MICROBIAL BIODIVERSITY IN THE RHIZOSPHERE OF VETIVER GRASS GROWN IN QUANG NAM PROVINCE AND DA NANG CITY

Nguyen Xuan Huong[2], Doan Chi Cuong[1,2*], Paul Truong[3], Vo Van Minh[1,2], Do Thu Ha[2]

[1] Environment and Biological Resource Teaching Research Team (DN-EBR) – Danang University

[2] Faculty of Biology and Environmental Sciences, University of Education – Danang University

[3] TVNI Technical Director, Brisbane, Australia, Email: p.truong@veticon.com.au

[*] Corresponding author: doanchicuong@gmail.com

Abstract - This paper reports the results of studies on the composition and distribution of soil microorganisms in soils taken above and around the rhizosphere of vetiver grass grown in some areas in Quang Nam and Da Nang. The composition and quantity of microorganisms in samples taken from areas planted with vetiver grass were greater than those without vetiver grass. In addition, greater quantity of microorganisms was found on the surface of vetiver roots than in zones further out in the rhizosphere; for example, the microorganism quantity in Phu Tho, Ai Nghia, Lien Chieu and Son Tra were in order of (168,1 – 14 – 12,3) x 10^6 CFU/g; (198,1 – 17,3 – 1,37) x 10^6 CFU/g; (31,8 – 2,8 – 0,27) x 10^6 CFU/g and (28,2 – 2,6 – 0,18) x 10^6 CFU/g, respectively. This increase in soil microbes surrounding Vetiver root zones is most likely due to excretion from the vetiver root system in term of nutrient and oxygen supply.

Key words – soil microbial diversity; distribution of soil microorganisms; vetiver grass; rhizosphere.

1. Introduction

Vetiver grass is a perennial grass with strong ecological adaptability and huge biomass (Dalton, Smith, & Truong, 1996). It was first introduced to soil conservation and land stabilization purposes in Fiji in the early 1950s, and promoted by the World Bank for soil and water conservation in India in the 1980s (Dalton et al., 1996). Because of these unique morphological, physiological and ecological characteristics, Vetiver is commonly known for cost-effectiveness in reducing soil erosion and controlling sedimentary pollution (Truong, Van, & Pinners, 2008) and for its tolerance to extreme soil conditions, including heavy metal contamination (Antiochia, Campanella, Ghezzi, & Movassaghi, 2007; Chen, Shen, & Li, 2004) as well as persistent organic pollutants (Paquin, Ogoshi, Campbell, & Li, 2002; Sarkar, Shakya, Makris, Datta, & Pachanoor, 2007).

Vetiver grass has a massive and complex root system, which can penetrate to the deeper layers of the soil (Mickovski & van Beek, 2009); owing to this characteristic is the favorable condition for soil microbial activity and the growth ability in different soil conditions (Punamiya et al., 2010). The objective of this study was to investigate the soil microbial biodiversity in rhizosphere of vetiver grass; this will further clarify the interpretation of contaminants removing mechanism of vetiver grass.

2. Material and Methods

2.1. Experimental sites

Vetiver grass (*Chrysopogon zizanioides*) was grown in Phu Tho and Ai Nghia (Quang Nam province), Lien Chieu and Son Tra (Danang city). The first and the second site was an alluvial soil of the central plain in Quang Nam province; the third was above the lake-banks in Lien Chieu District; and the fourth, a raddled soil in Son Tra Mountain.

2.2. Data collection

Soil control samples were collected before planting vetiver grass to determine chemical characteristics. Samples were taken at five points and mixed to form a representative soil sample for each site.

Soil samples adjacent to vetiver roots were collected from each site after one year of planting to analyse the chemical and biological characteristics.

Soil samples were taken according to method of ISO 10381-6:2009. Microbial population biodiversity (yeasts, fungi, actinomycetes, bacteria) of the rhizosphere soil samples were isolated and identified using the dilution plate count method in enrichment and selective media such as Gauze I, Ashbv, Pepton, Czapek (Dũng, 1978).

2.3. Analyzing chemical properties methods

Soil samples chemical characteristics from each site were analyzed using samples air-dried for 5 days and sieved to 2 mm. Nitrate nitrogen (N-NO_3), amonium nitrogen (N-NH_4), phosphorus (P_2O_5), potassium (K_2O), were determined using standard methods of soil analysis (Carter & Gregorich, 2008).

3. Results and discussion

3.1. The distribution of soil microorganisms in the areas without vetiver grass

The number of soil microbes and soil characteristics taken from the areas without vetiver grass in Da Nang city and Quang Nam province is presented at Table 1.

Table 1. *The composition and quantity of soil microbes in areas without vetiver grass*

No.	Sample Sites	Soil types	Soil characteristics						Soil microbes		
			Moisture	pH	NO^{3-}	NH^{4+}	P_2O_5	K_2O	Total aerobic	Total	Total

			(%)		mg/100g	mg/100g	mg/100 g		microorganisms (x 10^6 CFU/g)	fungi (x10^4 CFU/g)	actinomycetes (x 10^4 CFU/g)
1	Phu Tho	Sandy loam	35	4,8	4,45	2,15	7,01	5,83	46,5	35,0	5,6
2	Ai Nghia	Organic silt	50	6,8	5,0	3,03	6,8	5,56	58,3	34,8	9,0
3	Lien Chieu	Sandy soil	25	4,2	4,0	2,02	6,37	4,04	15,7	0,04	0,032
4	Son Tra	Clayey sand	23	3,5	0,3	0,4	0,8	0,48	12,1	0,025	0,01

The composition and the quantity of soil microorganisms were different from soil types. It is not depends on the concentration of soil nutrients, humidity but also soil pH.

Because of Ai Nghia are often consolidated annual by alluvial of Vu Gia River, so soil characteristics in this area are fertile, soils pH classified as neutral (pH = 6.8), moderate humidity (45%), and soil microbes thrive in comparison with other regions, namely: total aerobic microbes (58.3x10^6 CFU/g), total fungi (34.8x10^4 CFU/g), and total actinomycetes (9.0x10^4 CFU/g).

Soil in Phu Tho is characterized by low pH (pH=4.8), low humidity (35%) and silty sand, and soil microbes are not diverse than Ai Nghia region, namely: total aerobic microbes (46.5x10^6 CFU/g), total fungi (35x10^4 CFU/g), and total actinomycetes (5.6x10^4 CFU/g).

Soil in Lien Chieu district has a very low of pH (=4.2), the humidity (20%) and soil nutrient, and the quantity of soil microbes are lower than Ai Nghia and Phu Tho region, namely: total aerobic microbes (15.7x10^6 CFU/g), total fungi (0.04x10^4 CFU/g), and total actinomycetes (0.032x10^4 CFU/g).

Because clayed sands soil in Son Tra peninsula are mainly formed from granite, so soil characteristic in this area are very low capacity of water retention as well as the humidity (23,2%) and pH (=3.5), the quantity of soil microbes are lowest compared with the other region, namely: total aerobic microbes (12.1x10^6 CFU/g), total fungi (0.024x10^4 CFU/g), and total actinomycetes (0.01x10^4 CFU/g).

3.2. The distribution of soil microorganisms in the areas planted with vetiver grass

The number of soil microbes and soil characteristics taken from the areas planted with vetiver grass in Da Nang city and Quang Nam province is showed at Table 2.

Table 2. *The composition and quantity of soil microbes in areas planted with vetiver grass*

No.	Sample Sites	Soil types	Soil characteristics						Soil microbes		
			Moisture (%)	pH	NO^{3-} mg/100g	NH^{4+} mg/100g	P_2O_5 mg/100g	K_2O	Total aerobic microorganisms (x 10^6 CFU/g)	Total fungi (x10^4 CFU/g)	Total actinomycetes (x 10^4 CFU/g)
1	Phu Tho	Sandy loam	35	6,4	6,73	3,53	8,43	6,04	87,7	44,5	10,2

2	Ai Nghia	Organic silt	50	7,1	7,33	4,2	8,6	6,8	101,5	47,2	13,9
3	Lien Chieu	Sandy soil	25	5,3	5,25	4,03	7,4	5,15	25,9	0,3	0,12
4	Son Tra	Clayey sand	23	4,8	1,2	0,8	2,3	1,4	20,6	0,25	0,08

The composition and quantity of soil microbes in the areas planted with vetiver grass were increased compared with areas without vetiver grass. It depends on the of soil nutrients concentration, moisture as well as soil pH.

Soil pH, total bioavailable nitrogen, bioavailable phosphorus, and bioavailable potassium at Ai Nghia area fluctuated from 6.8 - 7.1; 8.03 - 11.52; 6.8 – 8.6; and 5.56 - 6.8, respectively. These are favorable conditions for the growth and development of soil microbes, therefore, the quantity of microbes have increased obviously and account for the highest number of microbes compared to other soil samples, namely: total aerobic microbes (from $58.3x10^6$ to $101.5x10^6$ CFU/g), total fungi (from $34.8x10^4$ to $47.2x10^4$ CFU/g), total actinomycetes (from $9.0x10^4$ to $13.9x10^4$ CFU/g).

At Phu Tho commune, soil pH, total bioavailable nitrogen, bioavailable phosphorus, and bioavailable potassium were fluctuated from 4.8 – 6.4; 6.6 - 10.26; 7.01 – 8.43; and 5.83 - 6.04, respectively. Because of these appropriate condition, there was a rise rapidly in the quantity of microbes, namely: total aerobic microorganisms (from $46.5x10^6$ to $87.7x10^6$ CFU/g), total fungi (from $35x10^4$ to $44.5x10^4$ CFU/g), total actinomycetes (from $5.6\ x10^4$ to $10.2x10^4$ CFU/g).

The sandy soil at Lien Chieu district has pH value, total available nitrogen, available phosphorus, and available potassium fluctuated from 4.2 - 5.3 ; 6.02 - 9.28, 6.37 - 7.4 ; and 4.04 - 5.15, respectively. Thus, it can be seen that the number of microorganisms has increased, in which: the total aerobic microbes, total fungi, and the total actinomycetes have increased $15.7x10^6$ - $25.9x10^6$CFU/g, $0.04x10^4$ - $0.3x10^4$ CFU/g, and $0.032x10^4$ - $0.12x10^4$ CFU/g, respectively.

In Son Tra peninsula, soil pH value increased from 3.5 to 4.8 while total available nitrogen, available phosphorus, available potassium fluctuated from 0.7 to 2.0, 0.8 – 2.3, 0.48 – 1.4, respectively, and the quantity of total aerobic microorganisms have incresed from $12.1x10^6$ to $20.6x10^6$CFU/g, total fungi have incresed from $0.025x10^4$ to $0.25x10^4$CFU/g, and the total actinomycetes have incresed from $0,01x10^4$ to $0,08x10^4$ CFU/g.

These results can be explained that the ability of root system to retaining and limited leaching nutrients into the deeper soil layers. Besides, the roots may have generated a number of enzymes, organic acids, which stimulate the growth and activity of soil microorganisms.

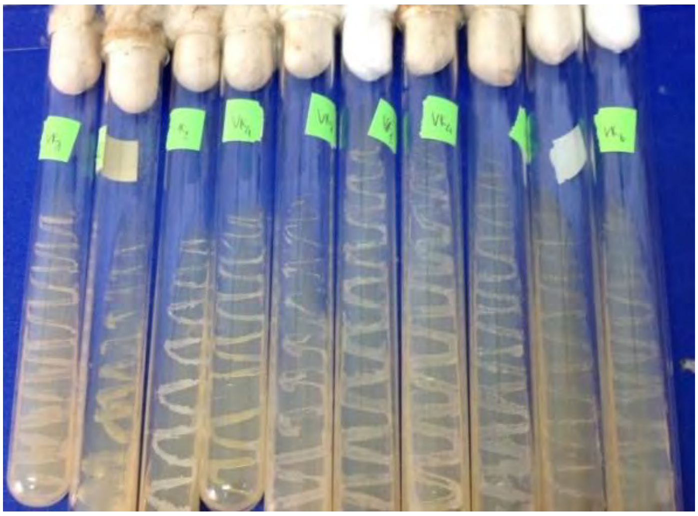

Figure 1. *Microbes were isolated from soil planted with vetiver grass*

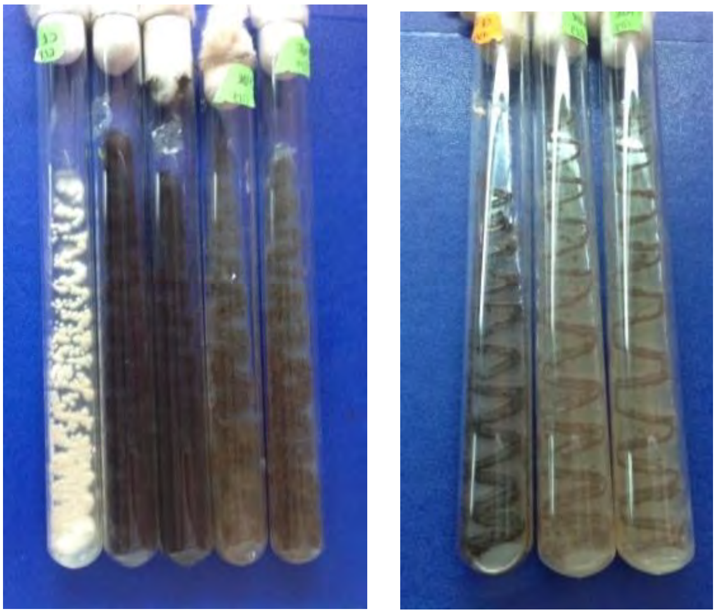

Figure 2. *Fungi, actinomycetes were isolated from soil planted with vetiver grass*

3.3. The distribution of microbes on the surface of roots, adjacent to roots and surrounding vetiver roots

The composition and the quantity of microbes on the surface of roots, adjacent to roots, and surrounding roots which sampled four eares at Quang Nam and Danang are showed in Table 3.

Table 3. *The composition and quantity of microbes*

No.	**Sample sites**	**Microbes (10^5 CFU/g)**		
		on the surface of roots (x10^6CFU/g)	adjacent to roots (x10^6 CFU/g)	surrounding roots (x10^6CFU/g)
1	Phu Tho	152,4	13,42	1,02

2	Ai Nghia	172,6	15,0	1,43
3	Lien Chieu	27,3	2,3	0,17
4	Son Tra	21,9	1,77	0,15

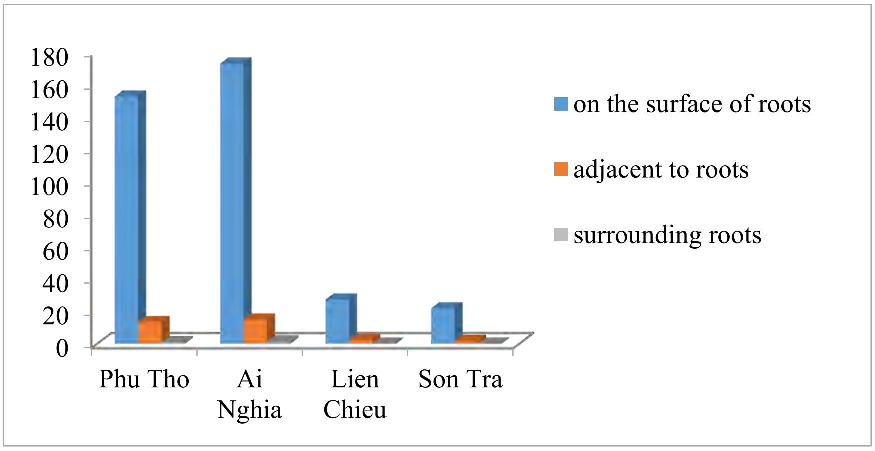

Figure 3. *The composition and the quantity of microorganisms on the surface of roots, adjacent to roots and surrounding roots are showed in table 3.*

In general, the number of microbes on the surface of roots is greatest in all samples, followed by microbes adjacent to roots and surrounding roots.

At Ai Nghia town, the amount of microbes on the surface of roots, adjacent roots and surrounding roots were 172.6x10^6 CFU/g; 15x10^6 CFU/g and 1.43x10^6 CFU/g, respectively, at Phu Tho commune were 152.4x10^6 CFU/g; 13.4x10^6 CFU/g and 1.02x10^6 CFU/g, respectively, at Lien Chieu district were 27.3x10^6 CFU/g; 2.3x10^6 CFU/g and 0.17x10^6 CFU/g, respectively, and in Son Tra peninsula were 27.3x10^6 CFU/g; 2.3x10^6 CFU/g and 0.17x10^6 CFU/g, respectively.

Lyunch (Lynch & Whipps, 1990) and Nannipieri (Nannipieri et al., 2008) explained that the organic compounds like carbonhydrate such as glucose, fructozo; amino acids such as loxin, serine, valine ...; and organic acids such as xitrit and vanic, vitamins are essenial nutrients for the growth of microbes in the root system.

Therefore, the highest quantity of microbes at Ai Nghia town are possible not only related to the vetiver root excretion but also affected by soil characteristics such as high levels of nutrients, the degree of soil aeration, moisture and appropriate pH values. In contrast, the quantity of microbes in the Son Tra Mountain have the lowest compared to the others.

4. Conclusion

From current study, we conclude that:

The distribution of microbes on the surface of roots of vetiver grass is the most abundant in comparison with the others which are adjacent as well as far surrounding roots.

The quantity of soil microbes in samples taken from areas planted with vetiver grass are greater than those without vetiver grass.

The distribution of microbes in vetiver root system are affected by root excretion and the relationship with soil nutrients contents and soil characteristics.

References

Antiochia, R., Campanella, L., Ghezzi, P., & Movassaghi, K. (2007). The use of vetiver for remediation of heavy metal soil contamination. *Anal Bioanal Chem, 388*(4), 947-956. doi: 10.1007/s00216-007-1268-1

Carter, M. R., & Gregorich, E. G. (2008). Soil Chemical Analyses and Soil Biological Analyses. In Y. K. Soon, W. H. Hendershot, E. Topp & C. A. Fox (Eds.), *Soil sampling and methods of analysis* (pp. 173-577). Canada: CRC Press.

Chen, Y., Shen, Z., & Li, X. (2004). The use of vetiver grass (Vetiveria zizanioides) in the phytoremediation of soils contaminated with heavy metals. *Applied Geochemistry, 19*(10), 1553-1565. doi: http://dx.doi.org/10.1016/j.apgeochem.2004.02.003

Dalton, P. A., Smith, R. J., & Truong, P. N. V. (1996). Vetiver grass hedges for erosion control on a cropped flood plain: hedge hydraulics. *Agricultural Water Management, 31*(1–2), 91-104. doi: http://dx.doi.org/10.1016/0378-3774(95)01230-3

Dũng, N. L. (1978). *Một số phương pháp nghiên cứu vi sinh vật học* (Vol. 1,2,3): NXB Khoa học kỹ thuật Hà Nội.

Lynch, J. M., & Whipps, J. M. (1990). Substrate flow in the rhizosphere. *Plant and Soil, 129*(1), 1-10. doi: 10.1007/BF00011685

Mickovski, S. B., & van Beek, L. P. H. (2009). Root morphology and effects on soil reinforcement and slope stability of young vetiver (Vetiveria zizanioides) plants grown in semi-arid climate. *Plant and Soil, 324*(1-2), 43-56. doi: 10.1007/s11104-009-0130-y

Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G., Renella, G., & Valori, F. (2008). Effects of Root Exudates in Microbial Diversity and Activity in Rhizosphere Soils. In C. Nautiyal & P. Dion (Eds.), *Molecular Mechanisms of Plant and Microbe Coexistence* (Vol. 15, pp. 339-365): Springer Berlin Heidelberg.

Paquin, D., Ogoshi, R., Campbell, S., & Li, Q. X. (2002). Bench-scale phytoremediation of polycyclic aromatic hydrocarbon-contaminated marine sediment with tropical plants. *International Journal of Phytoremediation, 4*(4), 297-313. doi: 10.1080/15226510208500089

Punamiya, P., Datta, R., Sarkar, D., Barber, S., Patel, M., & Das, P. (2010). Symbiotic role of Glomus mosseae in phytoextraction of lead in vetiver grass [Chrysopogon zizanioides (L.)]. *J Hazard Mater, 177*(1-3), 465-474. doi: 10.1016/j.jhazmat.2009.12.056

Sarkar, D., Shakya, K. M., Makris, K. C., Datta, R., & Pachanoor, D. (2007). High uptake of 2,4,6-trinitrotoluene by vetiver grass – Potential for phytoremediation. *Environ Pollut, 146*(1), 1-4. doi: http://dx.doi.org/10.1016/j.envpol.2006.06.020

Truong, P., Van, T. T., & Pinners, E. (2008). Part 5: Vetiver System for on-farm erosion control and other uses *Vetiver system applications* (pp. 61-86). The Vetiver Network International.