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Identification of *Pseudomonas fluorescens* using different biochemical tests

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Abstract

Pseudomonas fluorescens not only enhances the plant growth but also controls the fungal pathogens by production of anti fungal metabolites. The objective of this experiment was to identify *P. fluorescens* using different biochemical tests. This research was carried out in Plant Pathology Laboratory at Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. The result of this experiment indicated that *P. fluorescens* gave positive result for Catalase test, Gelatin liquefaction, Fluorescent pigment and Oxidase test but negative result for starch hydrolysis test. The colony of *P. fluorescens* was maximum in maize seed than that of rice seed after two hours of inoculation, whereas higher number of colony was found in rice seed than that of maize seed after twenty four hours of seed inoculation. This findings is useful for identifying colony of *P. fluorescens* per seed which is necessary for better seedling growth and effective biological control of pathogens.

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Keyword
Inoculation, Rice and maize seed, Serial dilution, *Pseudomonas fluorescens*

Introduction

Pseudomonas species encompasses a group of common rod shape, gram negative, one or more polar flagella providing mortality, aerobic in nature, non-spore farming, positive catalase test, positive oxidase test, nonpathogenic saprophytes that colonize soil, water and plant surface environments. It also secretes pyoverdine a fluorescent yellow green siderophore under iron limiting condition. Pseudomonas fluorescent also produces additional type of siderophore such as thioquindobactin (Scales et al., 2014). It is an obligate aerobe, except for some strains that can utilize NO_3 as an electron acceptor in place of O_2. It is motile by means of multiple polar flagella. *Pseudomonas fluorescens* has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources.

P. fluorescens is saprophytic fluorescent micro-organism, which are very common in soil around the rhizosphere of various plant species that could improve the plant vigour by incorporating free nutrient and plant growth hormone. In case of cereal crop, it help in

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increasing crop productivity by reducing soil borne disease and stimulating effect on plant growth.

A free living Plant growth promoting rhizobacteria (PGPR) *Pseudomonas fluorescens* are able to colonize around plant root and incorporate free atmospheric molecule into plant absorbable nutrient form along with improved plant growth and crop yield.

Iron chelation, antibiotic production, enhanced nutrient uptake and seedling emergence promotion and by plant phytohormone production. Siderophore are iron chelating compounds secreted by bacteria on or around the roots that affect the growth of the plants rhizobacteria. The PGPR, being more potent chelators, starve the deleterious rhizobacteria of their iron nutrient, thus protect the plants from the harmful effects of DRB, resulting in better growth and yield (Sakthivel et al., 2009).

Microbial inoculation in seed of crop increases the root biomass production and more colonization of beneficial micro-organism, which are interdependent with these considerations. This micro-organism is natural bio-controllers and improves soil fertility through its own effective Bio fertilizer potential PGPR stains. New research found that P. fluorescens predominant inhabitants of soil and aquatic environments. It plays in the very important role in the purification of pollutants of bioremediation from large chemical industry and secondary metabolites decay material and bio-control some harmful pathogen against crop.

Global food security was threatened by some crop disease and degrading soil fertility. Excess use of chemical fertilizer aiming increase crop yield reporting declining soil fertility lead to desert some place in the world. Root- associated soil bacteria would be the potential solution by improving soil condition and promoting defensive mechanism against various pathogens through improving plant growth mechanism.

The objective of this research was to identify *Pseudomonas fluorescens* using different biochemical tests and to identify the total number of its colony present in different seed at different time of inoculation.

Materials and Methods

Experimental Location dan Preparation

This experiment was conducted in Plant Pathology Laboratory at Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal in October 03, 2018. The liquid composition of *Pseudomonas fluorescens* were bring form the Agri-care and live-care pvt. Ltd., which was examined through different Bio-chemical test to assure the liquid component consist of bacteria *Pseudomonas fluorescens*.

Morphological characterization

Pure cultures of the selected isolates were streaked on King's B agar Petri plates separately for colony development.

Biochemical tests for *P. fluorescens*

For the identification of *P. fluorescens*, certain biochemical tests were conducted according to Bergey's Manual for Determinative Bacteriology.

Starch hydrolysis

Filter paper was dipped in a dry old culture suspension and was placed on Petri dishes containing starch agar medium and incubated for two days. The plates were than flooded

with one per cent iodine solution. A colorless halo around the growth and blue color in the rest of the plates showed utilization of starch by the microorganism.

Gelatin liquefaction

Filter paper discs were dipped in a day old culture suspension and were placed on Petri dishes containing gelatin nutrient agar medium. The Petri dishes were incubated at 300C for two days and then flooded with 12.5% $HgCl_2$ solution. The development of yellow halo around the growth indicates utilization of gelatin.

Fluorescent pigment

The test tubes containing sterilized Kings B medium were inoculated with the isolate of Pseudomonas sp. incubated for five days and observed. Yellowish green fluorescent pigment observed under UV light (365 nm) indicated positive results.

Catalase test

Capillary tubes (1mm outer isolated by 67mm in length0 were placed in a 50-ml beaker containing about 10 ml of 3% H_2O_2. A positive result is the rapid evolution of oxygen by bubbling. This phenomena is due to the breakdown of hydrogen peroxide H_2O_2 in to water and oxygen.

Oxidase test

Saturate Whatman no.1 filter papers with a 1% aqueous solution of Diemthyl-p-phenlyene- diamine oxalate. Development of red color within a 10 sec indicates a positive reaction.

Inoculation of Pseudomonas into a seed and Serial Dilution

The seed of Maize (Variety: Rampur Composite) and Rice (Variety: Masuli) were inoculated with *Pseudomonas fluorescens* at 2.5 ml kg^{-1} seed in 5% sugar solution. Let the seed for two hours soaked and secondly twenty four hours. Single seed was dipped into test tube containing a 10 ml of distilled water and shake the tube for one minute. One milliliter of diluted solution from first tube is transfer to second tube containing nine milliliter of distilled water. The process was continue up to six tube and solution made 1/1000000 diluted.

Material for Kings B medium

Proteose peptone (20g), K_2HPO_4 (1.5g), $MgSO_4.7H_2O_4$ (1.5g), Agar (15g), Glycerol (1.5 mL) and water (1 liter) were used in this experiment.

Results and Discussion

The entire test showed the presence of *Pseudomonas fluorescens* in the liquid. When the UV is ray was passed into *P. Fluorescens,* a shiny bluish yellow green fluorescent pigment was observed (Figure 1).

Much of the impetus in the study of “unusual” Gram-negative bacilli from clinical sources, was derived from King in Atlanta. The production of a fluorescent pigment on King’s medium B was characteristic of most isolates of *P. putida, P. fluorescens* and *P. aeruginosa. P. fluorescens* perform the best typical gluconate (almost always positive), urea (positive after

2-3 days), casein (positive), and gelatin (positive) reactions at room temperature (King and Phillips, 1978).

Non-fermenting, catalase-positive Gram-negative bacilli that grow on nutrient agar are often isolated in clinical laboratories. Biochemical techniques appropriate to a typical clinical microbiology laboratory, and for the most part described in Cowan and Steel's Manual for the identification of medical bacteria. On the basis of the oxidation-fermentation test and further tests for oxidase activity and motility, the organisms were allocated to one of several subgroups on the model of Cowan and Steel. However, it was found that the production of alkali in the oxidation-fermentation test was a very useful characteristic and had therefore differentiated it from the “no reaction" group of Cowan and Steel (King and Phillips, 1978).

Solubilization of mineral phosphates and mobilization of other essential nutrients by PGPR also helps in growth improvement of plants. The cell wall of plant cells are mainly composed of cellulose, which was embedded in an amorphous polysaccharide matrix of hemicelluloses, pectin and some glycols and proteins (Meyer and Abdallah, 1978). Also oxidized inositols served as non cellulosic cell wall components.

Table 1. Biochemical characteristics of *Pseudomonas fluorescens*

No.	Biochemical tests	Identification of *P. fluorescens*
1	Starch hydrolysis	Negative
2	Gelatin liquefaction	Positive
3	Fluorescent pigment	Positive
4	Catalase test	Positive
5	Oxidase test	Positive

Pseudomonas fluorescens gave positive result for Catalase test, Gelatin liquefaction, Fluorescent pigment and Oxidase test but negative result for starch hydrolysis test.

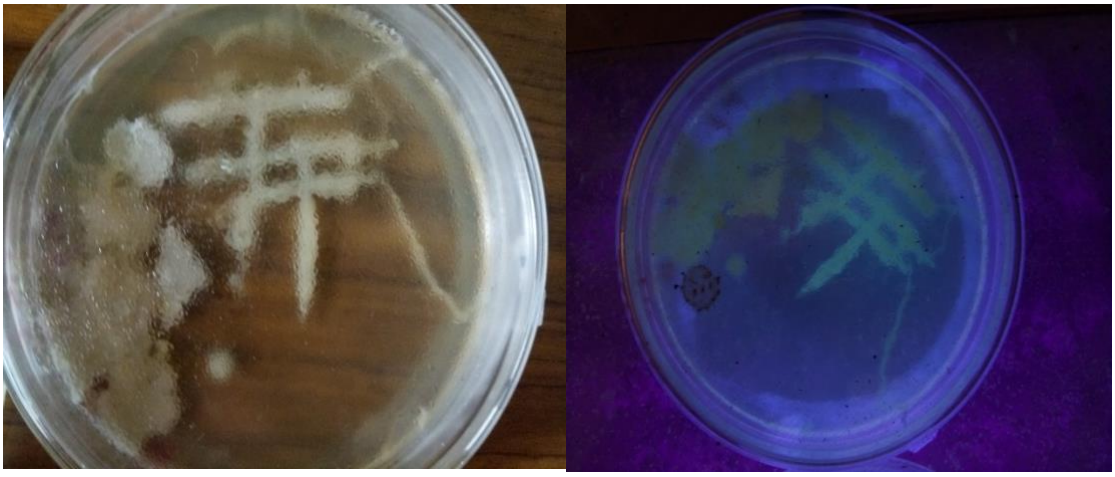

Figure 1. Fluorescent test under UV ray.

Colony of *P. fluorescens* per seed

After serial dilution to 10^6 of individual seed of Maize and Rice, which was let growth for one day in kings B medium (six media) at 35^0C. Colony of *P. Fluorescens* was found to be

higher after two hours of inoculation than that of twenty four hours. Similarly, the higher colony was found in maize seed than rice after two hours and vice-versa after twenty fours respectively. It was count on an average at two hours of inoculation was $2.17*10^8$(cfu/ml/seed) for rice and $1.81*10^8$ (cfu/ml/seed) for maize, whereas colony was count as $1.35*10^8$ (cfu/ml/seed) and $1.2*10^8$ (cfu/ml/seed) for maize and rice respectively. *i.e. cfu= colony forming units

Table 2. Colony of *P. fluorescens* per inoculated seed at different time period

Particulars	Time	
	2 hrs	24 hrs
Maize	$1.69*10^8$ cfu/ml/seed	$1.27*10^8$ cfu/ml/seed
	$1.94*10^8$ cfu/ml/seed	$1.53*10^8$ cfu/ml/seed
	$1.80*10^8$ cfu/ml/seed	$1.26*10^8$ cfu/ml/seed
Rice	$2.18*10^8$ cfu/ml/seed	$8.9*10^7$ cfu/ml/seed
	$2.12*10^8$ cfu/ml/seed	$1.28*10^8$ cfu/ml/seed
	$2.21*10^8$ cfu/ml/seed	$1.43*10^8$ cfu/ml/seed

*cfu=colony forming units.

The prevalence of pseudomonas spp. was 68% and 58% with mean count $9.02x10^4±2.87x10^4$ and $2.43x10^5±9.32x10^4$ for Damietta and kariesh cheese respectively. *Pseudomonas spp* were not detected in Feta cheese. The most prevalent spp was *p. fluorescens* (35.14% and 45.5%)

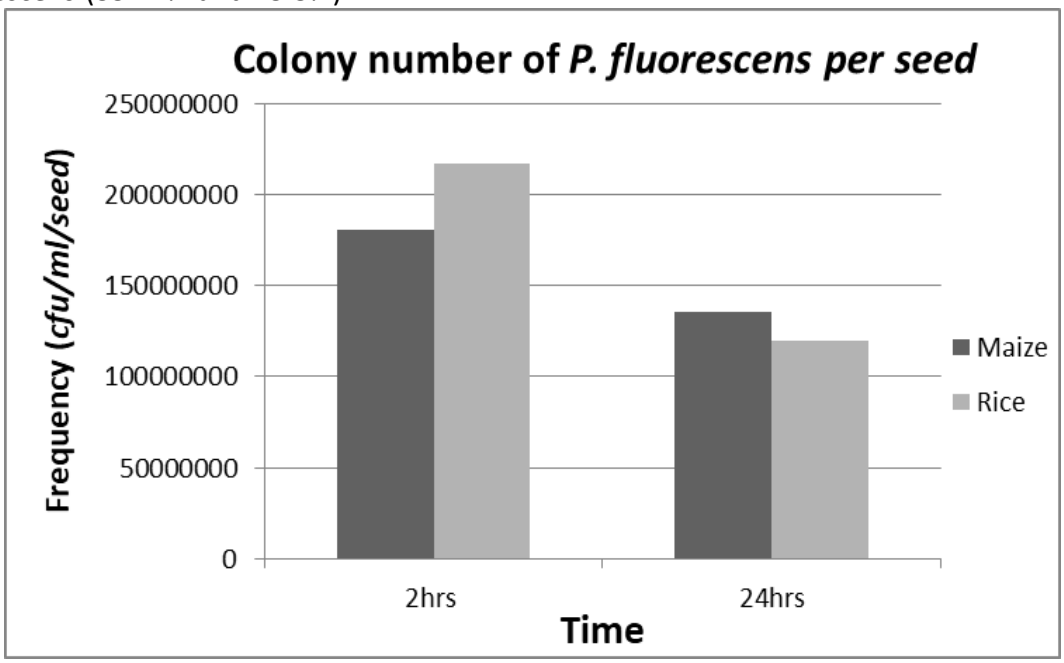

Figure 2. Colony of *P.fluorescens* per seed with respect to time.

while *P. aeruginosa* (21.26% and 15.2%); *P. putida* (27% and 18.2%) and *P. stutzeri* (16.21%and 21.21%) for Damietta and kariesh cheese respectively (El-Leboudy et al., 2015). Treatments, which were nominal target concentrations of SDP (as active ingredient) of 50, 100, 200, and 300 milligrams per liter (mg/L), were continuously applied for 24 hours (Luoma et al., 2015). Damietta cheesewere positive samples for pseudomonas spp. with mean count 9.02 x 104±2.8x104.

Conclusions

Pseudomonas fluorescens a Bio-control agent against various plant diseases was most effective after two hours of inoculation in larger size seed than that of small size seed, whereas soaking of inoculated small size seed for twenty four hours result grater colony per seed.

Aknowledgements

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