leaf and 97% in small leaf and isolate (B) shows 58% in large leaf and 63% in small leaf which indicates moderate level of pathogenicity. The extent of lesion caused by the isolate C (38% in large leaf and 36% in small leaf) and isolate D (37% in large leaf and 35% in small leaf) indicates low level of pathogenicity. Thus, this study has shown that isolate (A) sputum sample seems to be more pathogenic which has been collected from a patient, suffering from cystic fibrosis. Isolate (B) pus sample indicates moderate level of pathogenicity when compared to other two isolates (C and D) urine samples which indicates low level of pathogenicity. Thus, the pathogenicity among the clinical isolates varies. Thus plants can be used as a model for screening the pathogenic isolates. Further studies should be carried out for those four isolates to check whether it produces the same level of pathogenicity when inoculated in laboratory animals.

## Introduction

Bacterial pathogenicity is better understood by the use of animal model that is by artificially infecting the laboratory animals such as mice, rats, rabbits, guinea pigs and monkeys with human pathogenic bacteria. However there are problems inherent in the use of animal models such as the high cost, proper housing of the animals, risk of handling animals in the laboratory, and ethical issues. To overcome these problems, the use of invertebrates has been suggested (Chikaro *et al*., 2007), and in the past few

years, a number of different invertebrate host model system have been described. Experiments with plants, insects, protozoa, nematodes and slime moulds have recently come to the forefront in the study of pathogenic microorganisms (Eleftherios *et al*., 2007).

Among the alternative experimental models plants are fast, inexpensive, high throughput screening tool to identify putative virulence determinants (Rahme *et al*., 1997). Plant infection models provide a number of advantages in the study of human pathogenesis. This discovery has resulted in the development of convenient, cost effective, and reliable plant infection models to study the molecular basis of infection by human pathogens (Prithiviraj *et al*., 2004). Plants like thale cress, lettuce, tomato, onion; Chinese cabbage has been developed as plant models. Infectious diseases transmitted by bacteria are a major cause of mortality worldwide. Opportunistic pathogenic bacteria like *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are capable of infecting individuals who are affected by AIDS, extensive burn injury, cystic fibrosis, or are otherwise immunocompromised, have become a major concern in developed countries (Prithiviraj *et al*., 2004).

*Pseudomonas aeruginosa* is a gram negative, aerobic, rod-shaped bacterium with unipolar motility (Ryan *et al*., 2004). Like other members of the genus, *Pseudomonas aeruginosa* is a free-living bacterium, commonly found in soil and water. An opportunistic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants (Iglewski *et al*., 1996). It occurs regularly on the surfaces of plants and occasionally on the surfaces of animals. However, *P. aeruginosa* has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies track its occurrence as a nosocomial pathogen and indicate that antibiotic resistance is increasing in clinical isolates. *P. aeruginosa* is the type species of the genus *Pseudomonas* (Anzai, 2000). It secretes a variety of pegments, including pyocyanin (blue green) fluorescein (yellow green and fluorescent, now also known as pyoverdin) and pyorubin (red brown). Fluorescence under ultraviolet light is helpful in early identification of *P.aeruginosa* colonies. Fluorescence is also used to suggest the presence of *P.aeruginosa* in wounds.

It is often preliminarily identified by its pearlescent appearance and grape-like odor in vitro. Definitive clinical identification of *Pseudononas aeruginosa* often includes identifying the production of both pyocyanin and fluorescein. As an opportunistic pathogen of immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds and also causes other blood infections (Ikpeme *et al*., 2013). It is the most common cause of burn and external ear infections, and is the most frequent colonizer of medical devices (e.g. catheters). It cause community acquired pneumonias as well as ventilator associated pneumonias, being one of the most common agents isolated in several studies (Diekema *et al*., 1999). Chronic lung infection by *P. aeruginosa* is the severe complication in cystic fibrosis (CF) patients (Kolpen *et al*., 2014).

It is characteristically resistant to many antimicrobial agents owing to impermeability, multi-drug efflux and a chromosomal AmpC β-lactamase (More *et al*., 2015). One in ten hospital acquired infections is from *Pseudomonas*. It is also a common cause of post-operative infection in radical keratotomy surgery patients. It is

primarily a nosocomial pathogen and uses the virulence factors exotoxin A to ADP-ribosylate eukaryotic elongation factor 2 in the host cell, much as the diphtheria toxin does. Without elongation factors 2, eukaryotic cells cannot synthesize proteins and necrose. The release of intracellular contents induces an immunologic response in immunocompetent patients. Most *Pseudomonas* infections are both invasive and toxinogenic.

The ultimate *Pseudomonas* infection may be seen as composed of three distinct states: (1) bacterial attachment and colonization; (2) local invasion; (3) disseminated systemic disease. However, the disease process may stop at any stage. Particular bacterial determinants of virulence mediate each of these stages and are ultimately responsible for the characteristic syndromes that accompany the disease.

Aside from being an effective and deadly opportunistic human pathogen, *Pseudomonas aeruginosa* has been known to infect a number of plants. In plants, *P. aeruginosa* induces symptoms of soft rot in *Arabidopsis thaliana* (thale cress) and *Lactuca sativa* (lettuce) (Rahme *et al*., 1995; Walker *et al*., 2004). It is also a common colonizer of many fruits and green plants and can persist without causing disease symptoms (Cho *et al*., 1975).

It is a powerful pathogen in some insects like *Caenorhabditis elegans* (Mahajan-Miklos *et al*., 1999; Martinez *et al*., 2004) *Drosophila* (D'Argenio *et al*., 2001) and *Galleria mellonella* (Miyata *et al*., 2003). The association of virulence factors is the same for vegetal and animal infections (Rahme *et al*., 1995; Ausubel, 2000). This bacterium was demonstrated to employ a similar subset of virulence factors to elicit disease in animals and plants (Rahme *et al*., 1995; 1997). These findings established the validity of using a plant model to study pathogenesis of animal pathogens and led to the speculation that a plant model could be used to determine the pathogenicity of microorganisms.

In the current study *Pseudomonas aeruginosa* has been isolated from clinical samples and its pathogenicity has been screened on plant model to show that the pathogenicity varies between strains to strain. The plant model chosen was *Lactuca sativa* (lettuce) as it was widely accepted as a test for bacterial pathogenicity (Dinghra *et al*., 1985). Its pathogenicity has been tested on both the larger and smaller leaves. The assay with plant models seems to be faster and easier than animal models.

## Materials and Methods

### Collection of Clinical Samples

Samples of sputum (n = 2) from cystic fibrosis patients, urine (n = 4) from urinary tract infected patients and pus from burns and wounds of patients (n = 4) were collected from hospitals in Chennai (Apollo, Isabella, and Bilroth). All the samples were transported to the laboratory within one hour after collection for bacteriological analysis.

### Bacteriological Analysis

The samples were enriched in peptone water overnight. Cetrimide agar (Himedia) was used as selective medium for the isolation of *Pseudomonas aeruginosa*. The isolates were characterized by standard biochemical confirmation. After the identification, the isolates of *P. aeruginosa* were compared with a standard reference strain *P. aeruginosa* ATCC 27853 and stored at -70ºC for further studies.

## Screening of Pathogenic Strain of *Pseudomonas Aeruginosa* using Plant Model

### Bacterial Dose Preparation

Pure isolates of *Pseudomonas aeruginosa* were suspended in sterile phosphate buffer saline and diluted to standard concentration with an optical density of 1.0 at 600 nm. This suspension contained approximately $0.8 \times 10^9$ CFU/ml as determined by dilution and plating methods.

### Plant Model

### Collection of Lettuce Leaves

Lettuce (*Lactuca sativa*) leaves were purchased commercially. The leaves were then separated into outer larger leaves and inner smaller leaves, to use as model for pathogenic assay. The leaves were trimmed to uniform sizes in which larger leaves were of 10 cm and smaller leaves were of 6 cm, in length, measured from the base to the top of the midrib..

### Pathogenic Assay in *Lactuca Sativa*

Among the ten clinical specimens collected from pus, wound, sputum and urinary tract infections, bacterial analysis confirmed the presence of *Pseudomonas aeruginosa* in four isolates, one each from sputum and pus and two from urine samples. All the four isolates (A, B, C and D) were inoculated into separate smaller and larger lettuce leaves to determine the pathogenicity of *P. aeruginosa*.

### Preparation of Leaves for Inoculation

Leaves were detached and disinfected by washing them sequentially with tap water, 1% sodium hyphochlorite, sterile distilled water, 70% ethanol, and finally rinsed with sterile distilled water. Individual leaves were placed in sterile petriplates.

### Inoculation of Lettuce Leaves

Each leaf was inoculated with 10 µl of bacterial suspension $0.8 \times 10^9$ CFU/ml through the midrib using a hypodermic syringe. Two sets of control were maintained for large and small leaves. In the first, 10 µl of *Pseudomonas Putida* culture suspension was inoculated. In the second, 10 µl of sterile saline solution was injected. Inoculated leaves were incubated at 30°C in a humid chamber to avoid drying of the leaves. Symptoms of lesion due to infection were monitored daily for five days.

### Experimental Design

All the experimental and control inoculation were done in triplicates for each of the four isolates (A, B, C and D) for large and small leaves. The assignment of the leaves (small and large) to the petriplates and the inoculation (A, B, C, D and controls) was made randomly. The arrangement of the petriplates with inoculated leaves within the humid chamber was also done randomly.

### Measurement of the Lesion

The length of lesion which appeared as a streak of dark brown pigmentation along the length midrib of the inoculated leaf was measured to the nearest millimeter using a metric scale.

### Calculation of Extent of Lesion

In order to make the extents of lesion in leaves of two different sizes (10 cm and 6 cm) comparable the absolute lengths measured were converted to percentage of lesion in relation to the total length of the

leaf. The calculation was as follows

$$\text{Extent of lesion} = \frac{\text{Length of lesion (cm)}}{\text{Total length of leaf (cm)}} \times 100$$

**Statistical Analysis**

Mean and standard deviation were comparable for each of the triplicate values of the extent of lesion. One-way analysis of variance followed by Tukey's was used for the comparison of means of different groups. Two-way ANOVA with replication was used to analyse the interaction of the factors, size leaf and bacterial isolate.

**Results and Discussion**

**Isolation and characterization of *Pseudomonas aeruginosa***

The preliminary bacterial analysis of the ten clinical specimens from pus, wound, sputum and urinary tract infections specifically for *Pseudomonas aeruginosa*, showed that only four isolates were positive. Thus, the prevalence of *P. aeruginosa* in clinical samples was 40%. The microscopic and biochemical characteristics of *P. aeruginosa* in the four isolates were in complete agreement with those of standard, *P. aeruginosa* ATCC 27853.

**Pathogenicity**

All the four isolates inoculated in to the midrib of the two categories of leaves small, 6 cm (Fig. 1) and large, 10 cm (Fig. 2) produced visible lesions along with the midrib. However, there were significant differences among the four isolates and between the two leaf categories.

The extent of lesion caused by isolates A and B were significantly higher than those by the isolates C and D (Table 1; Fig. 3) between A and B, the former caused significantly higher lesions. The extent of lesion in smaller leaves due to isolate A or B inoculation was significantly greater than in the larger leaves. Isolate A caused significantly greater lesion isolate and the leaf size. That is, isolate A-small leaf combination have higher pathogenic lesion than other combination. The lesions caused by isolates C and D in both small and large leaves were in the range of 33 – 38% and the differences among them were not significant. The control large and small leaves inoculated either with *Pseudomonas putida* or sterile saline solution did not exhibit any pathogenic symptoms (Figs. 1 and 2).

**Statistical Analysis**

(Based on the data given in table 1)

**One way- ANOVA and multiple comparisons of means by Tukey's test**

**Inferences**

(a) The extent of lesion caused by isolate A (86% in large leaf and 97%in small leaf) is significantly greater than those by the other isolates $P<0.05$

(b) The extent of lesion caused by isolate A in the small leaf (97%) is significantly greater than that in larger leaf (86%) $P<0.05$

(c) The extent of lesion caused by isolate B 58% in large leaf and 63% in small leaf is significantly higher than those by isolates C and D $P<0.05$

(d) The extent of lesion caused by isolate B in smaller leaf (63%) is significantly greater than that in larger leaf (58%) $P<0.05$

(e) There is no significant difference among the means of the isolates C and D in both large and small leaves $P<0.05$

**ANOVA: Two factor with replication**

**Null Hypothesis Tested and Inference**

Leaf size: $H_0$:$\mu_{small}$; F:10.05;P=0.006;

$H_0$ Rejected; the extents of lesion in the large leaf and small leaf are significantly different.

Isolates: $H_0$ :$\mu_A = \mu_B = \mu_C = \mu_D$; F:757.841;P=$1.88^{-17}$.

$H_0$ Rejected; the extents of lesion caused the four isolates A, B, C and D are significantly different.

Interaction between leaf size and isolates:

$H_0$ interaction between leaf size and isolates

F: 12.02; P=0.0002; $H_0$ Reject. There is significant interaction between the two factors, leaf size and isolates.

The broad aim of the study was to develop a plant model to understand bacterial pathogenesis. Accordingly, leaves of the lettuce (*Lactuca sativa*) plant were chosen as the plant model and *Pseudomonas aeruginosa*, an opportunistic human pathogen was selected for infecting the leaves. Four different clinical isolates of *P. aeruginosa* were inoculated into two size categories of leaf (large, 10 cm and small 6 cm). Even though, all the isolates produced visible pathogenic effects along the length of the midrib of the leaf, the extent of pathogenic lesion significantly varied among the isolates and between the leaf sizes.

*Pseudomonas aeruginosa*, a ubiquitous gram negative bacterium, has been intensively studied as an opportunistic human pathogen (Britigan *et al*., 1997) and as the dominant pathogen infecting the lungs of cystic fibrosis patients (Pier *et al*., 1996). It is also responsible for 16% of nosocomial pneumonia cases (Wiblin *et al*., 1997), 12% of hospital required urinary tract infections, 8% surgical wound infections (Pollack *et al*., 1995) and 10% of blood stream infections (Gordon *et al*., 1998).

In the bacterial analysis of isolates from ten clinical specimens collected from sputum, pus, wound and urinary tract infections specifically for *Pseudomonas aeruginosa*, four isolates were confirmed as *P. aeruginosa* suggesting a prevalence rate of 40%. Agnihotri *et al*. (2004) reported a retrospective study of major aerobic bacterial isolates from pus/wound swabs taken from patients admitted to the burn unit at Govt. Medical College Hospital (Chandigarh, India). The study was carried out to determine the bacterial profile and antimicrobial susceptibility of the isolates and to describe the changes in trends over the study period. The pus/wound swabs yielded very high culture positivity (96%) for 665 total isolates. *Pseudomonas aeruginosa* was found to be the most common isolate (59%) followed by *Staphylococcus aureus* (17.9%), *Acinetobacter* spp (7.2%), *Klebsiella* spp (3.9%), *Enterobacter* spp (3.9%), *Proteus* spp (3.3%) and others (4.8%). *P. aeruginosa* was the predominant pathogen encountered suggesting that the prevalence rate of *P. aeruginosa* is higher, which is comparable to the result obtained in the current study.

Aside from being an effective and deadly opportunistic human pathogen pathogen, *Pseudomonas aeruginosa* has been known to infect a number of plants (Elrod and Braun, 1942). However, it was Dr. Rahme and co-workers, using the clinical isolate of *Pseudomonas aeruginosa* strain PA14 and the plant model *Arabidopsis thaliana* (thale cress) as well as *Lactuca sativa* (Lettuce), who first showed that this bacterium employs a similar subset of virulence factors

to determine or elicit disease in animals and plants (Rahme *et al*., 1997). They took advantage of the fact that in addition to produce visual disease symptoms on the plant leaf surfaces, similar to plant pathogenic bacteria *P. aeruginosa* was capable of multiplying rapidly in the apoplast, correlating with disease severity.

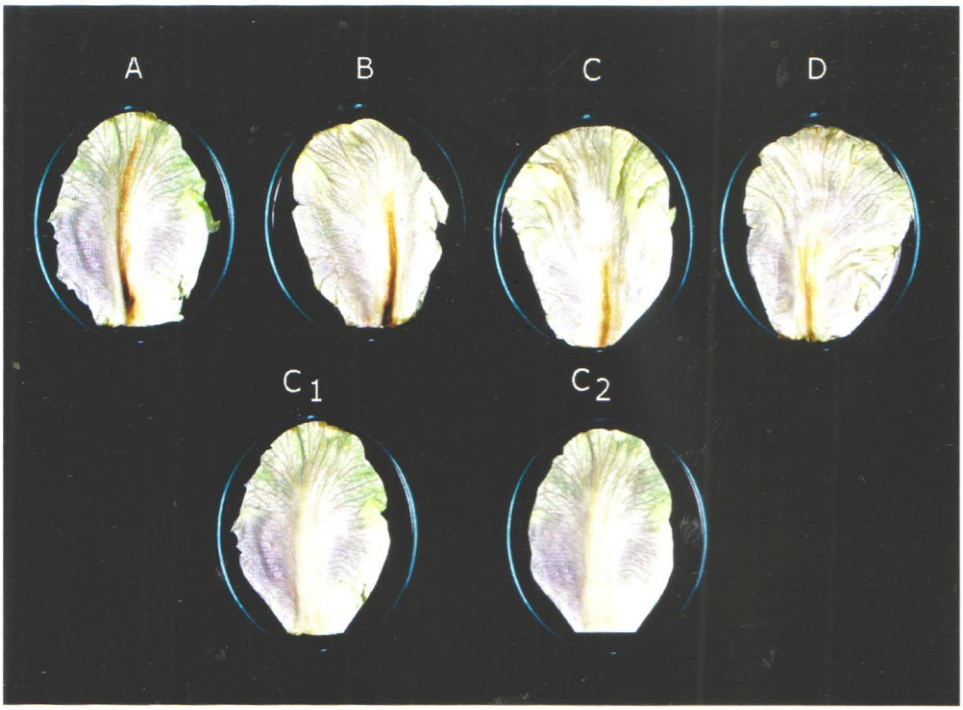
**Table.1** Extent of Pathogenic Lesion (in percent) Exhibited by the Two Size Categories of the Lettuce Leaves due to Inoculation of Four Clinical Isolates of *Pseudomonas aeruginosa*

| Isolate | Large Leaf (10 cm) | | | Mean ± SD | Small Leaf (6 cm) | | | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| | Leaf number in triplicate | | | | Leaf number in triplicate | | | |
| | 1 | 2 | 3 | | 1 | 2 | 3 | |
| A | 88.00 | 86.00 | 84.00 | 86.00 ± 2.00 | 100.00 | 96.67 | 95.00 | 97.22 ± 2.55 |
| B | 58.00 | 57.00 | 59.00 | 58.00 ± 1.00 | 63.33 | 65.00 | 61.67 | 63.33 ± 1.67 |
| C | 40.00 | 38.00 | 36.00 | 38.00 ± 2.00 | 40.00 | 33.33 | 35.00 | 36.11 ± 3.47 |
| D | 40.00 | 37.00 | 36.00 | 37.67 ± 2.08 | 38.33 | 33.33 | 33.33 | 35.00 ± 2.89 |

Note:

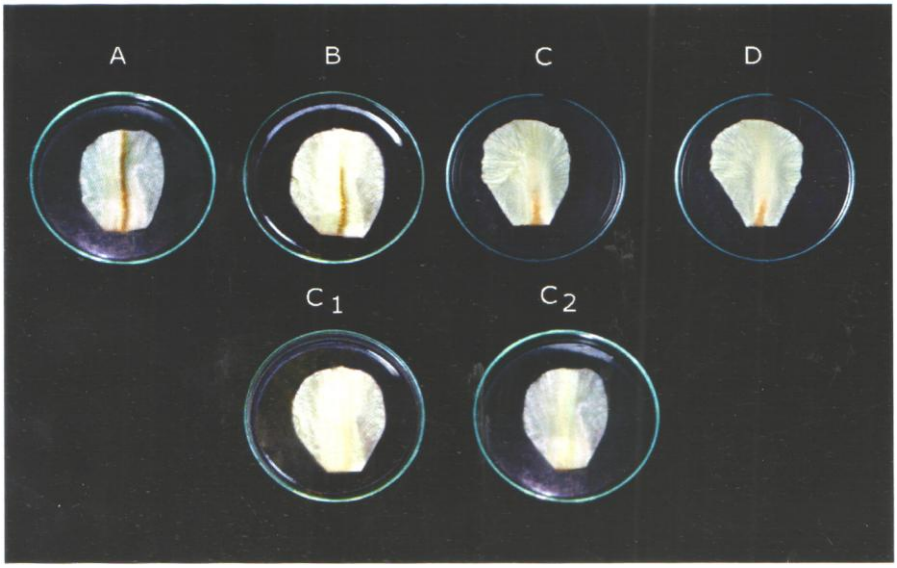
$$\text{Extent of lesion} = \frac{\text{Length of lesion (cm)}}{\text{Total length of leaf (cm)}} \times 100$$

**Fig.1** Larger Leaves



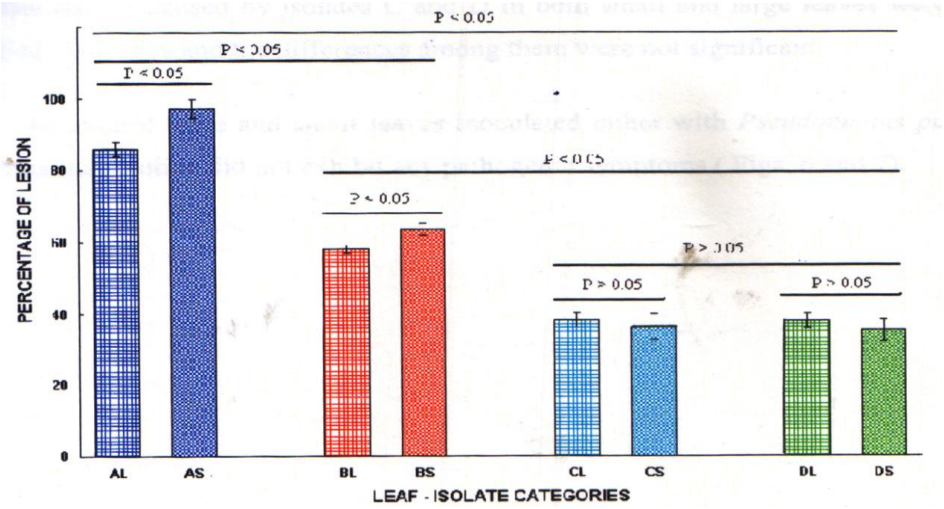

A - isolate A (sputum sample) shows larger length of lesion. B - isolate B (pus sample) shows moderate length of lesion. C and D - isolates C and D (urine samples) shows lower length of lesion. $C_1$ and $C_2$ - controls inoculated with *Pseudomonas putida and* sterile saline solution, no lesion was observed

**Fig.2** Smaller Leaves




A - isolate A (Sputum sample) shows larger length of lesion. B - isolate B (pus sample) shows moderate length of lesion. C and D - isolates C and D (urine samples) shows lower length of lesion. $C_1$ and $C_2$ - controls inoculated with *Pseudomonas putida* and sterile saline solution, no lesion was observed

**Fig.3** Extent of pathogenic lesion (in percent, Mean ±_SD) exhibited by the two size-categories of lettuce leaves due to inoculation with four clinical isolates of *Pseudomonas aeruginosa*




AL - Isolate A in large leaf, AS - Isolate A in small leaf, BL - Isolate B in large leaf
BS - Isolate B in small leaf, CL - Isolate C in large leaf, CS - Isolate C in small leaf
DL - Isolate D in large leaf, DS - Isolate D in small leaf
Statistical analysis- Multiple comparisons of means by Tukey's test following ANOVA

The plant model chosen in this study was lettuce (*Lactuca sativa*) as it was widely accepted as a test for bacterial pathogenicity (Dinghra and Sinclair, 1985) and the assay with lettuce leaves is faster and easier. This model was sufficiently sensitive to show disease symptoms after infection at low infectious doses and with a different clinical

isolates. In addition, the model system system was simple, rapid, and cost effective and required no special equipment.

The plant human pathogen model is also of evolutionary interest as it bridges the divide between plant and animal pathogenesis (Prithiviraj *et al*., 2004). In the present study, plant model is used to screen the pathogenicity of *Pseudomonas aeruginosa* isolated form different clinical samples like pus, sputum, wound and urine which causes cystic fibrosis, respiratory tract infections, wound infections, urinary tract infections etc.

Totally 10 clinical samples were collected, 4 isolates were identified as *Pseudomonas aeruginosa* and inoculated in the mid rib of the lettuce leaves and the lesion has been measured to determine pathogenicity. In the previous study, the diameter of the lesion has been measured (Martha Vivas-Flores and Diana Garnica, 2006). In the current study, the length of lesion has been measured. Few workers have tried to compare the virulence of wild and mutant type of *P. aeruginosa* in lettuce leaf model. Virulence was assayed in the lettuce model of infection and symptoms were monitored as hypervirulent, severe, moderate, weak, and none (no signs of infection) based on the extent of lesion (Melnie Filia Trault *et al*., 2006). Thus in the current study, the length of the lesion measured on lettuce leaf which indicates the level of pathogenicity as severe, moderate and low from the sputum (A), pus (B), and urine samples (C and D) is comparable to that reported by Filiatrault *et al*. (2006).

In previous studies, the pathogenicity of *Pseudomonas aeruginosa* has been compared between the clinical and environmental isolates (Martha Vivas-Flores and Diana Garnica, 2006). In the present study, the pathogenicity of *P. aeruginosa* has been compared between different clinical isolates, as the pathogenicity varies from strain to strain. It has long been recognized that *P. aeruginosa* strains isolated from sputum sample of CF (cystic fibrosis) patients differ from other *P. aeruginosa* strain, but it has not been fully appreciated how this can affect pathogenesis.

The inoculation has been done on both the larger outer leaves and smaller inner leaves. In previous studies, different modes of inoculation has been followed in plant models to determine the bacterial pathogenicity as the leaves were infiltered with a bacterial suspension (Rahme *et al*., 1995) using a 1 ml disposal syringe without a needle and in some experiments whole leaves or 4mm diameter leaf discs cut from the central portion of a leaf with a cork borer were immersed in bacterial suspension. Midrib route of inoculation may be considered as a more appropriate method for the assessment of pathogenicity. Hence in the present study, the midrib route of inoculation was chosen to delineate the pathogenicity of bacterial isolates.

The length of the leaf chosen in this study was 10 cm which is of larger in size and 6 cm which is of smaller in size to indicate that both the larger and smaller leaves can be used as a plant model to determine the pathogenicity of bacterial isolates.

In previous studies, workers have inoculated the pathogen *Xanthomonas campestris* pv. *vitans* in lettuce and they reported that lesion appeared on the leaves were brown which on later turns to black colour and the lesion may expand along the veins of the leaf (Sahin and Miller, 1997; Toussaint, 1999). In the present study after the inoculation and incubation in a humid chamber, the lesion that appeared on the larger and smaller leaves is brown in colour, which later on

turns to black color. These results are in agreement with those reported by the workers on the pathogen *Xanthomonas campestris* pv. *vitans* in lettuce.

Isolate (A) which has been inoculated in lettuce leaf shows the largest length of lesion (86%) which is considered to the more pathogenic. Isolate (B) shows the moderate level of pathogenicity (58%). Isolates (C and D) indicates the low level of pathogenicity (38% and 37%) in larger leaves respectively. As a control *Pseudomonas putida* and sterile saline solution has been inoculated in lettuce leaf, no lesion has been observed which seems to be non-pathogenic.

Same isolates when inoculated on smaller leaves of lettuce, same level of pathogenicity for each isolate has been observed. Isolate (A) shows 97%, isolate (B) shows (63%) and isolate (C and D) shows (36% and 35%) of lesion respectively. In previous studies, workers reported that *Pseudomonas aeruginosa* has been the predominant bacterium associated with pulmonary infection in patients with CF (cystic fibrosis)(Fick *et al*., 1981; Fitz Simmons, 1993; Govan and Deretic, 1996; Barth *et al*., 1998). In their study, *P. aeruginosa* was also found as an implicated organism in 82% culture positive patients and its rate of infection was significantly higher as compared to the other organisms in CF patients. The colonization with *P. aeruginosa* has been linked with pulmonary deterioration of CF patient resulting in an increased risk of hospitalization (Fick *et al*., 1981). They reported that, all the patients (n = 9) who required hospitalization were chronically infected with *P. aeruginosa*. Besides morbidity, *P. aeruginosa* has been associated with increased mortality (Fick *et al*., 1981; Fitz Simmons *et al*., 1993; Govan *et al*., 1992; Barth and Pitt, 1998). In their study all the 4 patients who died were chronically infected with *P. aeruginosa*. Thus significant higher rates of hospitalization and death were observed among patients chronically infected with *P. aeruginosa*. Thus in the current study the isolate which seems to be more pathogenic in plant model was the isolate (A) sputum sample which has been collected from a patient suffering from cystic fibrosis. Thus the results are comparable to those workers reported in their previous studies.

But in smaller leaf model the level of pathogenicity for the isolate (A) and isolate (B) seems to be more pathogenic in which the length of the lesion is greater than the larger leaf and this may be due to the smaller size of the leaf because, same concentration of the bacterial suspension which contained approximately $0.8 \times 10^9$ CFU/ml and same incubation time has been followed for both larger and smaller leaves. If the incubation time has been prolonged, the length of the lesion in larger leaf may also extend, and the level of pathogenicity may be increased for the isolate (A) and isolate (B). Thus, there is a significant interaction between the isolates and leaf size. But the lesions caused by isolated (C and D) in both smaller and larger leaves were in the range of 33-38% and the differences among them were not significant.

The results of this investigation demonstrated that sputum sample (A) which has been collected from the patient suffering from cystic fibrosis shows higher level of pathogenicity and pus sample (B) shows the moderate level of pathogenicity. Urine samples (C and D) when compared to sputum sample (A) and pus sample (B), the pathogenicity level is low when inoculated on both the larger and smaller leaves of

lettuce. Thus the pathogenicity of the bacterial isolates varies between strains. Further studies should be carried out for those four isolates to check whether it produces the same level of pathogenicity when inoculated in laboratory animals.

## References


Agnihotri N, Gupta V and Joshi R M. 2004. Aerobic bacterial isolates from burn wound infections and their antibiograms-a five year study. *Burns.* 30(3): 241 - 243.

Anzai Y, Kim H, Park J Y, Wakabayashi H and Oyaizu H. 2000. Phylogenetic affiliation of the Pseudomonads based on 16S rRNA sequence. *Int. J. Syst. Evol. Microbiol*. 50 (4):1563-89.

Ausubel F M. 2000. Arabidopsis Genome: A Milestone in Plant Biology. *Plant Physiol.* 124:1451-1454.

Barth A L, Pitt T L.1998. Microbial pathogens associated with Cystic fibrosis: special focus on *Pseudomonas aeruginosa*. *Braz.J.Infect.*Dis. 2:43-61.

Britigan B E, Rasmussen G T, Cox C D. 1997. Augmentation of oxidant injury to human pulmonary epithelial cells by the *Pseudomonas aeruginosa* siderophore pyochelin. *Infect. Immun*. 63:1071-1076.

Chikara Kaito and Kazuhisa Sekimizu. 2007. A Silkworm model of pathogenic bacterial infection. *Drug.Discov.Ther*. 1(2):89-93.

Cho J J, Schroth M N, Kominos S D, Green S K. 1975. Ornamental plants as carriers of *Pseudomonas aeruginosa. Phytopathology*. 65;425 - 431.

D'Argenio D A, Gallagher L A, Berg C A, Manoil C. 2001. Drosophila as a model host for *Pseudomonas aeruginosa* infection. *J.Bacteriol*.183:1466-1471.

Diekema D J, Pfaller M A, and Jones R N.1999. Survey of blood stream infections due to gram negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates. *Clin. Infect. Dis.* 29(3):595-607.

Dinghra O D and Sinclair J B.1985. Basic plant pathology methods. CRC Press, Boca Raton, FL.

Eleftherios Mylonakis, Artura Casadevall, and Ausubel Frederick. 2007. Exploiting Amoeboid and Non-Vertebrate Animal Model Systems to study the Virulence of Human Pathogenic Fungi. PLOS. *Pathog.* 3(7):10.

Elrod R P and Braun A C. 1942. *Pseudonmonas aeruginosa:* its role as a plant pathogen. *J. Bacteriol*. 44:633-645.

Fick R B Jr, Naegel G P, Matthay R A, and Reynolds H Y. 1981. Cystic fibrosis *Pseudomonas* opsonins. Inhibitory nature in an in vitro phagocytic assay. *J. Clin. Invest*.68(4):899 - 914.

Filiatrault M J, Picardo K F, Nagai H, Passador L and Iglewski B H. 2006. Identification of *Pseudomonas aeruginosa* Genes Involved in Virulence and Anaerobic Growth. *Infect. Immun*. 74(7): 4237-4245.

FitzSimmons SC. 1993. The changing epidemiology of cystic fibrosis. *J. pediatr*.122(1):1-9.

Gordon S M, Serkey J M, Keys T F, Ryan T, Fatica C A, Schmitt S K, Borsh J A, Cosgrove D M and Yared J P. 1998. Secular trends in nosocomial blood stream infections in a 55-bed cardiothoracic intensive care unit. *Ann. Thorac. Surg*. 65(1): 95-100.

Govan J R and Deretic V.1996. Microbial Pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkolderia cepacia*. *Microbiol. Rev.* 60:539-74.

Iglewski B H. 1996. *Pseudomonas* In: *Baron's Medical Microbiology,* 4th ed.

Univ of Texas Medical Branch.

Ikpeme E M, Enyi-Idoh K H, Nfongeh J F, Etim L B, Akubuenyi F C. 2013. Prevalence, antibiogram profile and cross transmission of Pseudomonas aeruginosa in a tertiary burn unit. Mal. J. Microbiol. 2013; 9: 116-9.

Kolpen M, Kühl M, Bjarnsholt T, Moser C, Hansen C R, Liengaard L, Kharazmi A, Pressler T, Høiby N, Jensen P Ø. 2014. Nitrous oxide production in sputum from cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. *PLoS One.* 9 (1):e84353.

Mahajan-Miklos, Tan S M V, Rahme L G, Ausubel F M. 1999. Molecular Mechanism of Bacterial Virulence elucidated using a *Pseudomonas aeruginosa- caenorhabditis elegans* pathogenesis model. *Cell.* 96(1):47-56.

Martha Vives-Florez and Diana Garnica. 2006. Comparison of Virulence between Clinical and Environmental *Pseudomonas aeruginosa* isolates. *International Microbiology*. 9:247-252.

Martinez C, Pons E, Prats G and Leon J. 2004. Salicylic acid regulates flowering time and links defence responses and reproductive development. *Plant. J.* 37(2):209-217.

Miyata S, Casey M, Frank D W, Ausubel F M d Drenkard E. 2003. Use of the *Galleria mellonella* caterpillar as a model host to study the role of the type III secretion system in *Pseudomonas aeruginosa* pathogenesis. *Infect Immun.* 71(5):2404-13.

More S, Raut S S, Gujar V M, Rathod V S, Rajhans V V, and Kale C D. 2015. Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated From Various Clinical Samples at a Tertiary Care Centre. *International Journal of Health Sciences and Research (IJHSR)*, 5 (1), 119-124.

Pier G B, Grout M, Zaidi T S, Olsen J C, Johnson L G, Yankaskas J R and Goldberg J B. 1996. Role of mutant CFTR in hyper susceptibility of cystic fibrosis patients to lung infections. *Science*. 271: 64 - 67.

Pollack M. 1995. *Pseudomonas aeruginosa*. *Principles and practice of infectious diseases*. 4th ed pp. 1980-2003.

Prithviraj B, Weir T, Bais H P, Schweizer and Vivanco J M. 2004. Plant models for animal pathogenesis. *Cellular Microbiology*. 7(3):315-324.

Rahme L G, Stevens E J, Wolfort S F, Shao J, Tompkins R G, Ausubel F M. 1995. Common virulence factors for bacterial pathogenicity in plants and animals. *Science*. 268:1899 -1902.

Rahme L G, Tan M-W, Le L, WWong S M, Tompkins R G, Calderwwood S B and Ausubel F M. 1997. Use of plant model hosts to identify *Pseudomonas aeruginosa* virulence factors. *Poc.Natl.Acad.Sci.USA*. 94:13245 - 13250.

Ryan K J and Ray C J. 2004. *Sherris Medical Microbiology*, 4th ed, Mc Graw Hill.

Sahin F and Miller S A. 1997. Identification of the bacterial leaf spot of lettuce, *Xanthomonas campestris* pv.*vitians* in Ohio and assessment of cultivar resistance and seed treatments. *Plants dis*. 81:1443-1446.

Toussaint V. 1999. Bacterial leaf spot, a new disease of lettuce in Quebec caused by *Xanthomonas campestris* pv.*vitians*. *Phytoprotection.* 80:121-125.

Walker B H, Holling C S, Carpenter S R, and Kinzig A. 2004. Resilience, adaptability and transformability in social-ecological systems. *Ecology and Society.* 9(2):5.

Wiblin R T. 1997. Nosocomial pneumonia: *Prevention and control of Nosocomial infection*. 3rd ed.pp. 807-819.