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ISOLATION AND CHARACTERIZATION OF *BACILLUS* SPECIES FROM SOIL IN NGERE TEA CATCHMENT AREA OF MURANG'A COUNTY, KENYA

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Abstract: **Bacteria are a very diverse group of organisms in soil, and major taxonomic groups are represented in most soils. The extent of the diversity of microorganisms in soil is seen to be critical to the maintenance of soil health and quality, since a wide range of bacteria are involved in the important soil functions. The objectives of this study were to isolate, characterise and identify groups of bacteria from *Bacillus* species that are associated with soil quality in tea growing areas of Ngere. 10 isolates were obtained using dilute nutrient broth agar and the bacterial morphology was identified as gram-positive rod-shaped bacteria, they grew well at pH ranging from 5 - 6.5 and temperature range of 25° C - 35° C. Based on different biochemical tests and sequence of 16S rDNA, the isolated bacteria were identified as belonging to genus *Bacillus*. Phylogenetic analysis of isolates D61, D19, and, D51, suggested that they were closely related to *Bacillus cereus* with 89-96% rDNA sequence analysis similarity while isolates D5, D16, S31, D70 and D2 were closely related to *Bacillus thuringiensis* with 83% - 96% rDNA sequence analysis similarity. Isolate S30 was closely related to *Bacillus subtilis* with 99% rDNA sequence analysis similarity and isolate D29 was closely related to *Bacillus mycoides* with 97% rDNA sequence analysis similarity. Given the low similarity values some isolates had to their closest relatives, chances are high that Ngere tea catchment area harbours a diverse population of as yet, uncultured and uncharacterized microbes.**

Keywords: **Soil quality, Tea, Bacteria, Phylogenetic analysis and *Bacillus* species.**

I. INTRODUCTION

Tea (*Camellia sinensis*) is a major cash crop in many developing countries, including China, India, Sri Lanka and Kenya (International Tea Committee, 2004). There are about 2.72 million hectares of land under tea cultivation globally (International Tea Committee, 2004) Tea grows in various latitudes from the sea level in Japan to 2700 m above mean sea level (amsl) in Olenguruone, Kenya and Gisovu, Rwanda (Owuor *et al*, 2008). The plant is widely adaptable to geographical areas with large variations in climate and physical features which affect rates of growth, yields and quality (Ng'etich et al., 2001). Tea grows well on highland well drained soils having a good depth, acidic pH in the range 4.5 to 5.5 and more than 2% organic matter (www.ktda**tea**s.com.Accessed on 6 July, 2011*).* *Bacillus* is described as aerobic or facultative anaerobic, gram positive, rod shaped, flagellated motile bacteria, catalase positive belonging to the division Firmicutes (Vargas *et al*., 2004). Soil microorganisms are important components of ecosystem functioning as they

determine the mineralization of soil organic matter and energy flow (Robertson and Groffman 2000; Palm *et al*. 2001; Robertson and Groffman, 2007). Microbial recycling of crop residues provides an important component to improve the soil organic matter pool and soil productivity in agricultural management systems, particularly in the tropics, where microbial soil organic matter turnover time is usually shorter as opposed to temperate agro-ecosystems (Manjaiah *et al*. 2000; Joergensen and Castillo 2001; Oelbermann *et al*. 2004). Majority of soil bacteria are heterotrophic in nature and derive their carbon and energy from complex organic substances/organic matter, decaying roots and plant residues (Glick, 1995). Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition (George *et al*., 1995; Timonen *et al*., 1996), plant health (Srivastava *et al.,* 1996; Filion *et al.,* 1999; Smith and Goodman, 1999), soil structure (Wright and Upadhyaya, 1998; Dodd *et al.,* 2000) and soil fertility (Yao *et al*., 2000; O'Donnell *et al*., 2001). Much of the recent studies have focused on tea production in relation to different ecological environment (Carr and Stephens, 1992) with little knowledge on soil microbial composition and their genetic diversity. This research focused on the presence of soil bacteria, their important as well as their diversity in small scale tea growing soils in Ngere tea catchment area of Murang'a County, Kenya

II. MATERIALS AND METHODS

2.1 Collection Of Soil Samples

Soil samples were obtained from Ngere tea catchment area. Cross-sectional study involving stratified random sampling was used. The study population was divided into strata based on the tea buying centres. From each stratum a random sample was collected. The soil samples were taken from a depth of 0-20cm and 20-40cm. A zigzag format of sampling was used; soil samples were mixed thoroughly to constitute a composite sample it was then transported intact at ambient temperature of 4^0 C in sealed polyethylene bags to the laboratory for processing. 1gm of soil was serial diluted in normal saline.

2.2 Isolation Of Soil Bacteria By Enrichment Techniques

Dilute Nutrient Broth Agar (DNBA) was used for the cultivation of microorganisms. Difco nutrient broth (DNB) consisted of Difco nutrient broth (BD Diagnostic Systems, Sparks, MD), at a concentration of 8 g per litre of distilled water. For solid media, 15 g of washed Difco technical agar (BD Diagnostic's systems) was prepared as described by (Joseph *et al.,* 2003). In order To adjust the medium pH to 6.0, 0.1M hydrochloric acid solution was added. The media were then autoclaved at 121° C for 15 minutes after which it was then dispensed into 90-mm-diameter polystyrene sterile plastic petri dishes. The freshly sieved soil was carefully mixed and pulverized with spatula on the larger piece of paper. 1 gram of soil was weighed on a sterile aluminium foil and transferred immediately to the conical flask containing 150 ml of normal saline (Janssen *et. al*, 2002). The flask was dispersed by stirring with Teflon-coated magnetic bars for 15 minutes at approximately 200 g. The soil suspension was then serial diluted where 1ml of the soil suspension was added to 9 ml test tube of normal saline. Dilution ratios included: 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. These preparations were mixed with a vortex mixer at approximately 150 rpm for 1 minute and 1ml of aliquots was rapidly transferred to other 9 ml tubes. For plate count experiments, 200 µl aliquots from different dilutions were transferred to petri dishes containing dilute nutrient broth agar (DNBA) and spread over the surface with a sterile glass spreading rod. This was followed by incubation at 25 ^{o}C for 24 to 72 hours in the dark. Sub culturing was done on dilute nutrient broth agar to isolate pure cultures.

2.3 Characterization of the Isolates

Preliminary characterization was performed using morphological and cultural characteristics as described by (Holt *et al.,* 1994). Morphological identification of the isolate was done under the dissecting and compound microscope to observe cell size, shape and arrangement characteristics after classical staining of bacteria (Bartholomew, 1962). 3% (w/v) KOH test (Gregersen, 1978) was used to determine gram characteristics of isolates. Biochemical tests that were also conducted included; citrate utilization, gelatine liquefaction, methyl red-Voges Proskauer, urease test, nitrate reduction test, motility at 25° C, starch hydrolysis, H_2S production, catalase test, oxidase test and indole production test. The identity of the isolates was however confirmed through molecular characterization. Total bacterial DNA was extracted according to the procedures described by, conventional phenolic extraction and isopropyl alcohol precipitation as described by (Marmur J, 1961). Bacterial 16S rRNA genes of the pure isolates were amplified and used as a template for amplification of 16S rRNA gene. PCR amplification was performed with a model gene Amp 9800 Eppendorf 96 thermal cycler (Applied

Biosystems), using universal primers pair combination of forward primer Bac 27F 5-'TAGAGTTTGATCCTGGCTCAG-3' and the reverse primer Bac1392-R 5-'GACGGGCGGTGTGTACA-3' according to the position in relation to *Escherichia coli* gene sequence (Lane,2001) PCR reactions were started by an initial activation of the enzyme at 94ºC for 5 min followed by 32 cycles consisting of denaturation (1 min at 94º C), annealing (1 min at 55º C) and extension (2 min at 72º C) and a final extension at 72º C for 10 min (Roux, 1995). The PCR product was visualized on a 1% agarose gel stained with ethidium bromide. PCR products were purified using the QIAquick PCR purification Kit protocol (Qiagen, Germany) according to manufacturer's instructions (Sambrook *et al*., 1989). Purified products were cycle sequenced by ABI prism big dye terminator Macrogen. The 16S rRNA gene sequences of the bacteria isolates were viewed and edited using Chromas software (www.technelysium.com.au). They were then aligned using CLASTAL W 1.6 software. To provide full sequences of about 1500 nucleotide bases the sequences were compared to sequences in the public databases with the BLAST search program on the National Center for Biotechnology Information (NCBI) Website (http://www.ncbi.nlm.nih.gov/) to find closely related bacterial 16S rRNA gene sequences. Mega 5 (Tamura *et al*., 2007) was also used to align and identify the closely related bacterial 16S rRNA gene sequences. The 16S rRNA gene sequences of the isolates and those of the closely related bacteria were then aligned and processed to produce Phylogenetic trees using MEGA software package (www.megasoftware.net).

III. RESULTS

3.1 Identification of the isolates

Morphological characterization was based on classical macroscopic techniques of colour, form, shape, and elevation of pure colonies. Most colonies were able to grow within 2-3 days of incubation at 25 °C. The colony morphology of the isolates ranged from, flat and filamentous or branching (**Table 1**). They were smooth or rough and the colour ranged from white to cream and brown (**Table 1**).The ability of the isolates to excrete extracellular enzymes was tested through hydrolysis of starch, and gelatine. The ability of the isolates to excrete intracellular enzymes was determined through tests on catalase reaction; urease, Voges-Proskauer, hydrogen sulphide production, nitrate reduction, methyl red, citrate utilization, oxidase, motility and triple sugar- iron test. The isolates deferred greatly on their ability to excrete various enzymes (**Table 2**).

Isolates further underwent molecular characterization. The amplified DNA yielded 1500 bps bands as show in **figure 1.** The **Table 3** below show Blast analysis of the partial sequences from the genus *Bacillus* within the Firmicutes in the domain bacteria with similarities between 83% and 99%. Among these were; *Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis* and *Bacillus mycoides* among others. Phylogenetic trees of the isolates from Ngere Tea catchment area constructed based on 16s rRNA gene NCBI libraries revealed in this study that ten isolates clustered into genus *Bacillus*, isolate D61, D19 and D51 were closely related to *Bacillus cereus* strain (HQ843838), while isolates D5, D16, S31, D70 and D2 closely related to *Bacillus thuringiensis* (JF895480), isolate D29 was grouped together with *Bacillus mycoides* and isolate S30 clustered together with *Bacillus subtilis* (**Figure 2**).

IV. DISCUSSION

Bacillus are described as aerobic or facultative anaerobic, gram positive, rod shaped, flagellated motile bacteria, catalase positive belonging to the division Firmicutes (Vargas *et al*., 2004) with a wide ecological diversity mostly saprophytic they are commonly found in soil, dust, milk, plant surfaces, a few are animal or insect parasites or pathogens. Many *Bacillus* species have been described including *Bacillus anthracis*, accusative agent of anthrax, *Bacillus cereus* which causes food poisoning, *Bacillus megaterium*, *Bacillus subtilis* and *Bacillus coagulans* notable food spoilers, causing ropiness in bread and related food. *Bacillus thuringiensis* is an important insect pathogen, and is sometimes used to control insect pests. Other species include; *Bacillus mycoides, Bacillus weihenstephanensis* among others (Weinstein and Colburn, 1950). *Bacillus* is the most abundant genus in the rhizosphere of soil, are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Gutierrez *et al*., 2003).

Phylogenetic analysis of isolates D61, D19, and, D51, suggested that they were closely related to *Bacillus cereus* with 89-96% rDNA sequence analysis similarity while isolates D5, D16, S31, D70 and D2 were closely related to *Bacillus thuringiensis* with 83% - 96% rDNA sequence analysis similarity. Isolate S30 was closely related to *Bacillus subtilis* with 99% rDNA sequence analysis similarity and isolate D29 was closely related to *Bacillus mycoides* with 97% rDNA

sequence analysis similarity (**Table 3**). Morphological and biochemical assignments of the isolates D16, D19 and D51 also suggested their close relatedness with *Bacillus cereus*; they are catalase positive, liquefied gelatine, citrate positive, Voges Proskauer positive and motile and had the ability to reduce nitrate to nitrite, and oxidase positive. Most isolates were negative with indole, methyl red test and hydrogen sulphide gas production (Boltyansika *et al*., 2004). Isolates D5, D16, S31, D70 and D2 were biochemically and morphologically closely related to *Bacillus thuringiensis* with the same characteristics as *Bacillus cereus* only that they had large rods upon gram staining. Isolate S30 was closely related to *Bacillus subtilis* it was catalase positive, motile and did not produce hydrogen sulphide gas. Isolate D29 closely related to *Bacillus mycoides* and it was a non- motile rod (**Table 2**). The ability of the isolates to reduce nitrate indicates their ability to produce enzyme nitrate reductase which reduces nitrates that the cell uses as a final hydrogen acceptor during anaerobic respiration to nitrites or free nitrogen gas and water (Harold, 2002). Some of the *Bacillu*s species have been classified as plant growth promoting Rhizobacteria (Probanza *et al*., 2002). There are a number of metabolites that are released by these strains (Charest *et al*., 2005) which strongly affect the environment by increasing availability of the plants (Barriusso *et al*., 2008). Naturally present in the immediate vicinity of plant roots, *Bacillus subtilis* is able to maintain stable contact with higher plants and promote their growth. *Bacillus megaterium* is very consistent in improving different root parameters (rooting, root length and dry matter content of roots) in mint (Kaymak *et al*., 2008). *Bacillus pumilus* can be used as a bio-inoculant for biofertilizer production to increase the crop yield of wheat variety in Mongolia (Hafeez *et al*., 2006). Soil bacteria isolates including *Bacillus cereus* UW 85 produces Siderophore and they can be used as efficient PGPR to increase the yield of the crop (Husen, 2003). *Bacillus megaterium* from tea rhizosphere is able produce Siderophore and thus it helps in the plant growth promotion and reduction of disease intensity (Chakroborty *et al*., 2006). Many soil microorganisms are able to solubilize phosphate through their metabolic activities exuding organic acids, which directly dissolve the rock phosphate, or chelating calcium ions that release Phosphate to the solution. About 95% of Gram-positive soil *bacilli* belong to the genus *Bacillus* (Garbeva *et al*., 2003). Members of *Bacillus* species are able to form endospores and hence survive under adverse conditions; some species are diazotrophs such as *Bacillus subtilis* (Timmusk *et al*., 1999),

V. CONCLUSION AND RECOMMENDATION

There is a diverse and complex prokaryotic community that is resident in Ngere tea catchment area. The biochemical properties of some isolates, like ability to reduce nitrate to nitrite, suggests their involvement in the nitrogen cycle within the tea soils. Most isolates from Ngere tea catchment area belong to plant growth promoting rhizobium (PGPR).

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REFERENCES

[1] Barriuso J, Solano BR., (2008). Ecology, Genetic Diversity and Screening Strategies of Plant Growth Promoting Rhizobacteria (PGPR). *Journal of Plant nutrition*: 1-17.

[2] Carr, M. K. V., & Stephens, W., (1992). Climate, weather and yield of tea. In K. C. Willson & M. N. Clifford (Eds.), Tea: Cultivation to Consumption (pp. 87–115). London: Chapman & Hall.

[3] Chakraborty U, Chakraborty B, Basnet M, (2006). Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium. Journal of Basic Microbiology*, 46 (Suppl 3): 186 – 195

[4] Charest MH, Beauchamp CJ, Antoun H., (2005). Effects of the humic substances of de- inking paper sludge on the antagonism between two compost bacteria and *Pythium ultimum*. FEMS *Microbiology Ecology*, 52 (Suppl 2): 219–227

[5] Dodd, J.C., Boddington, C.L., Rodríguez, A., Gonzalez-Chavez, C. and Mansur, I. (2000). Mycelium of *arbuscular mycorrhizal* fungi (AMF) from different genera: form, function and detection. *Plant Soil* 226: 131–151

[6] Filion, M., St-Arnaud, M. and Fortin, J.A., (1999). Direct interaction between the *arbuscular mycorrhizal* fungus *Glomus intraradices* and different rhizosphere microorganisms. *New Phytol*ogy 141: 525–533.

[7] Garbeva P, van Veen JA, van Elsas JD., (2003). Predominant Bacillus spp. in agricultural soil under different management regimes detected via PCR-DGGE. *Microbial Ecology*, 45 (Suppl 3): 302–316.

[8] Glick BR (1995). The enhancement of plant growth by free living bacteria. *Canadian Journal of Microbiology*, 41 (Suppl 2): 109–114.

[9] Gregersen T., (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. *European Journal of Applied Microbiology and Biotechnology* 5:123–127

[10] Gutiérrez Mañero FJ, Probanza A, Ramos B, Colón Flores JJ, Lucas García JA., (2003). Ecology, Genetic Diversity and Screening Strategies of Plant Growth Promoting Rhizobacteria (PGPR). *Journal of Plant Nutrition*, 26 (Suppl 5): 1101–1115

[11] Hafeez FY, Yasmin S, Ariani D, Mehboob-ur-Rahman Zafar Y, Malik KA., (2006). Plant growth-promoting bacteria as biofertilizer. *Agronomy for Sustainable Development*, 26: 143-150.

[12] Harold, J. B. (2002). Microbiological applications: a laboratory manual in general microbiology. 8th Edition 1- 478.

[13] Holt JG, Bergey H (1994). Bergey's Manual of Determinative Bacteriology, 9th ed. Williams and Wilkins, USA ISBN 978-0-683-00603-7 p. 182.

[14] Husen E., (2003). Screening of soil bacteria for plant growth promotion activities *in vitro*. *Indonesian Journal of Agricultural Sciences*, 4 (Suppl 1): 27-31

[15] International Tea Committee (2004). Annual Bulletin of Statistics.

[16] Joergensen, R.G., Castillo, X (2001). Interrelationships between microbial and soil properties in young volcanic ash soils of Nicaragua. *Soil Biology* and *Biochemistry*. 33: 1581–1589.

[17] Joseph, S. J., P. Hugenholtz, P. Sangwan, C. A. Osborne, and P. H. Janssen., (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. *Applied. Environment. Microbiology*. 69: 7210–7215

[18] Kaymak HC, Yarali F, Guvenc I, Donmez MF., (2008). The effect of inoculation with plant growth Rhizobacteria (PGPR) on root formation of mint (*Mentha piperita* L) Cuttings. *African Journal of Biotechnology,* 7 (Suppl 24): 4479-4483

[19] Lane H (2001). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) for studying soil microbial communities. *Applied. Environment. Microbiology*. 8: 353–373.

[20] Manjaiah, K.M., Voroney, R.P., Sen, U (2000). Soil organic carbon stocks, storage profiles and microbial biomass under different crop management systems in a tropical agricultural ecosystem. *Biolology.of Fertile. Soils* 32: 273–278

[21] Ng'etich, W. K., Stephens, W., & Othieno, C. O (2001). Responses of tea to environment in Kenya. 3. Yield and yield distribution. *Experimental Agriculture*, 37: 361–372.

[22] O'Donnell, A.G., Seasman, M., Macrae, A., Waite, I. and Davies, J.T (2001). Plants and fertilisers as drivers of change in microbial community structure and function in soils. *Plant Soil.* 232: 135– 145.

[23] Oelbermann, M., Paul Voroney, R., Gordon, A.M (2004). Carbon sequestration in tropical and temperate agroforestry systems: a review with examples from Costa Rica and southern Canada. *Agricultural. Ecosystem and Environ*ment 104: 359–377.

[24] Owuor, P. O., Obanda, M., Nyirenda, H. E., & Mandala, W. L., (2008). Influence of region of production on clonal black tea chemical characteristics. *Food Chemistry*, 108: 263–271

[25] Palm, C.A., Gachengo, C.N., Delve, R.J., Cadisch, G., Giller, K.E., (2001). Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource database. *Agricultural. Ecosystem and. Environment*. 83: 27–42.

[26] Probanza A, Lucas García JA, Ruiz Palomino M, Ramos B, Gutiérrez Mañero FJ, (2002). *Pinus pinea* L seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus* (*B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105). *Applied Soil Ecology*, 20 (Suppl 2): 75–84.

[27] Robertson, G.P., Groffman, P.M (2007). Nitrogen transformations. In: Paul, E.A. (Ed.), Soil Microbiology, Ecology, and Biochemistry. Academic Press, Oxford, United Kingdom, pp. 341–364.

[28] Roux, K. H. (1995). Optimization and trouble shooting in PCR, p. 53-62. In C. W.

[29] Sambrook, K. J., Fritsch, E. F. & Maniatis, and T., (1989) Molecular cloning: a Laboratory Manual, 2nd edition. Cold Spring Harbour, NY: Cold Spring Harbour

[30] Srivastava, D., Kapoor, R., Srivastava, S.K. and Mukerji, K.G., (1996). Vesicular arbuscular mycorrhiza—an overview. In: Mukerji, K.G. (Ed.), Concepts in Mycorrhizal Research. Kluwer Academic Publishing, Netherlands, pp. 1 –39.

[31] Tamura, K., Dudley, J., Nei, M. & Kumar, S., (2007) MEGA 5: Molecular Evolutionary Genetics Analysis (MEGA) software version 5.0. *Molecular Biology and Evolution* 24: 1596-1599.

[32] Timmusk S, Nicander B, Granhall U, Tillberg E., (1999). Cytokinin production by *Paenibacillus polymyxa*. *Soil Biology and Biochemistry*, 31 (Suppl 13): 1847–1852

[33] Timonen, S., Finlay, R.D., Olsson, S., Soderstrom, B., (1996). Dynamics of phosphorous translocation in intact ectomycorrhizal systems: non-destructive monitoring using a B-scanner. *FEMS Microbial Ecology*. 19: 171– 180.

[34] Vargas, V. A., Delgado, O. D., Kaul, R. H. and Mattiasson, B. (2004). Lipase-producing microorganisms from a Kenyan alkaline soda lake. *Biotechnology Letters,* 26: 81–86.

[35] Wright, S.F., Upadhyaya, A., (1998). A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* 198: 97–107.

[36] www.ktdateas.com/index2.php?option=com_content&do_pdf=1 Tea Growing Conditions & Husbandry (Accessed on 6 July, 2011)

[37] Yao, H., He, Z., Wilson, M.J., Campbell, C.D., (2000). Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. *Microbial Ecology.* 40: 223– 237.

APPENDIX. A

TABLES AND FIGURES

Table 1: Morphological characteristics of isolates obtained from Ngere tea catchment area

Isolate	Colony Characterization				Cell characterization	
	Colony colour	**Colony form**	**Colony elevation**	**Colony margin**	**Cell arrangement**	**Gram reaction**
D16an	Cream	Irregular	Flat	Ciliate	Large rods	Positive
D19	White	Irregular	Flat	Undulate	Small rods	Positive
D5	White	Irregular	Flat	Whip- like	Large rods	Positive
D61	Cream	Irregular	Flat	Branching	Small rods	Positive
D70	Brown	Irregular	Flat	Undulate	Large rods	Positive
D2	Brown	Irregular	Flat	Ciliate	Large rods	Positive
D29	Cream	Irregular	Flat	Undulate	Large rods	Positive
D51	Cream	Irregular	Flat	Ciliate	Short rods	Positive
S31	Cream	Irregular	Flat	Ciliate	Large rods	Positive
S30	White	Irregular	Flat	Entire	Large rods	Positive

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Table 2: Biochemical characteristics of the isolates obtained from Ngere tea catchment

Isolates	Biochemical Tests											
	Star	Indole	Cat	Nitrate	Mot	Urea	Cit	MR	VP	Gel	Oxid	H_2S
D70	+	-	+	+	+	+	+	-	+	+	+	+
D2	+	-	+	-	+	-	+	-	+	-	+	+
D29	+	+	+	-	-	-	+	-	+	-	+	-
D51	+	-	+	+	+	+	+	-	+	+	+	+
S30	+	-	+	+	+	+	+	-	+	-	+	+
S31	+	-	+	+	+	+	+	-	+	-	+	+
D16an	+	-	+	+	+	+	+	-	+	+	+	+
D5	+	-	+	+	+	+	+	-	+	+	+	-
D61an	+	-	+	+	+	+	+	-	+	+	+	+
D19	+	-	+	+	+	+	+	-	+	+	+	+

Key: (+) Positive, (-) Negative, MR: Methyl Red, VP: Voges-Proskauer, Cit: Citrate, Star: Starch, Gel: Gelatine, Cat: Catalase, Oxid: Oxidase, Mot: Motility and H_2S: Hydrogen Sulphide gas.

Table 3: Showing the blasted isolates, from Ngere tea catchment area nearest neighbours in the data bank and their percentage relatedness.

Isolate	**Length**	**Nearest relatives**	**Accession number**	**% similarity**
D61(bac27F)	770bp	*Bacillus sp.*DG5	JN208196	98%
		Bacillus cereus Wu2	JF267369	98%
		Bacillus cereus DYJK1-7	HQ483838	98%
		Bacillus cereus GTC 02932	AB592531	98%
		Bacillus cereus	AB592507	98%
D2 (bac 27F)	636bp	*Bacillus sp.*17	HQ600994	96%
		B. thuringiensis KU4	JF895480	96%
		B. thuringiensis BPRIST010	JF414763	96%
		B. cereus 13651EE	EU741099	96%
		B. thuringiensis S512Ba-14	HQ238549	96%
D29(bac 27F)	751 bp	*Bacillus* sp. MN3-10	JQ396537	97%
		B. mycoides NBRC 101228	AB679980	97%
		B. mycoides BCHMAC12	GU188897	97%
		B. mycoides GTC 02830	AB592539	97%
		B. weihenstephanensis GTC 02834	AB592543	97%

D5(bac 27F)	551bp	*Bacillus sp.*CM1	JN695713	93%
		Bacillus thuringiensis strain KU4	JF895480	93%
		Bacillus thuringiensis strain Z8B-46	HQ238660	93%
		Bacillus thuringiensis strain S512Ba *Bacillus thuringiensis Z5B-16*	HQ238549 HQ238510	93% 93%
D19(bac 27 F)	530 bp	*Bacillus sp.* Na2	G391509	95%
		Bacillus cereus strain X5	FJ763651	95%
		Bacillus cereus strain B1	EU857430	95%
		Bacillus cereus strain 1 *Bacillus cereus* strain 4W	FJ435113 DQ420187	95% 95%
D16 (bac 27F)	573bp	*Bacillus thuringiensis* clone C05	GU003833	93%
		Bacillus thuringiensis serotype H4a4b	DQ286358	93%
		Bacillus thuringiensis 41205	HM032789	93%
		Bacillus thuringiensis Bi29 Bacillus sp.JH2-2	HQ336299 JN880445	93% 93%
D51(bac 27F)	525 bp	*Bacillus sp.* PL49	HQ536223	86%
		Bacillus cereus strain SK	FJ2267613	86%
		Bacillus cereus strain APB2	HM046583	86%
		Bacillus cereus strain AIMST nn4 *Bacillus subtilis* strain CCTCC M2011162	HQ670579 JQ086379	86% 86%
S30 (bac 27 F)	748 bp	*Bacillus sp.* bE53	JQ612528	99%
		Bacillus cereus P14	JN700160	99%
		Bacillus anthracis Se07	JN700115	99%
		Bacillus subtilis CCTCC M2011162	JQ086379	99%
		Bacillus subtilis B7	JQ086378	99%
D70(bac 27 F)	676 bp	*Bacillus thuringiensis* KNUC2103	JN846923	94%
		Bacillus thuringiensis KNUC2102	JN084030	94%
		Bacillus thuringiensis RG17-11	AB677944	94%
		*Bacillus sp.*EE10m15	JN846923	94%
S31(bac27F)	497bp	*Bacillus sp.* SAP02-1	JN872500	83%
		Bacillus thuringiensis DSB8	JQ342872	83%
		Bacillus thuringiensis DSB4 *Bacillus thuringiensis* DSR5	JQ342868 JQ342656	83% 83%

Figure 1: PCR amplified 16SrDNA products from representative isolates among the isolated from Ngere tea catchment area using universal primers bac 27F and bac 1392R

Legend: Lanes 1(**D5***), 2(**D19***), 3(**D51***), 4(**D61**), 5(**D16***), 6(**S31***), 7(**D2***), 8(**D70***), 9(**S30***), 10(**D29***), (**C***) negative control and (**M***) M-1500 bp Molecular marker size.

*The figures within the brackets are the isolate numbers

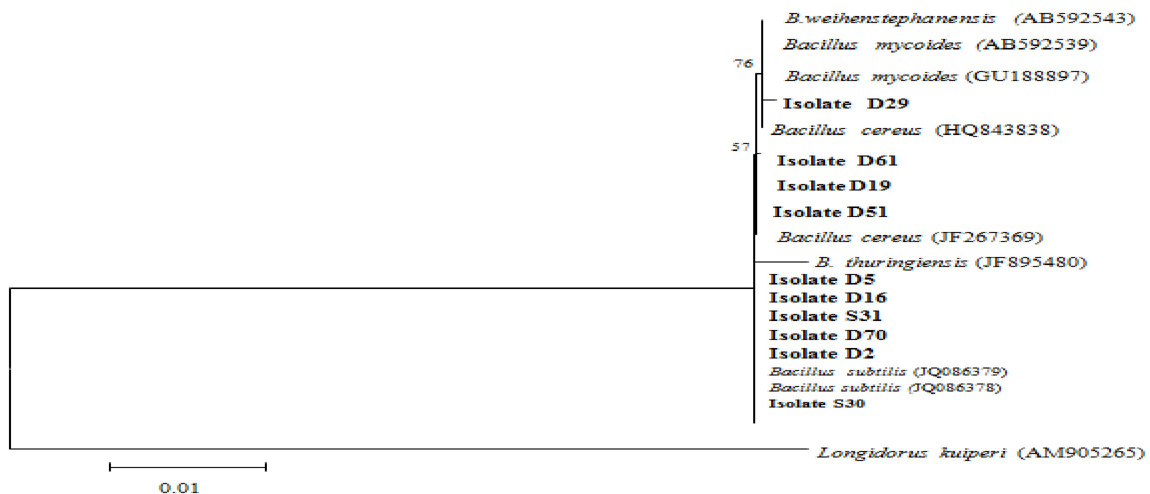

Figure 2: Neighbour- joining phylogenetic tree showing the evolutionary relationships of the taxa in the genus *Bacillus* of the isolates from Ngere tea catchment area. The scale bar indicates approximately 1% sequence difference. Numbers at nodes indicate bootstrap values of each node out of 100 bootstrap resampling. The gene sequence of *Longidorus kuiperi* AM905265 was used as an out-group