# INVENTORY OF MICROBIAL BIODIVERSITY IN SOME OIL PALM PLANTATIONS

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Land degradation and the loss of soil fertility caused by soil erosion due to land conversion is a major problem worldwide. Although the use of agrochemicals increases productivity, it may also cause detrimental effects to the environment. Currently, many oil palm plantations have turned to implementing sustainable agricultural management practices to reduce the negative impacts to the plantations, environment and public. In a sustainable management system, microbial diversity under the oil palm environment can be used for studying biodiversity and soil health because microbes are the fastest living organisms to react to environmental changes. Beneficial microbes can be efficiently integrated into the management practices without sacrificing productivity (Glick, 1995; Rosas *et al*., 2006).

Some microbes of importance to agriculture and agro-industries are listed below:

**Plant growth promoters.** Species of genera such as *Pseudomonas, Azospirillum, Bukholderia, Bacillus, Enterobacter, Rhizobium, Erwinia, Alcaligenes, Arthrobacter, Acinetobacter, Flavobacterium, Trichoderma, Serratia* and *Klebsiella.*

**Nitrogen-fixers.** Species of genera such as *Rhizobium, Actinobacterium, Brevibacillus, Azospirillum, Azotobacter* (such as *A. paspalii, A. chroococcum, A. vinelandii, A. agilis*), *Bradyrhizobium, Erwinia* and *Bacillus* (*e.g. B. sphaericus*).

**Phosphate-solubilizers.** Species of genera such as *Pseudomonas putida, Enterobacter agglomerans, Chromobacterium lividum, Klebsiella aerogenes, Enterobacter alvei* and *Staphylococcus* spp.

**Potassium-solubilizers.** Species of genera such as *Bacterium, Bordetella, Delftia, Dyella, Rhizobium, Sinomonas, Staphylococcus* and *Xanthomonas.*

**Converters of green wastes to organic fertilizer.** Species of genera such as *Bacillus, Actinomycetes,* and *Trichoderma.*

**Bioinsecticides.** *Bacillus thuringiensis, B. israelensis, B. sphaericus, Metarhizium anisopliae, Beauveria bassiana, Paecilomyces* spp., *etc.*

**Biofungicides.** *Burkholderia* spp., *Trichoderma* spp., *Stenotrophomonas maltophilia, Agrobacterium agropyri, Bacillus gladioli, Pseudomonas aeroginosa, Microbacterium testaneum, B. multivorans, etc.*

**Bioherbicides.** *Exserohilum longirostratum.*

**Biotechnology and food industry.** *Agrobacterium* spp., *Lactobacillus plantarum.*

## OBJECTIVES

The purpose of this inventory of microbial biodiversity is to facilitate the search for beneficial microbes for various purposes, such as:

- agro bio-products, *e.g.* compost, plant growth promoters or enhancers;
- alternatives to chemicals, *e.g.* bio-pesticides, bio-fertilizers and bio-remediation agents; and
- products for biotechnology and food industries.

## METHODOLOGY

Soil and degrading oil palm biomass resulting from different management practices, from different soil types and locations were sampled monthly for microbial analysis. Microbial count was conducted using the Pour Plate method, various enriched media were used for isolating the different groups of microbes. Isolates were identified using the 16S rDNA PCR-DGGE method. The National Centre for Biotechnology Information (NCBI) database and Mega4 software were applied for blasting the 16S rDNA sequence and constructing the phylogenetic tree of the microbes, respectively.

## RESULTS

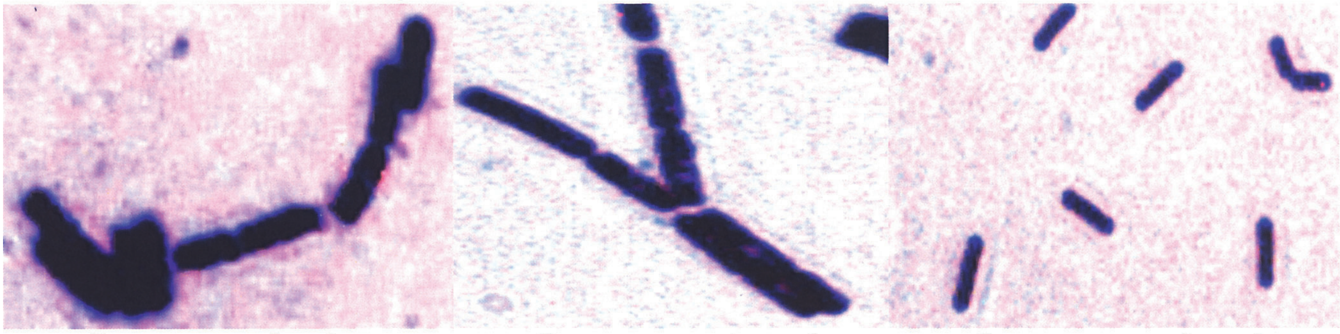
As of May 2010, some 2176 different microbe isolates comprising of nine taxonomies, 96 genera, 249 species and 685 strains have been identified, freeze-dried and stocked (*Table 1*). Microscopic structures of some of these microbes are shown in *Figures 1* and *2*. Microbial biodiversity at replanting of a second generation oil palm, at 12 months during biodegradation of the biomass, was more diversified (*Figure 3*) than in a secondary forest (*Figure 4*).

### TABLE 1. NUMBER OF SPECIES, STRAINS AND ISOLATES OF THE VARIOUS GENERA OF PROKARYOTES

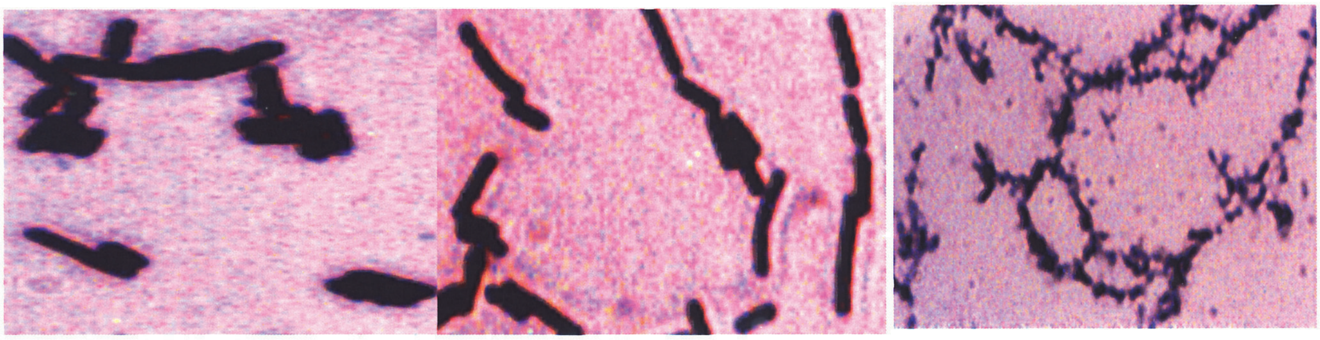
| No. | Taxanomy | Organism | Species | Strain | Isolate | Isolate/origin |
|---|---|---|---|---|---|---|
| 1 | α-Proteobacteria | *Acetobacter* sp. | 1 | 1 | 2 | 1C |
| 2 | β-Proteobacteria | *Achromobacter* spp. | 4 | 6 | 19 | 13 KM, 3T, 2 HP, 1C |
| 3 | β-proteobacteria | *Acidovorax* spp. | 3 | 3 | 9 | 7 TP, 1 KLM, 1 KM |
| 4 | Actinobacteria | *Actinobacterium* spp. | 1 | 2 | 2 | 1KM, 1T |
| 5 | Actinobacteria | *Actinomadura* | 1 | 1 | 1 | I TP |
| 6 | Actinobacteria | *Actinomyces* | 1 | 1 | 1 | 1 KM |
| 7 | Actinobacteria | *Actinomycetales* sp. | 1 | 1 | 1 | 1 KM |
| 8 | α-Proteobacteria | *Agrobacterium* spp. | 3 | 5 | 8 | 5 KM, 2 TP, 1T |
| 9 | β-Proteobacteria | *Alcaligenes* spp. | 2 | 4 | 9 | 6 BM, 3M |
| 10 | α-Proteobacteria | *Alpha proteobacterium clone* spp. | 2 | 3 | 8 | 8 KM |
| 11 | Actinobacteria | *Amycolatopsis* spp. | 3 | 3 | 4 | 4 TP |
| 12 | Firmicutes | *Aneurinibacillus* sp. | 1 | 1 | 2 | 2 KM |
| 13 | Bacteria | *Archaeon* sp | 1 | 1 | 1 | 1 HP |
| 14 | Actinobacteria | *Arthrobacter* spp. | 2 | 6 | 18 | 17 KM, 1 BM |
| 15 | Firmicutes | *Bacillus* spp. | 23 | 146 | 410 | 231 KM, 118 TP, 34 BM,12T, 13C,10B |
| 16 | γ-Proteobacteria | *Bacterium* spp. | 1 | 88 | 300 | 157 KM, 84 BM, 27 TP, 8HP, 3T, 3B |
| 17 | β-Proteobacteria | *Beta proteobacterium* | 1 | 3 | 5 | 3 TP, 1 BM, 2 KM |
| 18 | β-Proteobacteria | *Bordetella petrii* | 1 | 1 | 3 | 3C |
| 19 | α-Proteobacteria | *Bosea thiooxidans* | 1 | 1 | 2 | 2 KM |
| 20 | Firmicutes | *Brevibacillus* spp. | 7 | 11 | 45 | 31 KM, 8 TP, 6 BM |
| 21 | A-Proteobacteria | *Brevundimonas* spp. | 2 | 5 | 10 | 6 BM, 4KM, |
| 22 | β-Proteobacteria | *Burkholderia* spp. | 8 | 64 | 257 | 181 KM, 53 BM, 23 TP |
| 23 | α-Proteobacteria | *Caulobacter* spp. | 3 | 3 | 11 | 7 TP, 3 KM, 1 KM |
| 24 | Actinobacteria | *Cellulosimicrobium* sp. | 2 | 2 | 3 | 1 KM |
| 25 | Bacteroidetes | *Chitinophaga* spp. | 2 | 2 | 8 | 7 TP, 1 KM |
| 26 | β-Proteobacteria | *Chromobacterium* spp. | 2 | 2 | 4 | 4 BM |
| 27 | Bacteroidetes | *Chryseobacterium* spp. | 5 | 6 | 25 | 13 TP, 12 KM |
| 28 | γ-Proteobacteria | *Citrobacter* sp. | 1 | 1 | 4 | 3 KM, 1 HS |
| 29 | Firmicutes | *Clostridium* spp. | 4 | 4 | 4 | 3 TP, 1 KM |
| 30 | Firmicutes | *Cohnella* sp. | 1 | 1 | 1 | 1 KM |
| 31 | Actinobacteria | *Commamonas* sp. | 1 | 1 | 1 | 1 KM |
| 32 | β-Proteobacteria | *Cupriavidus* spp. | 3 | 5 | 21 | 13 KM, 8 BM |
| 33 | Actinobacteria | *Curtobacterium* sp. | 1 | 1 | 1 | 3 TP |
| 34 | β-Proteobacteria | *Delftia* spp | 7 | 9 | 24 | 11 KM, 9 TP, 4 BM |
| 35 | Bacteria | *Endosymbiont of Nilaparvata lugens* | 1 | 1 | 4 | 4 KM |
| 36 | γ-Proteobacteria | *Enterobacter* spp. | 2 | 3 | 7 | 4 T, 2 KM, 1 HS |
| 37 | γ-Proteobacteria | *Enterobacteriaceae bacterium* | 1 | 1 | 1 | 1 KM |
| 38 | Firmicutes | *Eubacterium* sp. | 1 | 1 | 1 | 1 BM |
| 39 | Bacteroidetes | *Flavobacterium* spp. | 4 | 7 | 15 | 8 TP, 7 KM |
| 40 | γ-Proteobacteria | *Frateuria aurantia* | 3 | 4 | 5 | 3 TP, 2 KM |
| 41 | Bacteria | *Fuel tank bacterium* | 1 | 6 | 13 | 11 BM, 2 C |
| 42 | γ-Proteobacteria | *Fulvimonas soli* | 1 | 2 | 2 | 2 TP |
| 43 | γ-Proteobacteria | *Gamma proteobacterium* | 1 | 3 | 9 | 7 T, 1 KM.1C |
| 44 | Actinobacteria | *Gordonia* sp. | 1 | 1 | 1 | 1C |
| 45 | β-Proteobacteria | *Herbaspirillum* sp. | 1 | 1 | 4 | 4 BM |
| 46 | Actinobacteria | *Intrasporangiaceae bacterium* | 1 | 1 | 3 | 3 KM |

| | | | | | | |
|---|---|---|---|---|---|---|
| 47 | β-Proteobacteria | *Janthinobacterium* spp. | 1 | 4 | 6 | 5 KM, 1 TP |
| 48 | Bacteria | *Kartchner Caverns bacterium* | 1 | 1 | 1 | 1 T |
| 49 | Actinobacteria | *Leifsonia* spp. | 3 | 7 | 33 | 33 KM |
| 50 | Firmicutes | *Lysinibacillus fusiformis* | 3 | 7 | 50 | 49 KM, 1 HS |
| 51 | γ-Proteobacteria | *Lysobacter* sp. | 1 | 1 | 1 | 1 TP |
| 52 | Firmicutes | *Marinibacillus* sp. | 1 | 1 | 1 | 1C |
| 53 | α-Proteobacteria | *Mesorhizobium* | 1 | 1 | 2 | 2 KM |
| 54 | α-Proteobacteria | *Methylobacterium* spp. | 6 | 20 | 38 | 29 BM, 7KM, 2 HS |
| 55 | Actinobacteria | *Microbacterium* spp. | 3 | 5 | 26 | 23 KM, 1 BM, 1TP,1C |
| 56 | Actinobacteria | *Micrococcaceae* spp. | 1 | 5 | 9 | 8 KM, 1 BM |
| 57 | Actinobacteria | *Micromonospora* sp. | 1 | 1 | 1 | 1B |
| 58 | microorganism | *microorganism clone* | 1 | 1 | 6 | 6 TP |
| 59 | β-Proteobacteria | *Mitsuaria chitosanitabida* | 1 | 1 | 1 | 1 BM |
| 60 | Actinobacteria | *Mobiluncus curtisii* | 1 | 1 | 2 | 1KM, 1BM |
| 61 | γ-Proteobacteria | *Moraxella* sp. | 1 | 1 | 1 | 1 KM |
| 62 | Actinobacteria | *Nocardia* spp. | 3 | 3 | 3 | 2 KM, 1 TP |
| 63 | α-Proteobacteria | *Novosphingobium* spp. | 1 | 1 | 13 | 13 BM |
| 64 | α-Proteobacteria | *Ochrobactrum* spp. | 3 | 4 | 9 | 4 KM, 3 TP, 2 BM |
| 65 | Bacteria | *Oxalobacteraceae bacterium clone* | 1 | 1 | 1 | 1 KM |
| 66 | Firmicutes | *Paenibacillus* spp. | 9 | 17 | 40 | 14 TP, 11 KM, 10 BM, 1 HS, 4C |
| 67 | β-Proteobacteria | *Pandoraea* spp. | 4 | 4 | 35 | 34 KM, 1 TP |
| 68 | γ-Proteobacteria | *Pantoea agglomerans* | 1 | 1 | 2 | 2 KM |
| 69 | α-Proteobacteria | *Paracoccus* spp. | 2 | 2 | 2 | I KM, 1 T |
| 70 | β-Proteobacteria | *Phenanthrene-degrading bacterium* | 1 | 1 | 1 | 1 KM |
| 71 | α-Proteobacteria | *Phenylobacterium* sp. | 1 | 1 | 1 | 1B |
| 72 | α-Proteobacteria | *Phyllobacterium* spp. | 3 | | 8 | 4 BM, 4 TP |
| 73 | γ-Proteobacteria | *Proteus* spp. | 3 | 5 | 7 | 7 KM |
| 74 | γ-Proteobacteria | *Providencia rettgeri* | 1 | 2 | 2 | 2 KM |
| 75 | β-Proteobacteria | *Pseudacidovorax* sp. | 1 | 1 | 5 | 3 KM, 2 KM |
| 76 | γ-Proteobacteria | *Pseudomonas* spp. | 13 | 56 | 168 | 108 KM, 33 BM, 17 TP, 6HS, 4T, 10C |
| 77 | β-Proteobacteria | *Rahnella* spp. | 2 | 2 | 95 | 94 KM |
| 78 | β-Proteobacteria | *Ralstonia* spp. | 3 | 7 | 8 | 3 KM, 2 BM, 3 BM |
| 79 | Bacterium | *Ramlibacter* sp. | 1 | 1 | 1 | 1 KM |
| 80 | α-Proteobacteria | *Rhizobium* spp. | 3 | 4 | 8 | 5 TP,2 BM,1 KM. |
| 81 | γ-Proteobacteria | *Rhodanobacter* sp. | 1 | 5 | 18 | 18 KM |
| 82 | Actinobacteria | *Rhodococcus* sp. | 1 | 1 | 2 | 2 KM |
| 83 | α-Proteobacteria | *Roseomonas* spp. | 2 | 2 | 2 | 2KM |
| 84 | Actinobacteria | *Sinomonas* sp. | 1 | 1 | 1 | 1 KM |
| 85 | Bacteroidetes | *Sphingobacterium* spp. | 2 | 7 | 34 | 26 KM, 2 BM,2 1T,1SP,2C |
| 86 | α-Proteobacteria | *Sphingomonas* spp. | 4 | 11 | 102 | 88 BM, 12 TP, 2 KM |
| 87 | Firmicutes | *Sporosarcina* sp. | 1 | 1 | 2 | 2 KM |
| 88 | Firmicutes | *Staphylococcus* spp. | 3 | 3 | 5 | 5 KM |
| 89 | γ-Proteobacteria | *Stenotrophomonas* spp. | 4 | 21 | 66 | 25 KM, 24 BM, 13 KM, 9 TP, 2 T |
| 90 | Actinobacteria | *Streptacidiphilus* sp. | 1 | 1 | 2 | 2 TP |
| 91 | Actinobacteria | *Streptococcus* spp. | 11 | 13 | 16 | 8 TP, 7 KM, 1 BM |
| 92 | Actinobacteria | *Streptomyces* spp. | 9 | 15 | 19 | 8TP, 3KM, 4BM, 4C |
| 93 | Bacteria | *Swine fecal bacterium* | 1 | 2 | 10 | 8 KM, 2 BM |
| 94 | Firmicutes | *Tissierella* sp. | 1 | 1 | 5 | 5 KM |
| 95 | β-Proteobacteria | *Variovorax* spp. | 4 | 7 | 17 | 8 KM, 6 TP, 1 KM, 1 BM |
| 96 | γ-Proteobacteria | *Xanthomonas* sp. | 1 | 1 | 1 | 1 BM |
| | | | **249** | **685** | **2176** | |

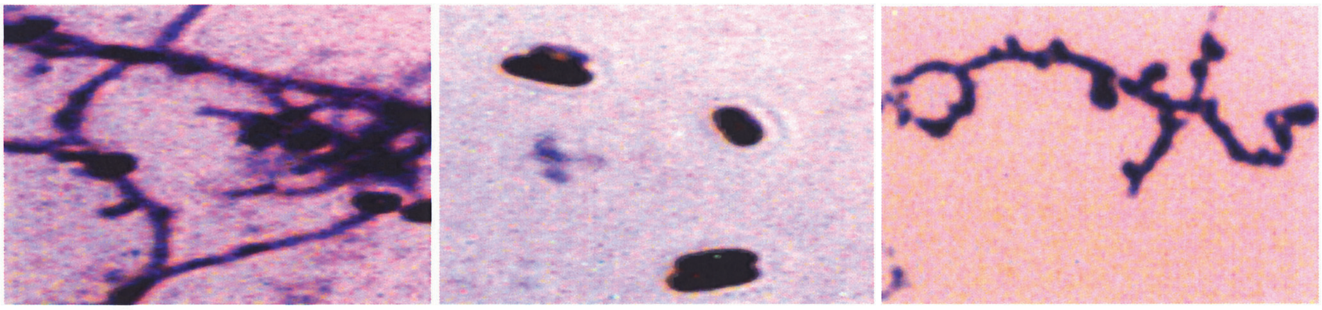
Note: Prokaryotes originating from oil palm plantations: KM = Keratong mineral soil, TP = Teluk Intan peat soil, BM = Belaga mineral soil, KLM = Kluang mineral soil, C = composting fronds, T = termites' guts sampled from oil palm plantation, SP = hot spring.



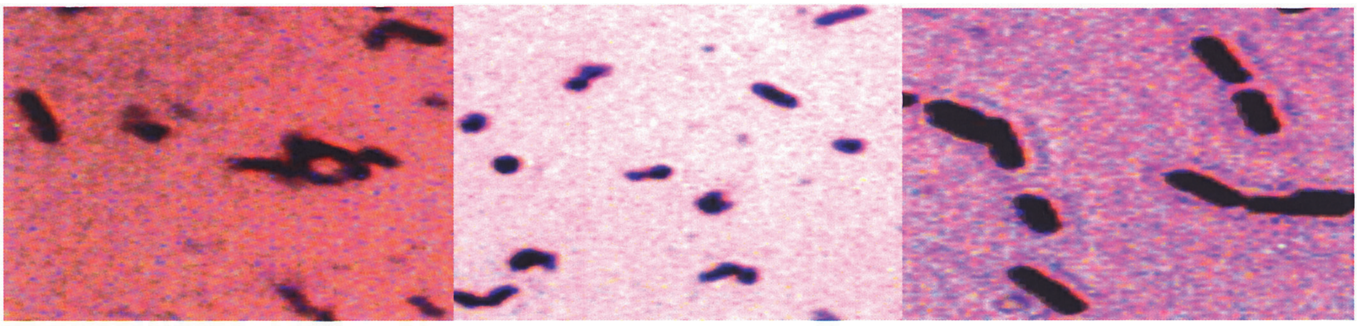
*Streptomyces ferralitis* *Streptomyces niveoruber* *Streptomyces rubrogriseus*



*Actinomycelales bacterium* *Amycolatopsis saalfeldensis* *Rhodanobacter GR18*

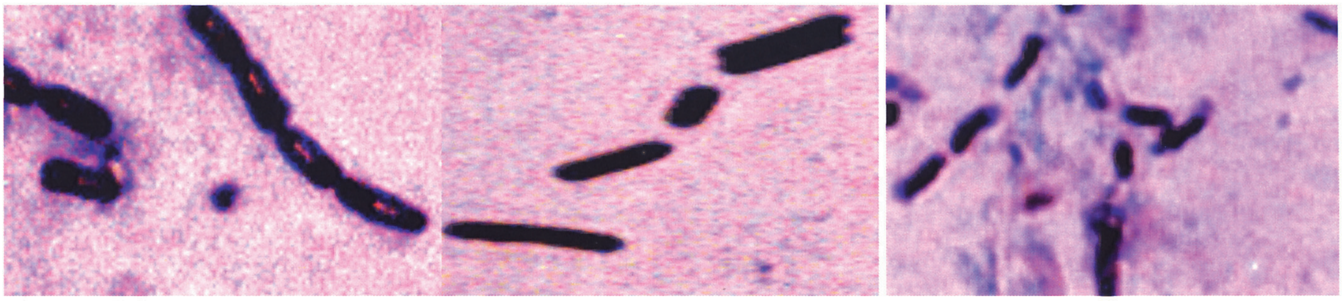


*Micromonaspora NN271* *Arthrobacter* *Streptomyces*

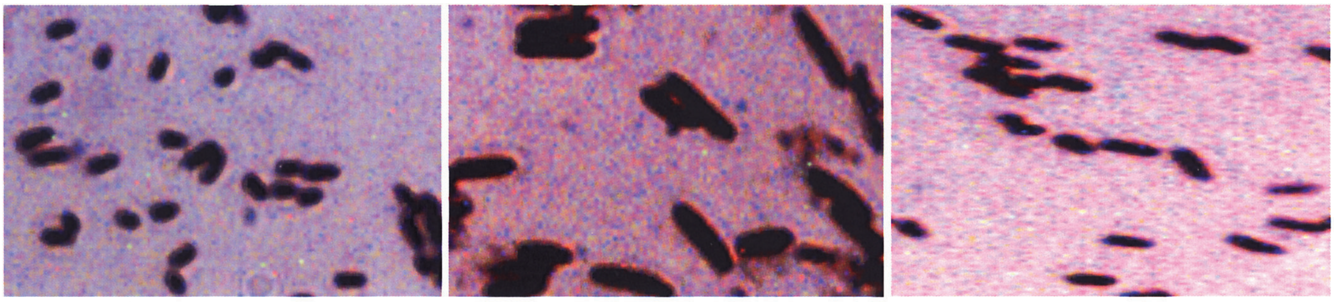


*Gordonia JDC2* *Cellulosimicrobium C10* *Streptomyces*

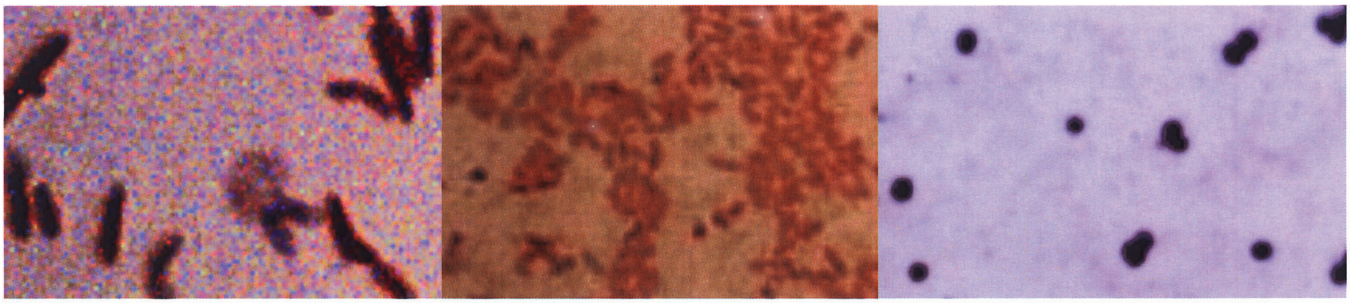
*Figure 1. Actinobacteria important for agro-industries. Magnification 4000 X.*



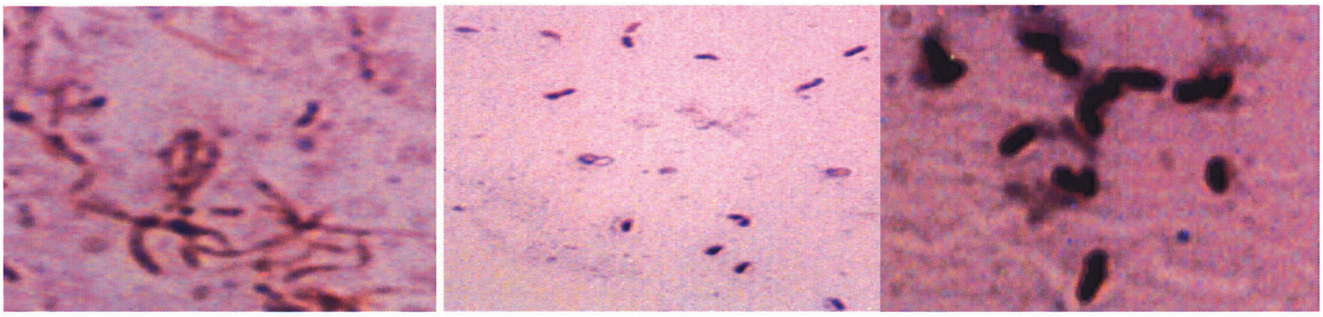
*Bacillus thuringiensis BAB-Bt2*
*B. thuringiensis serovar israelensis*
*Bulkholderia cepacia*



*Bacillus subtilis*
*Bacillus cereus*
*Peanibacillus*



*Pseudomonas trivalis*
*Klebsiella*
*Bacillus sphericus*



*Rhizobium KT204*
*Acetobacter lovaniensis*
*Xanthomonas BJQ-H4*

*Figure 2. Bacteria important for agro-industries. Magnification 4000 X.*

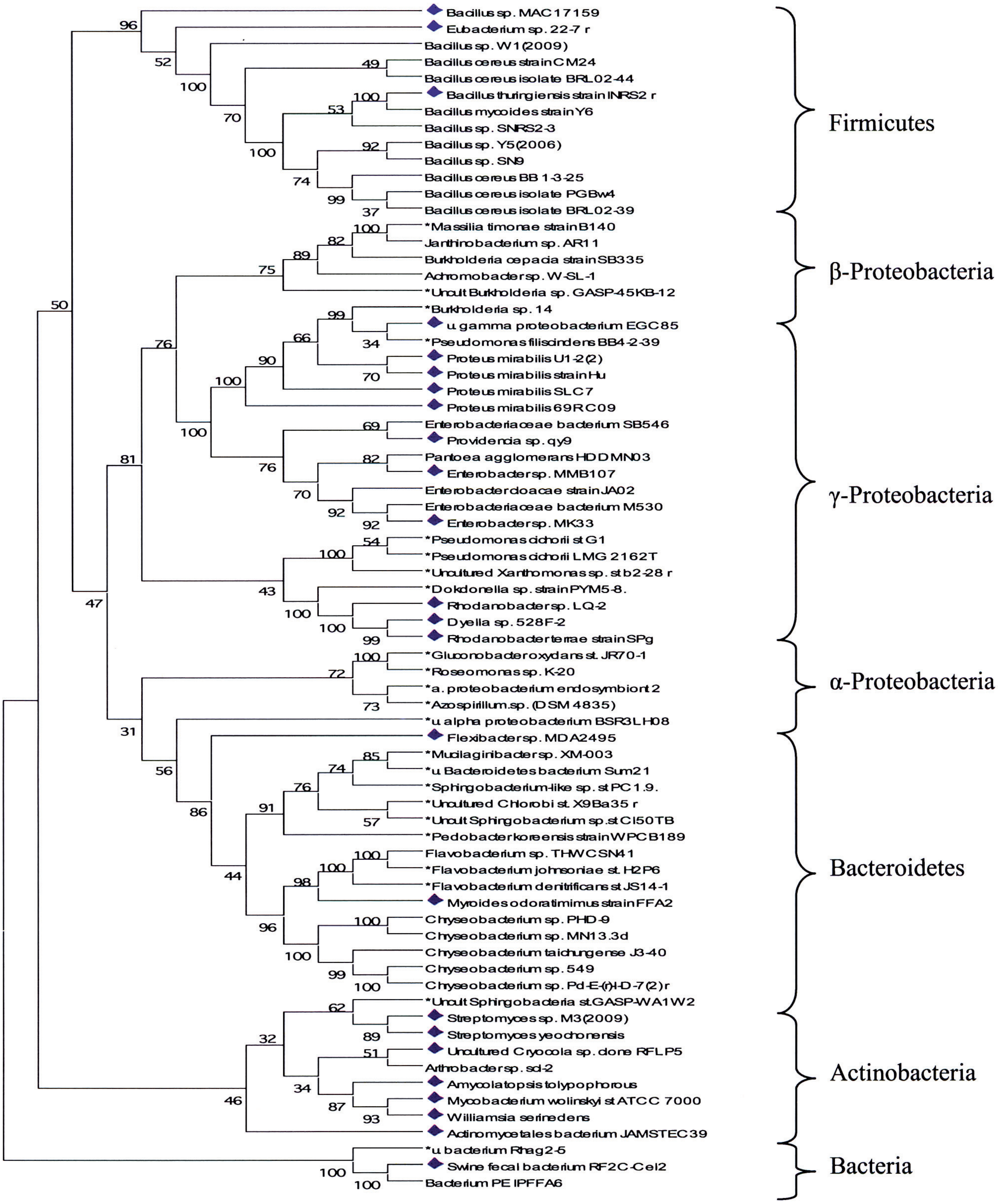


*Figure 3. Prokaryotes biodiversity in Keratong mineral soil at replanting.*

Note: Soil sampled at 12 months during biodegradation of oil palm trunks. The tree was constructed using the Kimura two-parameter algorithm and the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) are reported at each node. The scale bar indicates 0.02 substitution per nucleotide position. Legend: ♦ = phosphatase microbes and * = unculturable microbes. Taxonomic groups consist of seven taxonomies, 40 genera, 47 species and 72 strains.

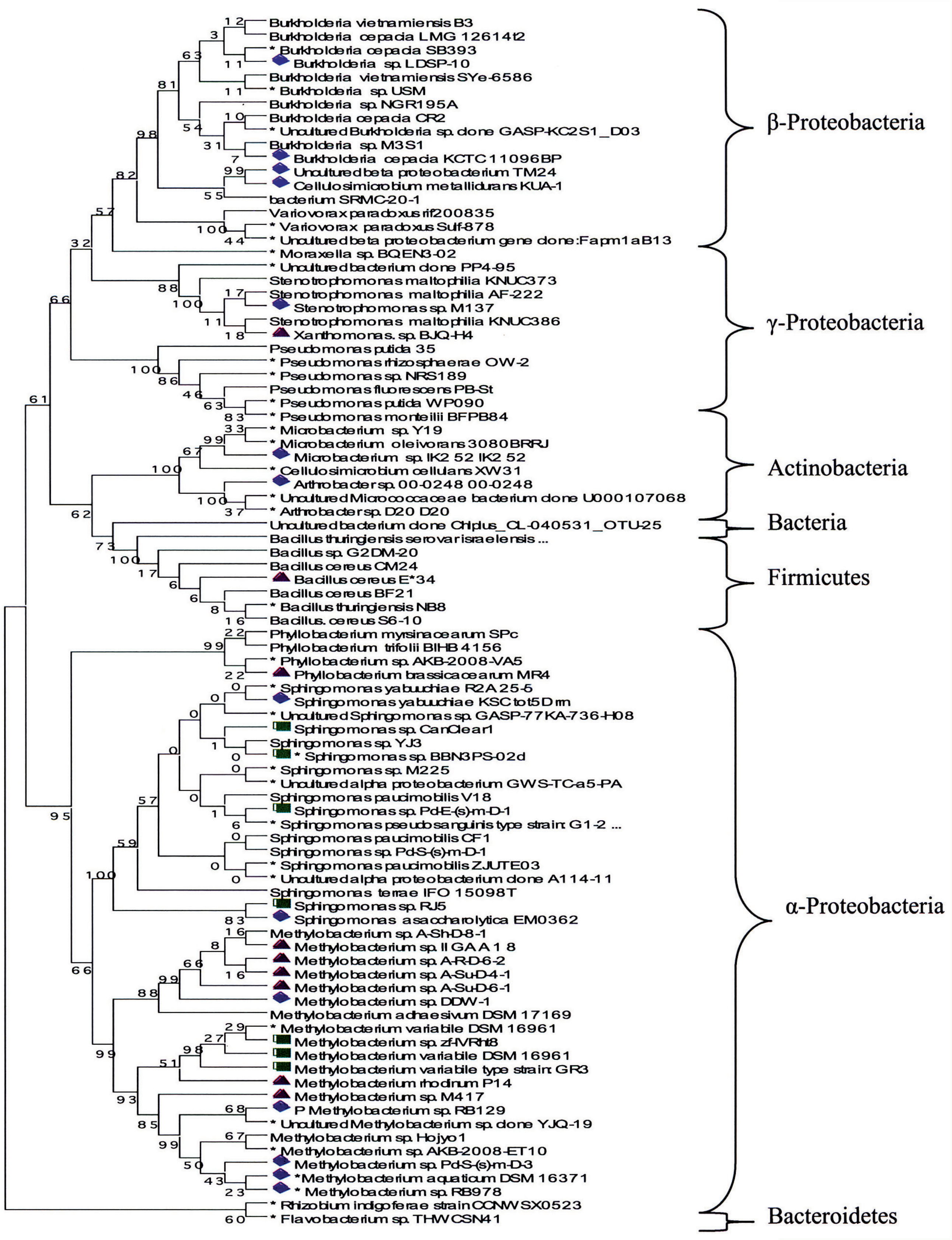


*Figure 4. Prokaryotes biodiversity in Belaga mineral soil in secondary forest.*

Note: The tree was constructed using the Kimura two-parameter algorithm and the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) are reported at each node. The scale bar indicates 0.02 substitution per nucleotide position. Legend: ■ = *nitrogenase microbes,* ● = *phosphatase microbes,* ▲= *k-solubilizing microbes and * = unculturable microbes.* Taxonomic groups consist of seven taxonomies, 23 genera, 50 species and 91 strains.

## BENEFITS

Microbial biodiversity can be selectively harnessed for use in agriculture, forestry, the environment, and for healthcare and food products.

The benefits include the following:

- safer alternatives to chemicals, *e.g.* as bio-fertilizers, bio-pesticides, bio-herbicides;
- producing agro products, *e.g.* compost, plant growth promoters or enhancers;
- valuable resources for bio-industries, *e.g.* microbial-based enzymes;
- bio-geochemical cycles of carbon, nitrogen, sulphur and other elements;
- recycling of wastewater, solid waste treatment; and
- bio-remediation of toxic wastes into non-toxic reusable products.

## CONCLUSION

A holistic approach and strategies for identification, conservation and optimization of the use of microbial biodiversity to enhance productivity, environment safety and quality of life are crucial for sustainable agriculture and bio-industries.

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