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ISOLATION AND CHARACTERISTICS OF FLUORESCENT PSEUDOMONADES' SPECIES OF PADDY FIELDS IN THE NORTH OF IRAN

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A collection of fluorescent pseudomonades isolated from rice rhizosphere was created. Bacteria were selected from farmer's fields in three provinces at north of Iran during the 2006 summer. By their morphological properties and the ability to fluorescent under UV light 111 cultures were identified as fluorescent species of pseudomonades. The isolates were characterized by biochemical and physiological traits too. Presence of oxidase, catalase, citrate and arginine hydrolyses and ability to ferment glucose were peculiar to all tested species; besides *Pseudomonas fluorescent* used trehalose and mesoinositol, *Pseudomonas putida* did not liquefy gelatine, and only *Pseudomonas aeruginosa* could grow at 41^0. Frequency of occurrence of *P. fluorescent*, *P. putida* and *P. aeruginosa* among tested fluorescent species was equal to 44.2%, 33.3% and 22.5% correspondingly.

Ստեղծվել է բրնձի ռիզոսֆերայից անջատված ֆլուորեսցենտ պսևդոմոնադների հավաքածո: Բակտերիաները անջատվել են հյուսիսային Իրանի երեք մարզերի ֆերմերային դաշտերից 2006 թ-ի ընթացքում: Այս բակտերիաների 111 կուլտուրաները մորֆոլոգիական առանձնահատկությունների և ուլտրամանիշակագույն ճառագայթների տակ ֆլուորեսցենցիայի ունակության հիման վրա նույնականացվել են որպես պսևդոմոնադների ֆլուորեսցենտ տեսակներ: Մեկուսացված բակտերիաները բնութագրվել են նաև իրենց կենսաքիմիական և ֆիզիոլոգիական հատկություններով: Օքսիդազների, կատալազների, ցիտրատի և արգինինի հիդրոլազների առկայությունը, ինչպես նաև գլյուկոզ խմորելու ունակությունը բնորոշ է բոլոր տեսակներին, այն դեպքում, երբ տրեգալոզ և մեզոինոզիտ յուրացնելու ունակությունը՝ *Pseudomonas fluorescent* տեսակին: Ժելատինը քայքայելու հատկությամբ օժտված է *Pseudomonas putida* տեսակը, իսկ 41° ջերմաստիճանային պայմաններում աճելու ունակությամբ օժտված է *Pseudomonas aeruginosa* տեսակը: Ուսումնասիրված ֆլուորեսցենտ բակտերիաներից *P. fluorescent, P. putida* և *P. aeruginosa* տեսակների հանդիպման հաճախականությունը համապատասխանաբար կազմել է 44.2%, 33.3%, 22.5%:

Создана коллекция флуоресцирующих псевдомонад, изолированных из ризосферы риса. Бактерии отобраны с фермерских полей трех провинций северного Ирана в течение 2006 года. 111 культур этих бактерий по морфологическим признакам и способности флуоресцировать в ультрафиолетовых лучах предварительно идентифицировны как флуоресцирующие виды псевдомонад.

Изоляты характеризовались и по своим биохимическим и физиологическим признакам. Наличие оксидазы, каталазы, гидролаз цитрата и аргинина и сбраживание глюкозы было свойственно всем видам, тогда как способность усваивать трегалозу и мезоинозитол характерна для *Pseudomonas fluorescent*, отсутствие способности разжижать желатину - для *Pseudomonas putida*, а рост при 41^0 - для *Pseudomonas aeruginosa*. Частота встречаемости *P. fluorescent*, *P. putida* и *P. aeruginosa* составляла соответственно 44.2%, 33.3%, 22.5% от изученных флуоресцирующих видов.

Identification of bacteria – rice rhizosphere – fluorescent pseudomonades

Saprophytic fluorescent pseudomonades include various species such as *Pseudomonas fluorescens, P. putida*, *P. aeruginosa* [4, 10, and 22]. All bacteria belonging to this group share the ability to produce soluble yellow-green pigments – pyoverdines, which act as siderophores for these bacteria [3, 14]. These microorganisms are considered to be rhizobacteria, since their density, propagation and activity are stimulated in the rhizosphere [1]. They are known also by the ability to synthesize a variety of other secondary metabolites [5, 6], which intensify the plant growth. Plant growth promotion can also be ascribed to other metabolites affecting the plant physiology such as formation of growth substances [18]. Consequently, some fluorescent pseudomonads can improve plant health and/or growth [2, 24]. Indeed, fluorescent pseudomonads are considered as potential biocontrol agents of soilborne diseases. Several studies have demonstrated their efficacy as microbial inoculants [1, 8, 20]. Most pseudomonads are free-living organisms in soil and water; they play an important role in decomposition, biodegradation, and the C and N circulations. The phrase “naturally-occurring organic compound cannot be degraded by some microorganisms" must have been coined to apply to members of the genus *Pseudomonas*, known for their ability to degrade hundreds of xenobiotic. However, they are usually unable to degrade some biopolymers in their environment, such as cellulose and lignin, and their role in anaerobic decomposition is minimal.

Considering the high diversity of fluorescent pseudomonades the different characteristics, which were recorded for various strains, are not surprising [15].

The identification of the members of the genus *Pseudomonas* is based on morphological, physiological, biological and genetic characteristics [13, 22, 25]. Determination of predominate fluorescent pseudomonads strains is important owing to their positive effects on plants [1, 5]. So we carry out these experiments to identify local strains in different rice fields in north Iran for the subsequent treatment of plantlets by the selected bacterial species before planting.

Material and methods: Fifty rice fields of different locations in north Iran including three provinces (Mazandaran, Gillan and Golestan) were selected for collection of rice rhizosphere samples. From the field of each site three hills of rice were randomly selected during summer seasons of 2006. Each sample including 1 kg rhizosphere soil (root + adhering soil) was transferred to the laboratory in an ice-box and stored at 4^0C.

The isolation of cultures was done by method of Koch on the Cetramid agar [17]. The plates were incubated at 28^0C for 48 hours. Colonies, which fluoresced under UV light (260nm), were selected and purified later on the King's B-agar medium [25].

The following general methods of morphological, physiological and biochemical tests were used for identifying *Pseudomonas species* [4, 10, 19, 22]: staining by Gram, determination of catalase and oxidase activities, gelatin liquefaction, growth at 41^0 [7, 23], arginine hydrolysis, utilizing citrate as a sole carbon and energy source, acid formation in carbohydrate (trehalose, mesoinositol and glucose) broths [7, 19, 21].

Results and Discussion: A microscopic study of bacteria showed that all isolates were gram negative. The bacteria isolates, tested under UV light, displayed fluorescence and had yellow, green, yellow-green, blue, brown, orange and white colors (Table 1).

Table 1. Percentages of isolates with different colors.

Provinces	Different colors displayed by isolates under UV light						
	Orange	White	Brown	Yellow & green	Green	Yellow	Blue
Mazandaran	3.9	13.8	17.6	3.9	19.6	19.6	21.6
Gillan	0	2.7	5.4	5.4	13.5	35.2	37.8
Golestan	0	8.7	17.4	0	26.1	21.7	26.1
Total	1.8	9.1	13.5	3.6	18.9	25.2	27.9

The majority of isolates had blue color. Then the color of colonies distributed in the following way: yellow > green > brown > white > yellow-green and, at the last position, orange color. Earlier for the characterization of the genus *Pseudomonas* the pigmentation was used as a generic trait, but this is not of great validity now. In fact the colonies and other cell masses always display some color due to the presence of normal cellular component. Thus, *P. stutzeri* is grouped with the non-pigmented species though the colonies of its many strains are dark brown due to the high concentration of cytochrome *c* in the cell (13). As for the chemical nature of pigments, blue color produced by some isolates, is due to the phenazine - blue pigment pyocyanine identified for *P. aeruginosa* [11]. Other phenazine pigments synthesized by fluorescent pseudomonades are green. Important pigments from the physiological and taxonomic standpoints are pyoverdines. They are typical yellow-green pigments of some fluorescent pseudomonades [13].

The isolates were characterized by biochemical and physiological traits too. Presence of oxidase, catalase, citrate and arginine hydrolyses and ability to ferment glucose was peculiar to all tested species (Table 2).

Table 2. Results of biochemical and physiological tests (%).

Tests	Reaction	
	Positive	Negative
Catalase formation	100	0
Oxidise formation	100	0
Arginine hydrolysis	100	0
Citrate utilization	100	0
Gelatin liquefaction	33.3	66.7
Growth at 41^0C	22.5	27.5
Glucose utilization	100	0
Trehalose utilization	44.2	55.8
Mesoinositol utilization	44.2	55.8

As for the ability to liquefy gelatin, some of the tested isolates were negative: according to the different classification systems such isolates belong to the species *P. putida* [4, 10]. Some isolates could grow at 41^0C. According to the different classification systems the ability to grow at this temperature is peculiar to *P. aeruginosa* [13]. Though all isolates were able to use glucose as single source of carbon and energy, some of them could use trehalose and mesoinositol too. Such species were identified as *P. fluorescens* (9, 12, 17, 19).

So based on the obtained results we can conclude that there were three predominant fluorescent pseudomonade species: some isolates were identified as *P. fluorescens* (they could utilize trehalose and mesoinositol) - 44.2 %; some species as *P. putida* (they could not liquefy gelatin) - 33.3 %; and other species as *P. aerugenosa* (able to grow at 41^0C) - 22.5 %.

Fluorescent pseudomonades were distributed in various provinces irregular. So in Mazandaran province fluorescent pseudomonades species were distributed respectively *P. putida* > *P. aeruginosa* > *P. fluorescens*; in Gillan province - *P. fluorescens*> *P. putida*>*P. aeruginosa*; the same order was established for Golestan province. But as it can be seen from Table 3, the percentage of species was specific for each province. In this table and at the Figure 1 the total number of tested species for three provinces was shown too.

Table 3. Total quantity (%) of tested species in three provinces

Provinces (number of samples)	*P. aeruginosa*	*P. putida*	*P. fluorescens*
Mazandaran (51)	33.3	37.3	29.4
Golestan (23)	17.3	26.1	56.5
Gillan (37)	10.8	32.4	56.8
Total (111)	22.5	33.3	44.2

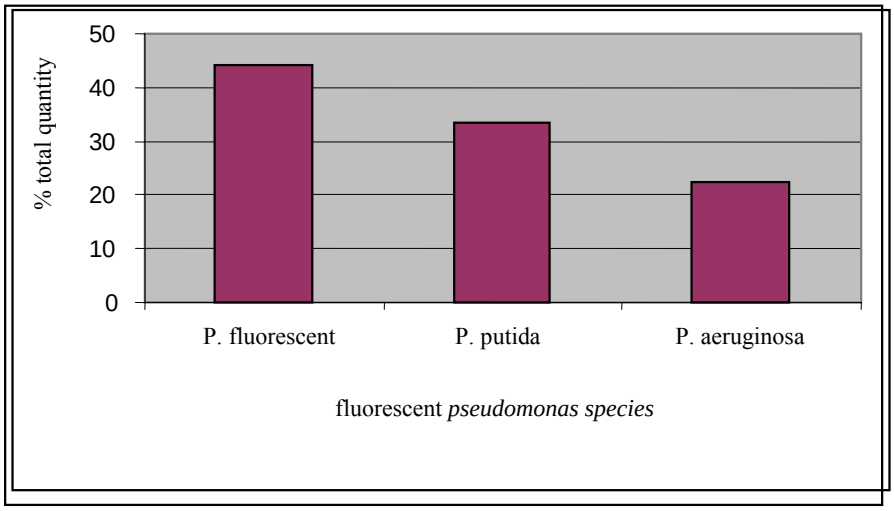

Fig. 1. Percentage of isolated bacterial samples from rice rhizosphere

The predominance in rice rhizosphere *P. fluorescens*, in comparison with others species, can be explained by its high competition ability and effective colonization. Some researchers reported the similar results. For example, Vlassak et al. (23) reported that dominating pseudomonad species of rice and banana rhizosphere in Sri-Lanka was *P. fluorescens.* On the other hand, Gardner et al. (7) - for citrus, Rasouli (17), Mozafar et al (16) - for many plants, particularly for wheat, reported that dominating fluorescent species was *P. putida*. Based on this research one can conclude:

1. For rapid identification of some fluorescent pseudomonades (*P. fluorescens, P. putida, P. aeruginosa*) it is sufficient to restrict oneself by the above mentioned tests. Final identification of these strains is possible only by using molecular analysis.

2. Number and distribution of fluorescent pseudomonad species varied at different places and identification of dominating species is important by other researchers and in the other geographical regions, where endemic bacteria with specific genotypes can be detected.

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