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Contribution to the Ecobiological Study of the *Pseudomonas fluorescens* Rhizobacteria

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ABSTRACT

The present work aimed at monitoring the abundance and diversity of indigenous *P. fluorescens* organisms in three different ecosystems. The number of culturable *P. fluorescens* organisms was counted on the King's medium B and identified by the use of the Analytical Profile Index (API 20 NE; bio Merieux), data are reported as CFU g^{-1}. Quantitative results have confirmed that *P. fluorescens* are successful root colonizers occupying different ecological niches, with the dominance of Biovar I (80%). *P. fluorescens* distribution were correlated significantly ($p<0.05$) with the majority of soil properties. Pearson's correlation coefficients of the relationship between *P. fluorescens* abundance and soil physicochemical properties indicated a significant ($p<0.05$) positive correlation. However, *P. fluorescens* abundance displayed a negative linear relationship with pH ($r = 0.62$) and clay ($r = 0.51$). No significant ($p>0.05$) relationships for the organic matter ($r = 0.13$, $p = 0.48$) and soil depth ($r = 0.099$, $p = 0.62$). The present findings suggested that the abundance of *P. fluorescens* may be strongly influenced by some abiotic and biotic factors.

Key words: *P. fluorescens,* abundance, ecosystems, correlated, soil, properties

INTRODUCTION

The fluorescent *Pseudomonas* species have attracted significant interest in the fields of biocontrol. Consequently, the production of microbial inoculants depends essentially on understanding the full mechanistic of their environmental adaptation and fitness. However, few comprehensive studies have described the abundance of this soil borne bacteria in the region of Mascara (Northern-Algerian West). *Pseudomonas* are a metabolically diverse and active group of bacteria and are an important part of the soil microbiota (Jayasinghearachchi and Seneviratne, 2010). They are one of the most important bacteria inhabiting the rhizosphere of diverse crop plants and have been frequently reported as Biological Control Agents (BCAs) (Costa *et al*., 2006). It has been shown that *P. fluorescens* strains are able to produce a wide range of active metabolites. The soil borne *Fluorescent pseudomonas* has been the subject of several studies in the past years. Because of their biocontrol activities, root colonizing and catabolic versatility. These mechanisms occurs through a synthesis of a great variety of compounds such as siderophores (Djibaoui and Bensoltane, 2005; Weller, 2007; Wensing *et al*., 2010; Reddy *et al*., 2010), phenazine (Saha *et al*., 2008), antibiotics (Haas and Keel, 2003; Farhan *et al*., 2010) and hydrogen cyanide (Bagnasco, 1997).

Number of rhizosphere bacterial strains belonging to *Fluorescent pseudomonads* have been used as seed inoculants to promote plant growth and increase yields (Vikram *et al*., 2007). Their bioaccessibility in soil and rhizosphere are complex but inter-related, the *P. fluorescens* surveys and activity in soils and rhizosphere are influenced by a great number of biotic and abiotic environmental factors. The ecological distribution of *P. fluorescens* in semiarid climate is less documented, presumably because of the limited knowledge of their abundance, activity and the factors that govern their abundance (Personal communication).

The present study was conducted in the region of Mascara (Northern-Algerian West). Thus, this study aimed to investigate the ecological distribution of *P. fluorescens* in different ecosystems and to elucidate the effect of soil properties on the abundance of these fluorescent communities to be used to control soil-borne phytopathogens.

MATERIAL AND METHODS

Soil sampling: This study was carried out during 2006/2007. It was conducted in three field sites characterizing different ecosystems: the experimental farm (agroecosystem), the forest of El-Zakour (forest ecosystem) and the plain of El- Kouayeur (humid ecosystem) located on the same geographic area of Mascara (Northern-Algerian West, 2°,11'W, 35°, 26 'N).

Soils were sampled in spring and summer from 9 sites (one profile per species (*Vicia* spp: C1-C2-C3, *Triticum* spp: C4-C5-C6, *Lens* spp: C7-C8-C9; *Pinus* spp: C10-C11-C12, *Asphodelus* spp: C13-C14-C15, *Tamarix* spp: C16-C17-C18; *Triticum* spp. (C19-C20-C21) *Cirsium* spp. (C22-C23-C24) *Hordium* spp. (C25-C26-C27).

Soil physical and chemical analysis: Air-dried samples were analysed for soil texture (determination of the percentage of sand, silt and clay by using either the pipette method), soil water, soil pH (code MA. 1010-pH 1.0), % OM (MA. 1010-PAF 1.0), % $CaCO_3$ (Scheibler calcimeter) and the Electrical Conductivity (EC).

Microbiological analysis: Plate dilution methods on different agar media were used for determining culturable *P. fluorescens* populations. Their number was counted on the *Pseudomonas fluorescens*-selective King's B medium (King *et al*., 1954). All microbial enumerations were carried out in duplicate. Data are reported as CFU g^{-1} dry soil. The isolates were identified follows Bergey's manual for bacteriology methods systematic (Kreig and Holt, 2001). Cell suspension of *P. fluorescens* was prepared by streaking them from in nutrient broth+10% glycerol stored at-80°C into Tryptic Soy Agar (TSA) plates and incubating at 25°C for 36 h to activate it and check for purity (Saravanan *et al*., 2004).

Statistical analysis: A Pearson correlation coefficients were calculated to determine which factors that govern *P. fluorescens* abundance. The relationship between soil physical, chemical characteristics and the microbial variables was investigated using Principal Components Analysis (PCA) using a traditional Euclidean distance for soil properties and microbial abundance (Legendre and Legendre, 1998). All data processing the Pearson correlation coefficients and the ACP were performed by STATISTICA (version 7).

RESULTS AND DISCUSSION

Microbiological results: Results of the microbial count varied depending on the vegetation and soil properties. Jha *et al*. (1992) found that biological activity and composition of soil microbes are

generally affected by many factors including physico-chemical properties of soil, temperature and vegetation. The diversity was assessed using the traditional selective plating and direct viable count. The 27 fluorescent isolates appeared similar to *P. fluorescens*. Has fluorescent yellow-green pigment produced one King's B, cetrimide agar but not on King's A medium? Has also positive catalase, lipase, arginine dihydrolase, gelatinase, urease and did not hydrolyse starch, they could grow at 4°C but not at 41°C?

The abundance of *P. fluorescens* varied over the three ecosystems (Fig. 1), this difference may be attributed to differences in sampling location and/or the time of sampling.

The highest density of *P. fluorescens* (3.15. 10^{10} ufc g^{-1}) was signaled in the rhizosphere of *Vicia* spp and *Triticum* spp in the agroecosystem ecosystem. This one was characterized by an important heterogeneous group of soil borne agents like *Erwinia* spp., *Xanthomonas* spp., *P. aeruginosa*, *Enterobacteriaceae* species, *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Pythium* spp., *Bacillus* spp. and *Streptomyces* spp.

Among the 70 fluorescents Pseudomonas isolates, 38.57 % belonged to *P. fluorescens* (Fig. 2a), with the dominance of Biovar I (80%) (Fig. 2b).

However, in the forest ecosystem this biodiversity and heterogeneity had decreased with the predominance of *Xanthomonas* spp. Moreover *P. fluorescens* was less abundant with a max of 2.7. 10^{9} ufc g^{-1} in the *Asphodelus* spp rhizosphere and absent in *Pinus* spp., *Tamarix* spp. rhizospheres. Within the humid ecosystem, a significantly higher level of indigenous *P. fluorescens* (2.7. 10^{11} ufc g^{-1}) was signaled in the rhizosphere of spontaneous *Triticum* spp., *Cirsium* spp. and *Hordium* spp.

As a consequence, *in situ* differences in *P. fluorescens* abundances among ecosystems are likely driver by variation in biotic and abiotic conditions. These fluorescents communities exhibited high rates in humid ecosystem soils (open system) but an average rate in the agro ecosystem. This is due probably to the occurrence of antagonism and competition for iron acquisition between *Leguminosae* siderophores that produce catechol-type (Sridevi *et al*., 2008), *Graminaceae* phytosiderophores and *P. fluorescens* siderophores (Zhang *et al*., 1997; Rengel and Romheld, 2000). Furthermore, lowest colonization levels of *P. fluorescens* in forest ecosystem should be related to the forest management strategy *in situ* (absence of fertilization end amendment).

Miller (1993) and Tuitert *et al*. (1998) reported even that among 105 bacteria isolates, 40 of Gram negative belonged to the species of the *P. fluorescens*. In addition (Frey *et al*., 1997;

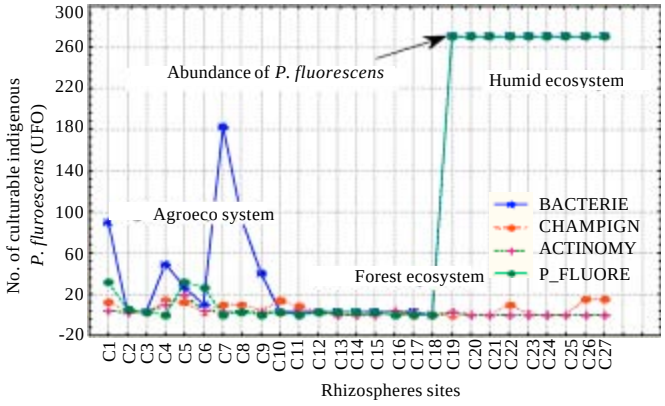

Fig. 1: *P. fluorescens* abundance

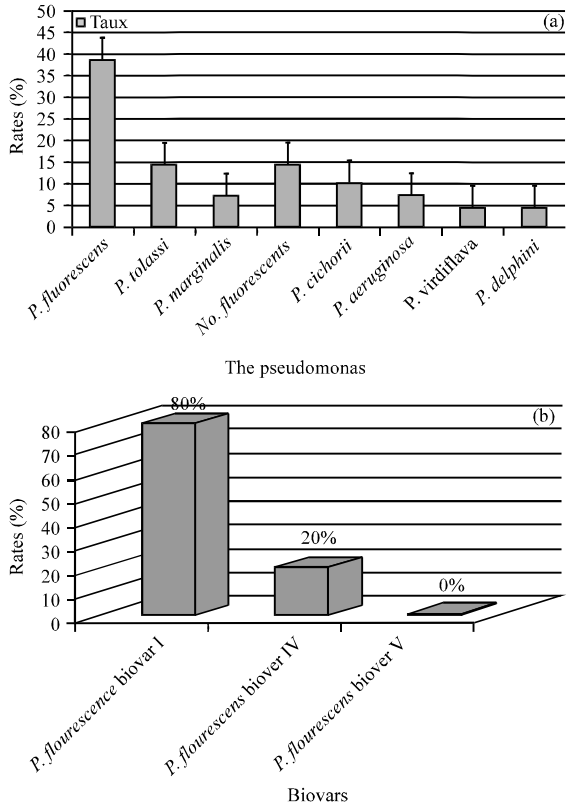

Fig. 2 (a-b): Pseudomonas isolates rates and biovar distribution

Timonen *et al*., 1998; Parret and De Mot, 2000) demonstrated that these species are the most common bacteria in the soils with a rate of 78% of the total aerobic community but in the forest soils this group represented only 12% of the total aerobics bacteria. These results are in agreement with Naina *et al*. (2006) reports which recovered that these fluorescents population were amongst the most abundant populations in soil and in the rhizosphere. Although, the sites prospected, *P. fluorescens* encompasses arguably the most diverse and ecologically significant group of species. They have the ability to fit into a large variety of niche environments, including the association with plant hosts with a remarkable degree of physiological and genetic adaptability.

Physicochemical results: Figure 3 showed that the results of soil physicochemical properties varied depending on the ecosystem type. Soil pH affects the solubility of soil minerals, the availability of plant nutrients and the activity of microorganisms, pH values ranged between 7.5 and 8.5. The highest values were signaled in agro ecosytem and the humid ecosystem soils. The electrical conductivity occur either naturally or as a result of inappropriate soil use and management and the humid ecosystem station tended to be more saline (1163 μs cm^{-1}). Soil Organic Matter was higher in the forest ecosystem station but lowest in the agro ecosystem station. These values can be explained by the fact that any OM fraction incorporated was quickly assimilated by microorganisms present especially by the most pathogens (i.e., presence of competition). The dominating textural classes are that of clay and silty clay (44%). On the basis of our results it appeared that the presences of *P. fluorescens* were important in the silty clay soils of the humid station. These data are in agreement with previous results. Clay-sized particles are

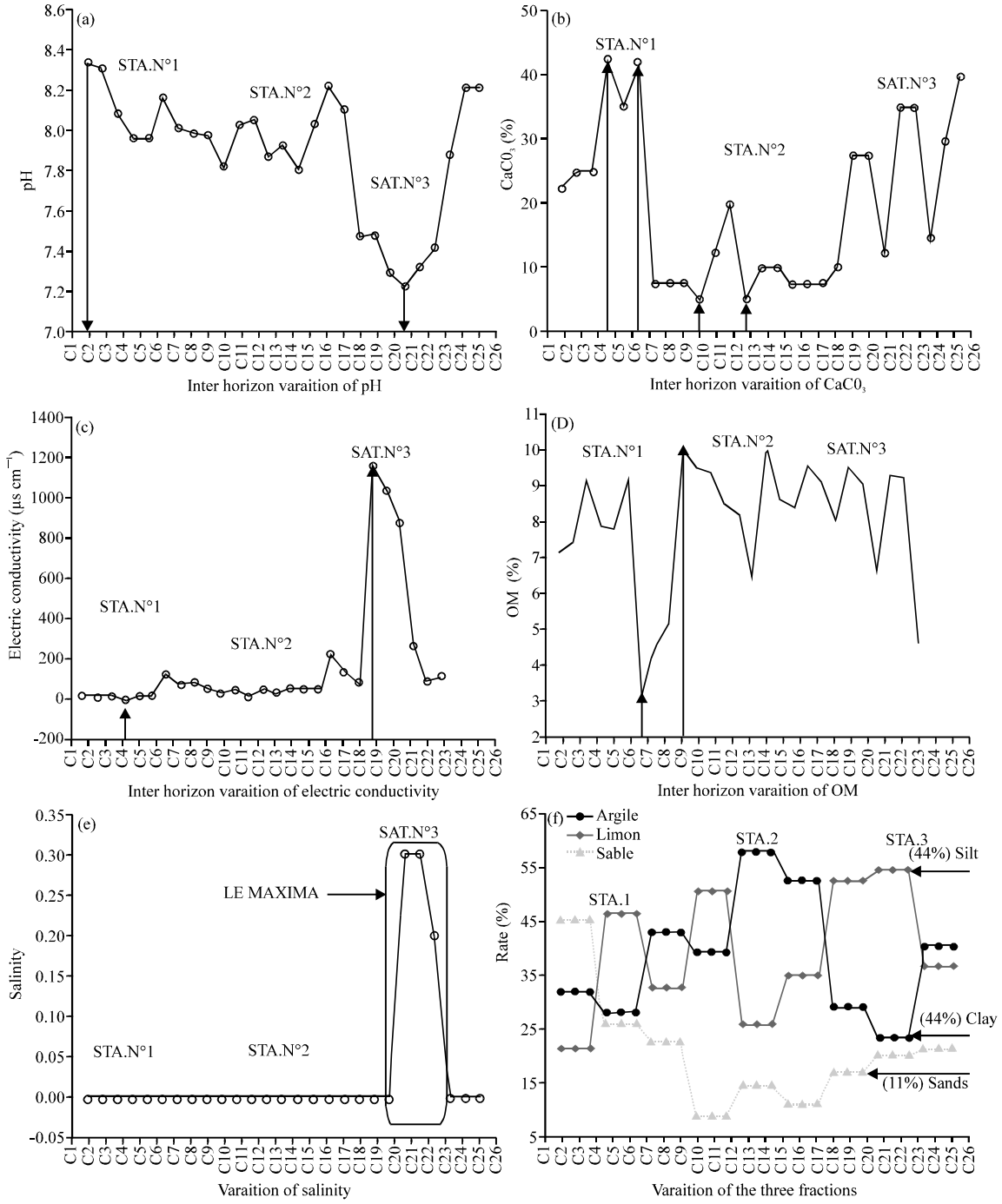

Fig. 3 (a-f): Soil physicochemical properties

thought to protect organic matter through adsorption and aggregation, shelter soil microorganisms from predation (Elliott *et al*., 1980).

Pearson's correlation coefficients of the relationship between *P. fluorescens* abundance and soil physicochemical properties are presented in Fig. 4. A significant ($p = 0.005$) positive correlation existed between soil pH and *P. fluorescens* abundance. However no correlation ($p>0.05$) with $CaCO_3$

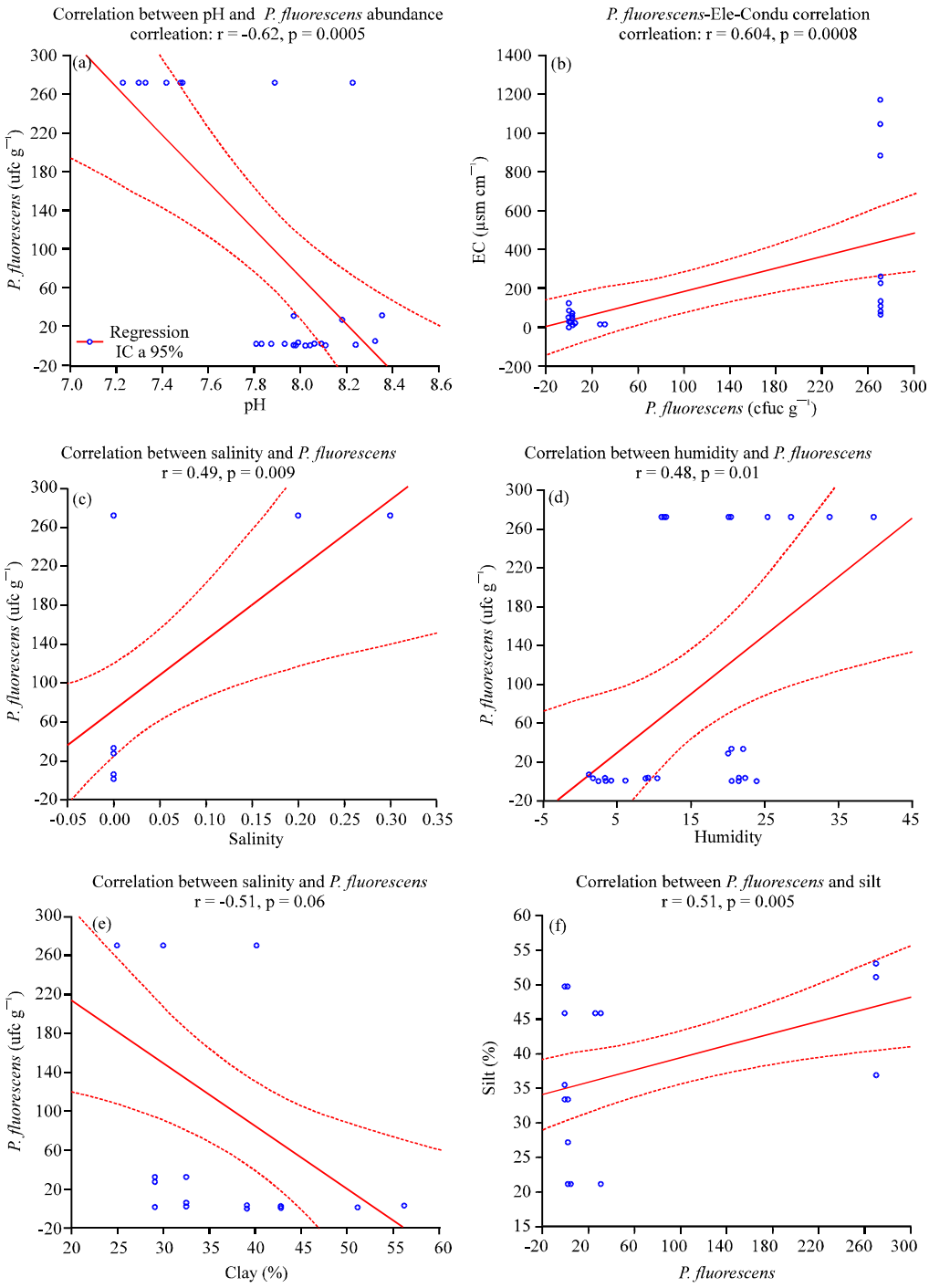

Fig. 4 (a-f): Significant correlation between of *P. fluorescens* abundance and soil parameters

factor, certain authors recognized that this one had a propriety to replace the hydrogen ions and to exhibit atypical high acidity. *P. fluorescens* abundance showed a significant relationship ($p<0.05$) with the electrical conductivity since this rhizobacteria are considerate as a siderobacteria capable to reduce Fe^{+3} (Tansupo *et al.*, 2008; Berti and Thomas, 2009).

Even so, *P. fluorescens* signaled no significant (p>0.05) relationships for the OM (r = 0.13, p = 0.48) (Fig. 5a) and soil depth (Fig. 5b) (r = 0.099, p = 0.62).The reduction of organic matter content observed in the soils could also be a cause of reduced soil enzyme activity (i.e., class of OM not hydrolysable). These results are in agreement with previous report of (Namour, 1999) focused on the OM kinetic.

Principal Component Analysis (PCA) (Fig. 6) was conducted on the soil physicochemical variables as well on *P. fluorescens* abundance to determine how these variables are interrelated. Cluster analysis performed on PCA record scores identifies five groups. The ordination diagrams showed that Axis 1 distinguished G4 and G5 groups of characterized objects belonging to the agroecosytem and forest ecosystem soils. On the positive pole Axis 2 distinguished a group of characterized objects G1 and G3 belonging to the humid ecosystem and G2 belongs to the forest ecosystem.

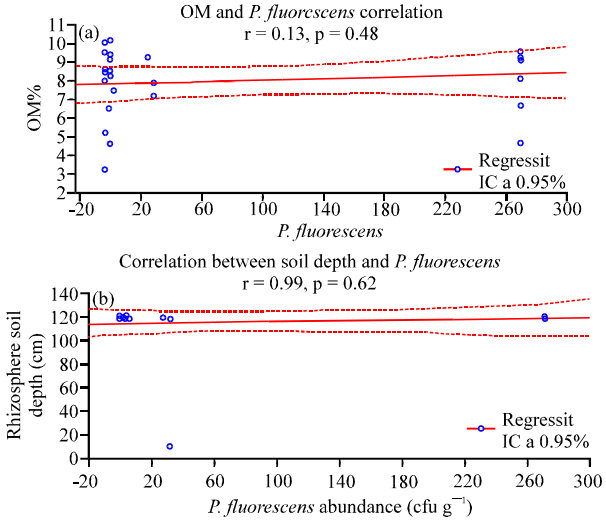

Fig. 5 (a-b): *P. fluorescens* abundance correlations with OM[g] and soil depth[h]

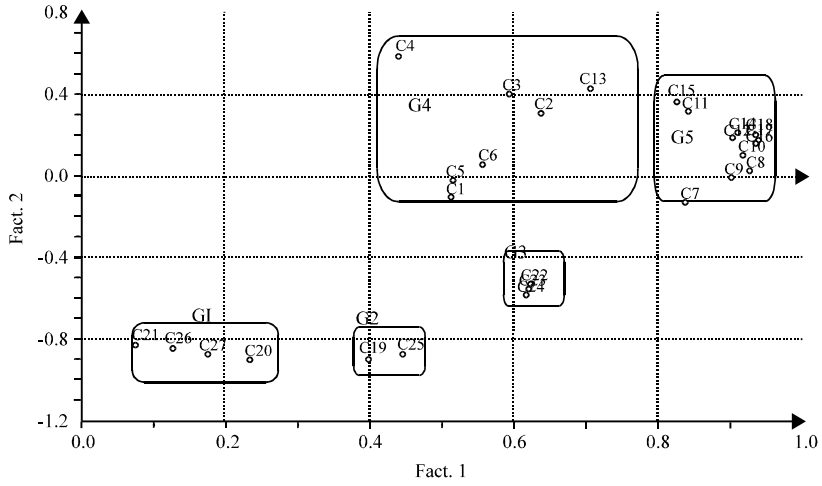

Fig. 6: Principal component analysis of the *P. fluorescens* abundance

CONCLUSION

A relatively full picture of the interaction between different soil properties and *P. fluorescens* abundance can be inferred from the description of rhizosphere biota based on the abundance using qualitative and quantitative evaluation of these communities. Such studies are required to completely understand the ecological role of *P. fluorescens*. On the basis of our results we conclude that *P. fluorescens* encompasses arguably the most abundant and ecological significant group of bacteria. They had a considerably higher occurrence in the rhizosphere soil independing on the plant type. Due to their significant correlation with the majority of soil properties, we concluded that *P. fluorescens* is capable to compete for any ecological niche.

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