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Short communication

PHYSICOCHEMICAL FACTORS AFFECTING PERSISTENCE OF *FRANCISELLA TULARENSIS* IN SOIL

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ABSTRACT

Francisella tularensis (FT) is gram negative, non-motile, capsulated and intracellular zoonotic fastidious bacteria, causing tularemia in both animals and human. Soil texture and heavy metals play an important role in survival and persistence of microbes in environment. In the present study, association of physical and chemical composition of soil was determined in 74 FT positive and negative sites in seven districts of Punjab, Pakistan. Soil samples were analyzed for macro and micronutrients including magnesium, copper, chromium, nickel, manganese, cobalt, lead, cadmium, sodium, iron, calcium, nitrogen, phosphorus and potassium by ammonium bicarbonate-diethylenetriaminepenta acetic acid (DTPA) method using atomic absorption spectrophotometer. Soil pH, moisture, clay, silt, sand, soluble salt and organic matters were determined using standard protocols. A significant difference was found in silt (21.49±14.36), clay (69.68±19.34), soluble salt (3.96±2.89), organic matters (7.32±3.95), nitrogen (0.37±0.20), nickel (1.06±0.23), cadmium (0.37±0.18), magnesium (1.51±3.44), lead (3.51±0.85) and zinc (0.56±0.19) in soil collected from both FT positive and negative sites. There was a non-significant difference in pH (8.21±1.93), sand (10.75±7.73), moisture (11.34±6.94), phosphorus (15.98±16.07), copper (0.23±0.18), chromium (0.38±0.97), manganese (1.99±9.15), iron (7.95±80.42), calcium (75.53±720.29), sodium (49.66±48.06) and potassium (43.59±37.66) in soil samples collected from all districts. In conclusion, physicochemical factors of soil play some role in persistence of FT in environment.

Keywords: *Francisella tularensis*, soil texture, heavy metals, spectrophotometer.

INTRODUCTION

As a habitat of microorganisms, soil is very diverse and complex on the planet earth and serves as home of 25 per cent of biodiversity (Powlson *et al*. 2001; Jeffery and van der Putten 2011). Soil is multilayered and has complex environment of mineral and organic ingredients that may be present either in gaseous, solid or liquid forms (Baumgardner 2012). Due to variety in the composition of soils, physical parameters, agricultural cultivation practices, and crops have resulted in vast diversity of microbial flora (Berg *et al*. 2005). Soil borne pathogens may be Euedaphic pathogens that are true soil organisms such as *Bacillus anthracis*, *Clostridium botulinum*, *Listeria monocytogenes*, *Francisella tularensis* and *Yersinia enterocolitica*, etc. The soil transmitted organisms capable of surviving in soil but not true organisms are *Coxiella burnetii*, *Entamoeba histolytica*, *Salmonella enterica and Pseudomonas aeruginosa*, etc (Jeffery and van der Putten 2011).

Francisella tularensis cause tularemia disease and as compared to other zoonotic pathogens, is having wide host range including mammals, birds, reptiles, fishes, rodents and arthropods such as fleas and ticks, etc (Beran 1994). *Francisella tularensis* is category A select biowarfare agent (Wong *et al*. 2003) and is highly infectious and can be transmitted to human though many sources including infected animal, contaminated foods or water, tick or insects bites, and infected aerosol (Goodman *et al*. 2005; Chitadze *et al*. 2009).

In soil borne pathogens, different macro and micro elements based nutrients availability in soil play an important role in survival of bacteria. Redox potential reactions due to these metals, many give out important roles as cofactors in enzymes and about 30–45 per cent of known enzymes are metalloproteins which perform function under availability of metal co-factor (Klein and Lewinson 2011). However, during infection, different hosts also synthesize some metal chelating proteins and resulted in restriction of the availability of essential metals from invading pathogens (Wakeman and Skaar 2012). In the present study, physico-chemical factors of soil were studied including pH, moisture, silt, clay, total soluble salt, phosphorus (P), copper (Cu), chromium (Cr), calcium (Ca), nickel (Ni), manganese (Mn), iron (Fe), cobalt (Co), lead (Pb), cadmium (Cd), sodium (Na), magnesium (Mg), potassium (K), nitrogen (N), and organic matter both in positive and negative soil for FT.

This study emphasized the role of soil's physical and chemical factors in persistence of FT in soil.

MATERIALS AND METHODS

Soil sampling: Approximately 250-300 grams of soil sample was taken from *Francisella tularensis* positive (n=74) and negative (n=74) sites (unpublished data) using a portable electronic weighing balance (Mughal, Pakistan). The sample was properly labeled and transported to Department of Plant Sciences, Quaid i Azam, University, Islamabad.

Soil Analysis: The pH of soil was measured by preparing 1:1 (soil:water) suspension followed by filtration through Whatman No. 42 filter paper and was determined through digital pH meter (Committee *et al*. 1978). Percent moisture of soil (10g) was determined using standard protocol. Each sample was dried in an oven for 100h at 72ºC (McLean 1982). Textural class of each soil sample was determined in terms of percentage by weight of each soil fraction (Robert and Frederick 1995). Total soluble salt was determined using recommended procedure (Magistad *et al*. 1945). The soil samples were analyzed for macro and micronutrients for Mg, Cu, Cr, Ni, Mn, Co, Pb, Cd, Na, Fe, Ca, N (Fierer *et al*., 2001), P (Brown 1998), K by following developed method of Ammonium Bicarbonate DTPA (Soltanpour and Schwab 1977) and reading was taken for Phosphorus on concentration mode at 880nm wavelength on spectrophotometer (HITACHI U – 1500) (Oslen *et al*., 1954) as well as copper, chromium, calcium, nickel, manganese, iron, cobalt, lead, cadmium, sodium, magnesium, potassium (Soltanpour and Schwab 1977) and nitrogen (Fierer *et al*. 2001) was measured on atomic absorption SpectrAA-100 atomic absorption spectrophotometry (Varian, spring vale Australia). Organic matter of each soil sample was measured in mg per kg (Nelson and Sommers 1982).

Statistical analysis: The presence or absence of a pathogen in relation to soil chemistry was determined through independent student t distribution (T-test). $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

In present study, physico-chemical variation was found in both *Francisella tularensis* (FT) positive and negative soil. Silt, clay, soluble salt, organic matters, N, Ni, Cd, Mg, Pb and Zn concentration was found significantly different ($p<0.05$) in *Francisella tularensis* positive and negative sites as shown in Table 01. Different metals including Fe, Cu, Mn and Zn are involved in many important biological processes and they are ubiquitously found in all organisms as constituents of proteins, including enzymes, storage proteins and transcription factors (Hood and Skaar 2012). Silt concentration was found 6 mg/kg which is more in positive point as compared to negative point and overall range and average concentration was observed as 1.00-81.00 and 17.04±10.84, respectively. Clay content in positive soil was 3 mg/kg which is significantly higher as compared to negative soil with range and average concentration in both positive and negative soil 9.00-99.00 and 79.64±14.11, respectively. *Burkholderia pseudomallei* showed successful growth from soil containing clay with six months interval for up to 30 months (Thomas and Forbes-Faulkner 1981). Previous studies showed that clay is the finest fraction of soil and contacting inorganic compounds which increases chances of bacterial survival (Marshall 1975). Clays and silts have more organic matter due to increase in surface area as compared to sandy fraction and enhances chances of survival of microorganisms (Burton Jr 1982). Range and average concentration of soluble salts was found 0.00-19.60 and 4.58±2.92, respectively with 4.9 mg/kg significantly higher concentration in FT positive points as compared to negative points. Organic matters (6.40±4.14) were found 1.69 mg/kg significantly high in FT positive areas as compared to negative areas. *Francisella tularensis* has great affinity towards organic matter in soil for survival requirements (Dennis *et al*. 2001). Earlier study showed that soil, content of clay, N, and organic matter are positively correlated with survival of *Azospirillum brasilense* (Bashan and Vazquez 2000). Positive sites for FT showed 0.08 mg/kg higher concentration for nitrogen (0.32±0.20) from negative sites. Nickel (1.67±0.35) and cadmium (0.59±0.25) was observed 1.93 and 0.21 mg in higher concentration in positive sites with range 0.64-2.83 and 0.00-1.19, respectively. Previous study showed that aerobic heterotrophic populations of bacteria are more sensitive to different metal groups Ni, Cd followed by Cu, Hg, Mn, Cr and minimum to Zn (Ahmad *et al*. 2005). Concentration of magnesium (Ave=3.46±6.74) was found 13.48 mg/kg more in FT positive sites as compared to negative sites with range 0.10-31.50. During the dry season, Mn content in soil was found 91.58 mg/ kg more in *Burkholderia pseudomallei* positive sites as compared to negative sites (Suebrasri *et al*. 2013). Magnesium plays important role in prolonging survival rate of bacteria during starvation and cold shock by stabilizing bacterial ribosomes and permeability mechanism of bacteria (Leadbetter and Poindexter 2013). Lead (Av=5.53±1.24) and zinc (Ave=0.89±0.25) was found 1.04 and 0.13 gm per kg in negative sites as compared to positive sites, respectively. Lead is not very toxic for microorganisms and Pb tolerant bacteria has been reported (Kanwal *et al*. 2004). Some studies showed that zinc is involved in bacterial gene expression, various cellular reactions and metabolism including DNA replication, glycolysis, pH regulation and the biosynthesis of amino acids, extracellular peptidoglycan and acts as a cofactor of virulence factors (Outten and O'Halloran 2001). Zinc in excess amount also resulted in toxicity to cells via inhibition of key enzymes and thus most of bacteria has a delicate system to keep in balance zinc metal for carrying out essential cellular functions and prevent from zinc toxic effects (Wang *et al*. 2012; Porcheron *et al*. 2015). The ability of bacterial colonization and pathogenicity depends upon availability and uptake of these essential elements. Heavy metals such as Hg, Cd, Zn, Ni, Cu and Ag showed toxic effects at higher concentration and resulted in cell death leading physiological functions (Kanwal *et al*. 2004). This study showed a great importance of physical and chemical factors in persistence of FT in soil and there is a need to further investigate that how these factors affect the persistence of FT in field conditions and what factors are pressing the pathogenicity of the organism.

Table 01. Soil physico-chemical factors and their association with persistence of soil borne *Francisella tularensis*.

Soil analyate	*F.tularensis* positive soil Mean±SD	*F. tularensis* Negative soil Mean±SD	Range	T test
pH	8.37±1.91	8.05±1.94	0.67-13.20	0.310
Sand	10.67±9.51	10.82±5.47	1.00-81.00	0.905
Silt	17.04±10.84	25.94±16.04	1.00-81.00	0.000
Clay	79.64±14.11	59.71±18.78	9.00-99.00	0.000
Soluble salt	4.58±2.92	3.33±2.73	0.00-19.60	0.008
Moisture	10.55±7.16	12.12±6.66	0.40-28.60	0.169
Organic matter	6.40±4.14	8.25±3.55	0.40-19.60	0.004
Nitrogen	0.32±0.21	0.43±0.19	0.02-0.98	0.001
Phosphorus	17.86±17.39	14.09±14.51	0.04-104.40	0.154
Nickel	1.67±0.35	1.52±0.34	0.64-2.83	0.012
Cadmium	0.59±0.25	0.49±0.25	0.00-1.19	0.016
Copper	0.32±0.22	0.34±0.28	0.01-2.01	0.549
Chromium	0.41±0.21	0.72±2.05	0.00-18.06	0.186
Manganese	0.89±1.23	5.06±19.22	0.01-162.57	0.065
Iron	1.09±0.82	20.87±169.95	0.25-1463.10	0.319
Calcium	23.51±11.32	2.02E2±1527.84	0.55-13167.93	0.314
Magnesium	3.46±6.74	1.10±2.32	0.10-31.50	0.005
Lead	5.53±1.24	4.96±1.31	2.49-8.96	0.007
Sodium	69.84±74.71	77.84±70.25	0.21-214.95	0.504
Zink	0.89±0.25	0.75±0.33	0.16-1.85	0.006
Potassium	79.39±66.29	81.98±74.12	9.89-455.00	0.823

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