

Chapter 13

Microbial Diversity of Brazilian Biomes

Thiago Bruce, Alinne de Castro, Ricardo Kruger,
Cristiane C. Thompson, and Fabiano L. Thompson

Introduction

Brazil is a megabiodiverse country with approximately 20% of the total number of described species on the planet (Mittermeier et al. 2004). It has the largest tropical forest cover (>6 million km², corresponding to approximately 30 times the area of the United Kingdom or approx. 20 times the area of Germany) and one of the largest marine realms (>4 million km²) of the planet. The diversity of biomes (e.g., the Amazon and Atlantic Forests, Southern Plains or Pampas, Cerrado (a savanna-like vegetation), Pantanal (wetlands), Caatinga, coral reefs, oceanic islands, mangroves, salt marshes, coastal environments, and deep sea; (IBGE 2004)) allows for the diversification of a variety of life forms. Between 1999 and 2009, more than 1,200 new species of plants and vertebrates were discovered only in the Amazon region, corresponding to one new discovery every 3 days (WWF 2010). There is indeed a considerable amount of literature on the biodiversity of plants and animals in Brazil. Their diversity is used as a basic parameter in the implementation of management actions on priority areas for conservation and protection (Myers et al. 2000). Studies on the microbial diversity are comparatively much scarcer. For instance, only recently the microbial diversity of Amazon and Cerrado soils and the marine realm have been studied in a systematic way. The aim of this chapter was to establish an overview on the microbial diversity studies (N=150) carried out in Brazil mainly in

T. Bruce • F.L. Thompson (✉)

Laboratory of Microbiology, Institute of Biology, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
e-mail: fabiano.thompson@biologia.ufrj.br

A. de Castro • R. Kruger

Laboratory of Enzymology, University of Brasilia (UNB), Brasilia, Brazil

C.C. Thompson

Laboratory of Microbial Genetics, Institute Oswaldo Cruz (IOC-FIOCRUZ),
Rio de Janeiro, Brazil

the last 5 years, including taxonomic studies based on cultured microorganisms and culture-independent studies based on molecular fingerprints and 16S rRNA clone libraries. The chapter focuses mainly on prokaryotes with environmental (e.g., coral holobionts), agricultural (e.g., crop promoting), and biotechnological importance (e.g., bioremediation).

Microbial Diversity and Polyphasic Taxonomy

Taxonomy deals with the classification (creation of new taxa), identification (allocation of isolates into known taxa), and nomenclature (Vandamme et al. 1996). The polyphasic approach is based on the integration of phenotypic, genotypic, and phylogenetic information in order to establish reproducible, informative, and useful classification and identification schemes. In total, at least 18 bacterial species, 32 fungal species, and 12 protozoan species have been described in Brazil in the last 5 years (Tables 13.1 and 13.2). Taxonomic descriptions of protozoan species are scarce and have been based mainly on morphologic parameters (Siqueira-Castro et al. 2009; Dias et al. 2010a), whereas new bacterial and fungal species descriptions have applied a polyphasic approach. The new taxa were isolated from different sources and localities in Brazil. The diversity of *Vibrios* associated with coral holobionts showed that this group is a regular component of the microbiota (Alves et al. 2010). Studies have shown that *Vibrios* are abundant in the coral *Mussismilia* microbiota (Fig. 13.1) and play important ecological functions such as nitrogen fixation (Alves et al. 2010; Chimetto et al. 2008). The coral holobiont *Mussismilia* is endemic in Brazil and comprises about 70% of the reef structures (Leão and Kikuchi 2005). *Vibrio harveyi*, *Vibrio rotiferianus*, *Vibrio campbellii*, *Vibrio alginolyticus*, *Vibrio mediterranei*, and *Vibrio chagasii* are the most abundant species in coral holobionts (<http://www.taxvibrio.lncc.br>) (Chimetto et al. 2009). Several new *Vibrio* isolates appear to be highly virulent to corals (Alves et al. 2010; de O Santos et al. 2011). New species of marine bacteria associated with corals were also recently described (Chimetto et al. 2011; Chimetto et al. 2010a).

Toward a Genomic Taxonomy

Whole-genome sequence launched microbial taxonomy into a new era, with the possibility of establishing systematics on the basis of complete genomes (Coenye et al. 2005). Currently, there are 1,532 complete prokaryotic genome sequences and 4,726 genome projects in progress (National Center for Biotechnology Information, NCBI, <http://www.ncbi.nlm.nih.gov/sites/genome>). About 36 genomes are deposited in NCBI by Brazilian projects. Twenty-one genomes are published. There are 52 *Leptospira* genomes from FIOCRUZ that were sequenced by J. Craig Venter Institute. In addition, several genomes of plant growth promoting bacteria have been studied (<http://www.bnf.lncc.br/>).

Table 13.1 List of descriptions of new taxa from terrestrial environments

Species	Biome	Local	Approach	Sample	References
Bacteria					
<i>Burkholderia mimosarum</i>	Atlantic Forest	Southeast Brazil	Polyphasic	Root nodules of plant (<i>Mimosa pigra</i> and <i>M. scabrella</i>)	(Chen et al. 2006)
<i>Paenibacillus ritogradensis</i>	Rhizosphere	Rio Grande do Sul	Polyphasic	Rhizosphere suspensions of wheat (<i>Triticum aestivum</i>)	(Beneduzi et al. 2010)
<i>Stenotrophomonas pavanii</i>	Sugarcane plants	São Paulo	Polyphasic	Stems of a sugarcane (<i>Saccharum officinarum</i>)	(Ramos et al. 2010)
<i>Streptomyces lunalinharesii</i>	Cerrado	Central Brazil	Polyphasic	Acid orthic ferral soil	(Souza et al. 2008)
<i>Burkholderia sabiae</i>	Agriculture	Brazil	Polyphasic	Legume tree (<i>Mimosa caesalpinifolia</i>)	(Chen et al. 2008)
<i>Metschnikowia cerradonensis</i>	Cerrado	Tocantins	Polyphasic	Flowers (<i>Ipomoea carnea</i>) and beetle (<i>Conotelus beetle</i>)	(Rosa et al. 2007a)
<i>Burkholderia nodosa</i>	Atlantic Forest	Southeast Brazil	Polyphasic	Root nodules (<i>Mimosa bimucronata</i> and <i>Mimosa scabrella</i>)	(Chen et al. 2007)
Candidatus <i>Magnetoglobus multicellularis</i>	Araruama lagoon	Rio de Janeiro	Morphology and 16S rDNA gene	Hypersaline coastal lagoon	(Abreu et al. 2007)
<i>Azothobium doeberineriae</i>	Root	Minas Gerais and Rio de Janeiro	Polyphasic	Root nodules of woody species (<i>Sesbania virgata</i>)	(de Souza et al. 2006)
<i>Burkholderia ferrariae</i>	Iron ore	Minas Gerais	Polyphasic	High-phosphorous iron ore suspension	(Valverde et al. 2006)

(continued)

Table 13.1 (continued)

Species	Biome	Local	Approach	Sample	References
<i>Burkholderia silvaticantica</i>	Sugarcane and maize field	Rio de Janeiro	Polyphasic	Rhizosphere	(Perin et al. 2006)
<i>Candida queiroziae</i>	Atlantic Forest	Minas Gerais	D1/D2 domains of the LSU rDNA and morphology	Rotting wood and wood-boring insects	(Santos et al. 2011)
<i>Lachancea mirantina</i>	Fermentation process	Pernambuco	D1/D2 of the 26S rDNA gene, ITS region and EFA1 gene	Fermentation process for cachaça production	(Pereira et al. 2011)
<i>Wickerhamiella pagnoccae</i> and <i>Candida jalapaonensis</i>	Cerrado	Tocantins	ITS/5.8S rDNA and the D1/D2 region of the LSU rRNA, and morphology	Nectar of flower bracts of plants (<i>Heliconia psittacorum</i>)	(Barbosa et al. 2011)
<i>Ophiocordyceps rufipedis</i> , <i>O. balzani</i> , <i>O. melanotici</i> , and <i>O. novogranadensis</i>	Atlantic Forest	Minas Gerais	Morphology	Carpenter ants	(Evans et al. 2011)
<i>Hypocnemella verrucospora</i>	Atlantic Forest	até tem mata atlantica	Morphology	Decayed wood of the angiosperm tree (<i>Piptadenia gonoacanthae</i>)	(Coelho et al. 2010)
<i>Trichosporon chiarellii</i>	Ant nest	São Paulo	D1/D2 domain and the ITS1–5.8S rDNA–ITS2 region and morphology	Leaf-cutter ant (<i>Atta capiguara</i>)	(Pagnocca et al. 2010)

<i>Candida golutbevii</i>	Pantanal	Mato Grosso do Sul	D1/D2 domain of the LSU rDNA gene and morphology	Flower of <i>Ipomoea</i> sp.	(Rosa et al. 2009a)
<i>Candida aechmeae</i> and <i>Candida vrieseae</i>	Itapuã Park	Rio Grande do Sul	D1/D2 domain of the LSU rDNA gene and morphology	Leaves of bromeliads (<i>Aechmea recurvata</i> and <i>Billbergia nutans</i>) and tank water of the bromeliad <i>Vriesea gigantea</i>	(Landell et al. 2010)
<i>Spathaspora arborariae</i>	Atlantic Rain Forest and Cerrado	Minas Gerais	D1/D2 domains of the LSU rDNA and morphology	Rotting wood	(Cadete et al. 2009)
<i>Wickerhamomyces queroliae</i> and <i>Candida jatapaonensis</i>	Cerrado	Tocantins	D1/D2 domains of the LSU rDNA and morphology	Larvae of <i>Anastrepha mucronata</i> from ripe fruit of <i>Peritassa campestris</i> and flowers of <i>Centropogon cornutus</i>	(Rosa et al. 2009b)
<i>Cryptococcus bromeliarum</i>	Pedreira Beach in Itapuã Park	Rio Grande do Sul	D1/D2 domains of the 26S rDNA, ITS region, morphology and phenotype	Bromeliads <i>Vriesea procerata</i> , <i>V. friburgensis</i> , and <i>Tillandsia gardneri</i>	(Landell et al. 2009)
<i>Candida materiae</i>	Atlantic Forest	Minas Gerais	D1/D2 region of the LSU rDNA and morphology	Rotting wood samples	(Barbosa et al. 2009)
<i>Moniliella fonsecae</i>	Ipuca Forest fragment	Tocantins, Lago Verde	ITS/5.8S rDNA and the D1/D2 region of the LSU rRNA, and morphology	Flowers (<i>Byrsonima orbigniana</i>)	(Rosa et al. 2009c)

(continued)

Table 13.1 (continued)

Species	Biome	Local	Approach	Sample	References
<i>Pseudocercospora cryptostegiae-madagascariensis</i>	Plant	Minas Gerais	Morphology	Leaf spots of plant (<i>Cryptostegia madagascariensis</i>)	(da Silva et al. 2008)
<i>Trichosporon insectarium</i>	Cheese	Brazil	D1/D2 region of the LSU rDNA and physiology	Artisanal cheese	(Fuentefria et al. 2008)
<i>Aspergillus brasiliensis</i>	Soil	Brazil	ITS-1/5.8S rDNA and ITS-2, AFLP analysis and morphology	Soil	(Varga et al. 2007)
<i>Candida floscolorum</i> and <i>Candida floris</i>	Atlantic Forest and Pantanal	São Paulo and Mato Grosso do Sul	D1/D2 region of the LSU rDNA, morphology and physiology	Flowers of <i>Heliconia velloziana</i> , <i>H. episcopalis</i> and <i>Ipomoea</i> sp.	(Rosa et al. 2007b)
<i>Farysizyma itapuenis</i> , <i>F. setubalensis</i> , and <i>F. taiwanian</i>	Itapuã Park	Rio Grande do Sul	Polyphasic	Leaf of the <i>Bromeliads</i> <i>Dyckia</i> sp., <i>Tillandsia gardneri</i> , <i>F. gemini-flora</i> , <i>Vriesea friburgensis</i> and <i>V. procera</i>	(Inácio et al. 2008)
<i>Candida heliconiae</i> C. <i>picinguabensis</i> and <i>C. saopaulonensis</i>	Atlantic Forest	São Paulo	D1/D2 region of the LSU rDNA, morphology and physiology	Water of flower bracts of <i>Heliconia velloziana</i>	(Ruivo et al. 2006)
Protozoa <i>Trypanosoma serpintis</i>	Pantanal	Mato Grosso do Sul	Morphometrical, ultrastructural and SSU rRNA, and gGAPDH genes	Snake (<i>Pseudoboa nigra</i>)	(Viola et al. 2009)

Table 13.2 List of descriptions of new taxa from aquatic environments

Species	Biome	Local	Approach	Sample	References
Bacteria					
<i>Marinobacterium coralli</i>	São Sebastião Channel at Preta beach	São Paulo	Polyphasic	Coral (<i>Mussismilita hispida</i>)	(Chimetto et al. 2011a)
<i>Vibrio communis</i> sp. nov.	São Sebastião Channel at Grande, Preta and Portinho beach, and Abrolhos Bank	São Paulo and Bahia	Polyphasic	Corals and zoanthids (<i>M. hispida</i> , <i>Phyllogorgia dilatata</i> , <i>Palythoa caribaeorum</i> , and <i>P. variabilis</i>)	(Chimetto et al. 2011b)
<i>Vibrio variabilis</i> and <i>V. marinus</i>	São Sebastião Channel at Grande, Preta and Portinho beach	São Paulo	Polyphasic	Zoanthids (<i>Palythoa caribaeorum</i>)	(Chimetto et al. 2011)
<i>Marinomonas brasiliensis</i>	São Sebastião Channel at Grande beach	São Paulo	Polyphasic	Coral (<i>M. hispida</i>)	(Chimetto et al. 2010a)
<i>Photobacterium jeanii</i>	Abrolhos Bank and São Sebastião at Portinho and Preta beach	Bahia and São Paulo	Polyphasic	Corals and zoanthids (<i>Phyllogorgia dilatata</i> and <i>Palythoa caribaeorum</i>)	(Chimetto et al. 2010b)
<i>Limnohabitans australis</i>	Pond	São Paulo	Polyphasic	Freshwater pond	(Hahn et al. 2010)
Fungi					
<i>Kabatana rondoni</i>	Amazonian Fish	Amazonia	Ultrastructural and molecular	Fish (<i>Gymnorhamphichthys rondoni</i>)	(Casal et al. 2010)
<i>Potaspora morhaphis</i>	Amazon River	Pará	Light microscope, ultrastructural, and 18S rDNA	Fish (<i>Potamorhaphis gutianensis</i>)	(Casal et al. 2008)
Protozoa					
<i>Myxobolus brycon</i>	Pantanal	Mato Grosso do Sul	Morphology and ITS ultrastructural	Fish (<i>Brycon hilarii</i>)	(Azevedo et al. 2011)

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Table 13.2 (continued)

Species	Biome	Local	Approach	Sample	References
<i>Myxobolus oliveirai</i>	Pantanal	Mato Grosso do Sul	Morphology, ultrastructural and 18S rDNA gene	Fish (<i>Brycon hilarii</i>)	(Milanin et al. 2010)
<i>Myxobolus scitades</i>	Poti River	Piauí	Light and electron microscopy	Fish (<i>Sciades herzbergii</i>)	(Azevedo et al. 2010)
<i>Henneguya hemiodopsis</i>	Poti River	Piauí	Ultrastructural	Fish (<i>Hemiodopsis microlepes</i>)	(Azevedo et al. 2009a)
<i>Chloromyxum riorajum</i>	Atlantic coast	South Atlantic coast of Brazil	Light and electron microscopy, and phylogenetic	Fish (<i>Rioraja agassizii</i>)	(Azevedo et al. 2009b)
<i>Loma psittaca</i>	Amazon River	Pará	Light and electron microscopy, and phylogenetic	Fish (<i>Colomesus psittacus</i>)	(Casal et al. 2009a)
<i>Myxobolus heckelii</i>	Tocantins River	Pará	Light and electron microscopy	Fish (<i>Centromochlus heckelii</i>)	(Azevedo et al. 2009c)
<i>Myxobolus cordeiroi</i>	Pantanal (Aquidauna and Miranda River)	Mato Grosso	Morphology, electron microscopy and molecular	Catfish (<i>Zungaro jahu</i>)	(Adriano et al. 2009)
<i>Chloromyxum menticirrii</i>	South Atlantic coast of Brazil (Barra da Lagoa)	Santa Catarina	Light and electron microscopy	Fish (<i>Menticirrhus americanus</i>)	(Casal et al. 2009b)
<i>Henneguya rondoni</i>	Amazon River	Pará	Light and electron microscopy	Fish (<i>Gymnothamphichthys rondoni</i>)	(Azevedo et al. 2008)
<i>Myxobolus metynnis</i>	Amazon River	Pará	Light and electron microscopy	Fish (<i>Metynnis argenteus</i>)	(Casal et al. 2006)

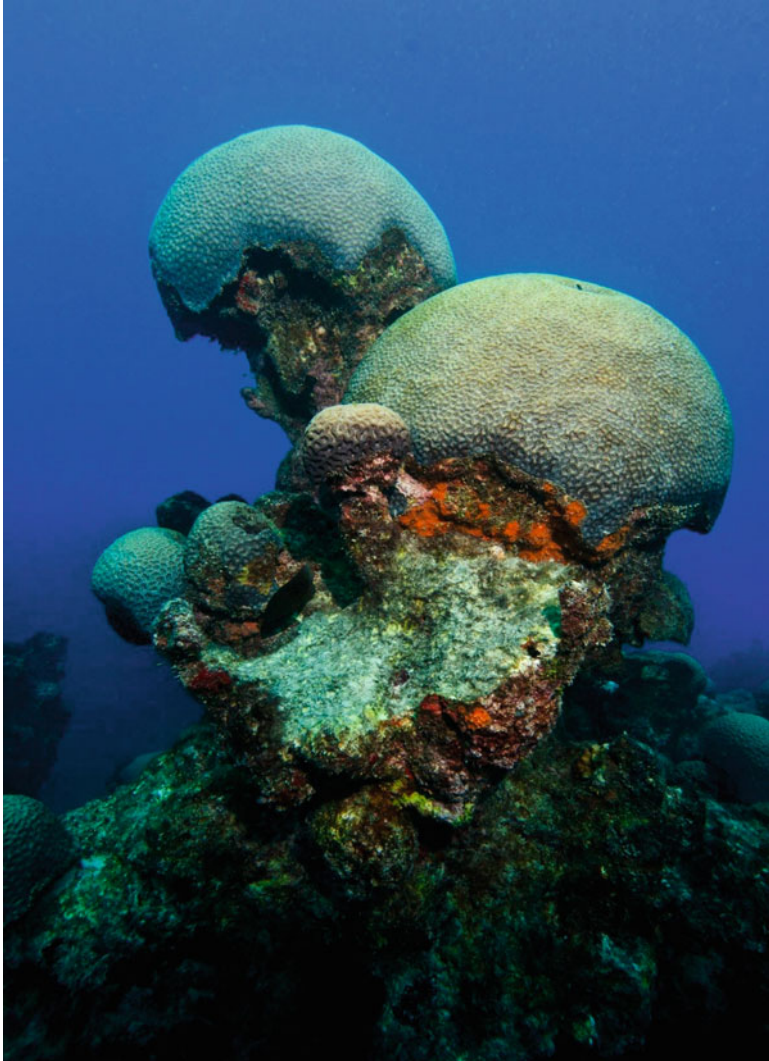


Fig. 13.1 Colonies of *Mussismilia braziliensis* *Mussismilia hispida*, and other associated organisms of the Abrolhos reef bank (Photo by Pedro Meirelles)

Genomic taxonomy is defined on the basis of comparative genomics, including multilocus sequence analysis (MLSA), Supertree, average amino acid identity (AAI), average nucleotide identity (ANI) genomic signatures, codon usage bias, metabolic pathway content, and core and pan genome analyses. The main goal of the genomic taxonomy is to extract information that can be used in order to establish a solid approach for microbial identification and classification (Thompson et al. 2009). Recent studies have developed in silico DNA–DNA hybridization (DDH)

methods in order to access whole genome similarity (Auch et al. 2010). Our studies suggest that a microbial species can be defined on the basis of several genomics features: A microbial species may be defined as a group of monophyletic strains that share >95% similarity in MLSA, >95% AAI, and <10 Karlin's dissimilarity signature (Thompson et al. 2009). We anticipate that these features will be used in the future for online identification and authentication of different types of culturable microbial taxa (Ribeiro et al. 2009). Approaches have been developed in the last three decades in order to analyze the yet uncultured microorganisms. These approaches do not require cultivation in traditional culture media.

Studying the Microbial Diversity by 16S rRNA Library Sequences: A First Attempt

The first culture-independent studies carried out in Brazil date back to the late 1990s (Borneman and Triplett 1997). Sequencing by the Sanger method was used extensively until recently to determine genetic diversity after cloning of DNA directly from an environmental sample (Pace et al. 1985; Schmidt et al. 1991). This approach was widely applied for the study of different Brazilian biomes (Tables 13.3 and 13.4). The first studies using pyrosequencing to study the (marine) microbial diversity are now appearing (Thompson et al. 2011; Roesch et al. 2007).

Microbial Diversity in Brazilian Forests

At least one study has been undertaken in each major Brazilian forest biome (Table 13.2). The Amazon is one of the main hot spots of global biodiversity. Yet attention to the microbial biodiversity was extremely rare in the last decades (Borneman and Triplett 1997). In the western Amazon region, a soil type, known as "Terra Preta" (meaning black soil), is able to stably accumulate organic matter (Lima et al. 2002). This type of soil has been produced and managed successfully by pre-Columbian indigenous communities (about 800 years ago) (Mann 2002). This soil is characterized by high fertility and carbon storage. These characteristics make this region interesting for the investigation of microbial growth promoters in plants. Growth induction occurs by synthesis of microbial compounds (e.g., vitamins, antimicrobials) which facilitates nutrient uptake. In total, 14 phyla were identified in Terra Preta, and only 9 phyla were identified on adjacent unmodified soils, with a prevalence of *Acidobacteria* based on 16S rRNA gene sequences (Kim et al. 2007). The Terra Preta soil type had species richness 25% higher than in the forest soil. Clear differences were observed between the structure of microbial communities in Terra Preta soil type and adjacent unmodified soils because the last one has been identified with a predominance of Crenarchaeota (Jesus et al. 2009; Cenciani et al. 2009; Grossman et al. 2010). This archaeal group makes a significant contribution to

Table 13.3 List of culture-independent studies in terrestrial biomes

Biome	Local	Molecular approach	Samples	References
Agricultural soil	Paraná	16S rRNA PCR and DGGE 16S rRNA gene	Soil	(Nakatani et al. 2011)
Mangrove	Bahia	16S rRNA PCR, DGGE, and clone library sequencing	Rhizosphere and sediment	(Peixoto et al. 2011)
Cerrado	Mato Grosso do Sul	rRNA ITS PCR and 16S rRNA sequencing	Soil	(Alves-Prado et al. 2010)
Atlantic Forest	Rio de Janeiro	16S rRNA clone library sequencing	Soil	(Bruce et al. 2010)
Mangrove	São Paulo	16S rRNA PCR, DGGE, and clone library sequencing	Sediment	(Dias et al. 2010b)
Atlantic Forest	Paraná	16S rRNA clone library sequencing	Soil	(Faoro et al. 2010)
Mangrove	Rio de Janeiro	16S rRNA gene pyrosequencing	Rhizosphere	(Gomes et al. 2010b)
Mangrove	Rio de Janeiro	16S rRNA PCR, DGGE, and clone library sequencing; <i>ndo</i> PCR, DGGE, and clone library sequencing	Rhizosphere	(Gomes et al. 2010a)
Amazon	Manaus	16S rRNA PCR, DGGE 16S rRNA gene, and clone library sequencing and clone T-RFLP analysis	Soil	(Grossman et al. 2010)
Sugarcane field	São Paulo	16S rRNA and <i>gyrB</i> gene sequencing	Rhizosphere and root	(Luvizotto et al. 2010)
Mangrove	São Paulo	<i>dsrB</i> and <i>mcrA</i> PCR, DGGE, and clone library sequencing	Sediment	(Taketani et al. 2010b)
Mangrove	Bahia	16S rRNA PCR, DGGE, and clone library sequencing	Sediment	(Taketani et al. 2010a)
Amazon	Amazonas	16S rRNA T-RFLP and clone library sequencing; <i>amoA</i> real-time PCR and clone sequencing	Soil	(Taketani and Tsai 2010a)
Transgenic eucalyptus cultivar	São Paulo	16S rRNA quantitative real-time PCR, PCR, DGGE, and clone library sequencing	Rhizosphere	(Andreote et al. 2009)
Amazon	Rondônia	16S rRNA PCR and DGGE analysis	Soil	(Cenciani et al. 2009)

(continued)

Table 13.3 (continued)

Biome	Local	Molecular approach	Samples	References
Amazon	Amazonas	16S rRNA T-RFLP and clone library sequencing	Soil	(Jesus et al. 2009)
Amazon	Amazonas	16S rRNA clone sequencing	Root nodules	(Lima et al. 2009)
Atlantic Forest	Bahia	16S rRNA PCR, DGGE, and clone library sequencing	Soil	(Maciel et al. 2009)
Caatinga	Sergipe	16S rRNA PCR, ARDRA, DGGE, and sequencing	Rhizosphere	(Monteiro et al. 2009)
Atlantic Forest	Rio de Janeiro	<i>rpoB</i> and <i>gyrB</i> quantitative real-time PCR and 16S rRNA gene and clone library sequencing	Soil sediment	(Rodrigues et al. 2009)
Atlantic Forest	Rio de Janeiro	<i>rpoB</i> PCR and DGGE analysis	Soil	(Aboim et al. 2008)
Transgenic tobacco cultivar	São Paulo	16S rRNA PCR, ARDRA, DGGE, and sequencing	Rhizosphere/rhizoplane	(Andreote et al. 2008)
Wheat field	Rio Grande do Sul	<i>nifH</i> PCR, RFLP, and 16S rRNA sequencing	Rhizosphere and bulk soils	(Beneduzi et al. 2008a)
Rice field	Rio Grande do Sul	<i>nifH</i> PCR, RFLP, and 16S rRNA sequencing	Rhizosphere and bulk soils	(Beneduzi et al. 2008b)
Mangrove	Rio de Janeiro	16S rRNA PCR, DGGE, and clone library sequencing	Sediment	(Gomes et al. 2008)
Maize field	Rio Grande do Sul	<i>nifH</i> clone library sequencing	Rhizosphere, roots, and stem	(Roesch et al. 2008)
Cerrado	Minas Gerais	<i>rpoB</i> PCR, DGGE, and sequencing	Rhizosphere	(Coelho et al. 2007)
Amazon	Amazonas	16S rRNA clone library sequencing	Soil	(Kim et al. 2007)
Sugarcane field	São Paulo	16S rRNA BOX PCR and <i>RecA</i> gene sequencing	Rhizosphere and root	(Mendes et al. 2007)
Soybean cultivar	Paraná	16S rRNA PCR, DGGE; RFLP and BOX-PCR analysis	Soil and node	(Pereira et al. 2007)
Sugar cane field	Rio Grande do Sul	16S rRNA gene pyrosequencing	Soil	(Roesch et al. 2007)
Atlantic Forest	Paraná and São Paulo	<i>nifH</i> gene BOX-PCR and 16S rRNA sequencing	Roots	(Albino et al. 2006)

(continued)

Table 13.3 (continued)

Biome	Local	Molecular approach	Samples	References
Mangrove	Rio de Janeiro	16S rRNA T-RFLP and sequencing	Sediment	(Brito et al. 2006)
Maize field	Rio de Janeiro	16S rRNA PCR and DGGE analysis	Rhizosphere	(Costa et al. 2006)
Cerrado	São Paulo	ITS rRNA PCR, DGGE, and clone sequencing	Soil	(de Oliveira et al. 2006)
Agricultural soil	PR, RS, DF, AM, MG, and GO	16S rRNA ERIC-REP PCR, RAPD PCR, RFLP, and clone library sequencing	Soil	(Hungria et al. 2006)
Atlantic Forest	São Paulo	16S rRNA clone library sequencing	Phyllosphere	(Lambais et al. 2006)
Mangrove	Rio de Janeiro	16S rRNA gene sequencing	Rhizosphere	(Maciel-Souza et al. 2006)
Atlantic Forest	Rio de Janeiro	16S rRNA gene sequencing	Soil	(Souchie et al. 2006)
Pantanal	Mato Grosso do Sul	16S rRNA PCR and ARDRA analysis	Roots	(Brasil et al. 2005)
Maize cultivar	Minas Gerais	<i>rpoB</i> PCR, DGGE, and clone library sequencing	Soil	(da Mota et al. 2005)
Amazon	Amazonas	16S rRNA gene sequencing	Sediment	(MdF et al. 2005)

the soil nitrogen cycle by means of ammonia oxidation (Taketani and Tsai 2010a; Taketani and Tsai 2010b). So far, microbial isolation and taxonomic characterization have not been performed in this soil in order to find potential new microbes that could be used in soil fertilization (Miransari 2011; Adesemoye et al. 2009; Beneduzi et al. 2008a). Clearly, research aiming at the microbial biodiversity in the Amazon forest has just begun to disclose the potentially huge untapped microbial diversity reservoir. The Atlantic Forest is also a main hotspot of diversity, but only 10% of its original cover is still preserved (Myers et al. 2000). Soil microbial community diversity of the Atlantic Forest in the states of Rio de Janeiro, Paraná, and São Paulo was analyzed. There was a clear dominance of novel *Acidobacteria* (Faoro et al. 2010; Bruce et al. 2010). Bacteria such as *Enterobacteriaceae* and *Bacillus* sp. and mycorrhizal fungi such as *Aspergillus* sp., *Glomus macrocarpum*, and *Glomus etunicatum* were identified and appeared to perform key functions for the health of soil ecosystems of the Atlantic Forest (Souchie et al. 2006). Atlantic Forest soil microbes may reduce sulfates and promote remediation of contaminated environments by heavy metals (Muyzer and Stams 2008). Sulfates of metals (e.g., cadmium, cobalt, copper, iron, nickel, and zinc) are highly soluble, while the corresponding metal sulfides have low solubility, allowing the precipitation and removal of contaminants by soil microbes. Mangrove ecosystems are included in the Atlantic

Table 13.4 List of culture-independent studies in aquatic biomes

Biome	Local	Molecular approach	Sample	References
Cabo Frio upwelling region	Rio de Janeiro	16S rRNA PCR, DGGE, and clone sequencing	Water	(Cury et al. 2011)
Guanabara Bay	Rio de Janeiro	16S rRNA pyrosequencing	Water	(Thompson et al. 2011)
Conceição Lagoon	Santa Catarina	16S rRNA PCR and DGGE analysis	Water	(Fontes et al. 2011)
Abrolhos Bank	Bahia	<i>pyrH</i> gene sequencing	Coral mucus	(Alves et al. 2010)
Abrolhos Bank	Bahia	16S rRNA PCR and clone library sequencing	Coral	(de Castro et al. 2010)
Campos Basin	Rio de Janeiro	16S rRNA PCR and clone library sequencing	Water	(Korenblum et al. 2010)
Tartaruga Beach	Rio de Janeiro	16S rRNA PCR and clone library sequencing	Coral	(Lins-de-Barros et al. 2010)
Guaeca Beach, Ilhota da Prainha, and Toque-Toque Island	São Paulo	ITS rRNA PCR, ARDRA, and sequencing	Sponges, ascidians, and algae	(Menezes et al. 2010)
Cagarras Archipelago and Praia Vermelha Beach	Rio de Janeiro	16S rRNA PCR and sequencing	Sponges	(Fontana et al. 2010)
Guanabara Bay and coastline	Rio de Janeiro	16S rRNA and <i>amoA</i> clone library sequencing	Seawater and sponges	(Turque et al. 2010)
Sepetiba Bay	Rio de Janeiro	16S rRNA PCR and clone library sequencing	Water	(Almeida et al. 2009)
Caboclo Island and Tartaruga Beach	Rio de Janeiro	16S rRNA PCR, DGGE, and clone library sequencing	Sponge <i>Aplysina fulva</i> specimen	(Hardoim et al. 2009)
Paraná River	Paraná and Mato Grosso do Sul	16S rRNA PCR and clone library sequencing	Water	(Lemke et al. 2009)
Abrolhos Bank	Bahia	16S rRNA PCR and clone library sequencing	Coral mucus	(Reis et al. 2009)
Aruama Lagoon	Rio de Janeiro	<i>nifH</i> and 16S rRNA gene sequencing	Water	(Clementino et al. 2008)
Domestic wells and river	Pará	<i>RecA</i> PCR and RFLP analysis	Water	(Dall'Agnol et al. 2008)
Guanabara Bay	Rio de Janeiro	16S rRNA clone library sequencing	Water, sediment, and sponges	(Turque et al. 2008)
Guanabara Bay	Rio de Janeiro	16S rRNA clone library sequencing	Water	(Vieira et al. 2008)
Itanhaém River	São Paulo	16S rRNA PCR and clone library sequencing	Water	(Carvalho et al. 2007)

Campos Basin	Rio de Janeiro	16S rRNA PCR, ARDRA, and clone library sequencing	Oil	(Sette et al. 2007)
Guanabara Bay	Rio de Janeiro	ITS rRNA DGGE and 16S rRNA clone library sequencing	Water	(Clementino et al. 2007)
Guanabara Bay estuary	Rio de Janeiro	16S rRNA PCR, DGGE, and clone library sequencing	Water	(Vieira et al. 2007)
Mangrove	Ceará	16S rRNA PCR and DGGE analysis	Water	(Sousa et al. 2006)

Forest biome, commonly found in transitional zones between terrestrial, marine, and freshwater for over 6,000 km of coast. Mangroves are considered to be nurseries and diverse, yet these ecosystems are subject to intense anthropogenic impact due to their proximity to areas extensively urbanized and industrialized (Amado-Filho et al. 2008). In January 2000, mangrove areas of the Guanabara Bay suffered a major environmental disaster due to leak of about 1.3 million gallons of oil (Gabardo et al. 2000). The characterization of bacterial diversity and bioremediation by mangrove inhabiting microorganisms provided new ideas for improving the management of these environments. The microbial communities of the mangrove reflect the spatial variation of sediment composition (Peixoto et al. 2011; Taketani et al. 2010a). The increased abundance of plasmids (e.g., INCP-1a, 1b-INCP, INCP INCP-7 and-9) and functional genes (naphthalene, and extradiol dioxygenases intradiol) involved in hydrocarbon degradation were identified in the microbial communities after exposure to oil (Gomes et al. 2010a). Apparently, high levels of oil were significantly associated with *Betaproteobacteria*, whereas the levels of polycyclic aromatic hydrocarbons were associated with *Actinobacteria*. Groups involved in aerobic and anaerobic oil degradation, including *Alteromonadales*, *Burkholderiales*, *Pseudomonadales*, and *Rhodobacteriales Rhodocyclales*, *Marinobacter*, *Alcanivorax*, *Microbulbifer*, *Sphingomonas*, *Micrococcus*, *Cellulomonas*, *Dietzia*, and *Gordonia* were detected in impacted areas (Gomes et al. 2008; Brito et al. 2006). Unimpacted mangrove areas had a community dominated by *Alphaproteobacteria*, *Gammaproteobacteria*, and *Acidobacteria*, while minor components of communities were identified as *Betaproteobacteria* and *Actinobacteria* (Dias et al. 2010b). Low concentrations of oxygen and high concentrations of organic matter created conditions favorable for the establishment of anaerobic organisms. Sulfur-reducing bacteria and methanogenic Archaea were identified at different depths of sediment. The distribution patterns of genes related to these metabolic processes (sulfate reductase – *dsrB* and methyl-coenzyme M reductase – *mcrA*) showed a greater diversity at greater depths (Taketani et al. 2010b).

Studies on the Cerrado fields (Cerrado is a savanna-like field, which are being used for crops such as soybeans, corn, and coffee (Marris 2005)) have focused on microbial groups that play roles in nutrient cycling in agricultural systems. Chemical fertilization often results in unexpected harmful environmental effects, including leaching of nitrate into ground water, surface runoff of phosphorus and nitrogen run-off, and eutrophication of aquatic ecosystems. Biological nitrogen fixation mediated by microbes allows a significant reduction of nitrogen fertilizer input. *Paenibacillus* diversity is more influenced by the type of farming than by nitrogen fertilization (Coelho et al. 2007). Subpopulations were identified by molecular methods, representing a useful tool to monitor the effect of agricultural practices (de Oliveira et al. 2006). Fungal communities had 50% loss of diversity in soils used for soybean crops (de Castro et al. 2008). This finding reinforces the need for preservation of Cerrado in order to avoid microbial diversity losses due to the transformation of the original grassland soils. Currently, the Cerrado biome has only about 20% of its original cover and is therefore among the hotspots for conservation priority (Myers et al. 2000).

Microbial Community Diversity in Marine Environments

Coastal waters, upwelling systems, coral reefs, and a hypersaline lagoon were analyzed in the last years (Table 13.2, Figs. 13.2 and 13.3). The first studies on the marine realm focused on the analysis of the coral holobiont *Mussismilia* (Fig. 13.3). The genus *Mussismilia* comprises three species (*M. hispida*, *M. braziliensis*, and *M. hartii*). One of the first studies that investigated the microbial diversity of this endemic coral, using 16S rRNA gene sequences, was conducted by Reis et al. (2009) (Reis et al. 2009). Our analysis revealed the dominance of members of the *Alphaproteobacteria* in healthy

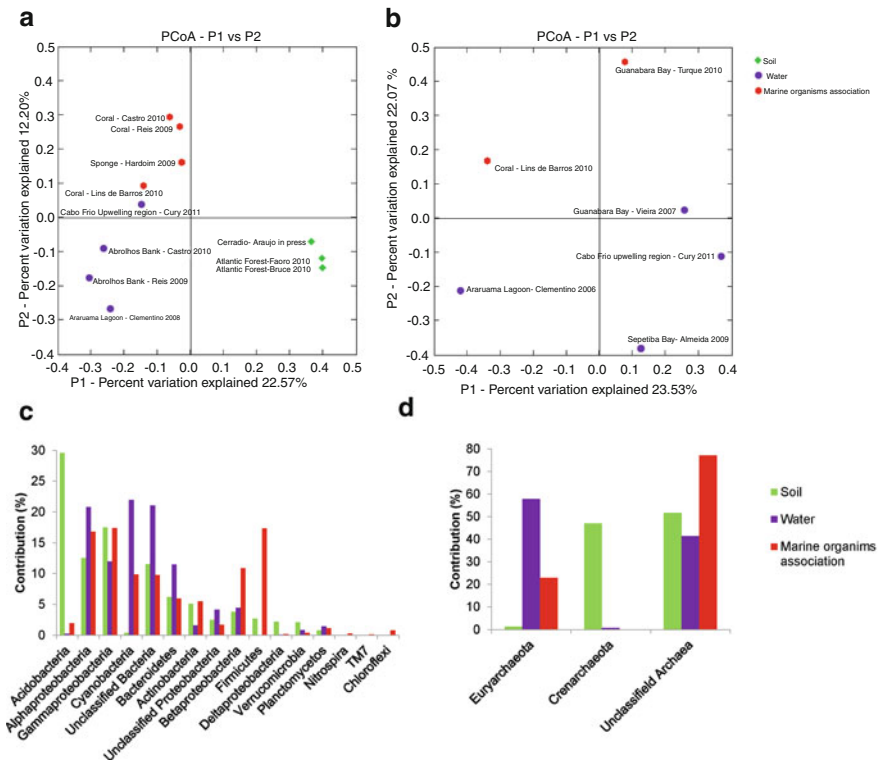


Fig. 13.2 Upper: Principal coordinate analysis (PCoA) of the unweighted pairwise using Fast UniFrac distance matrix. Each point corresponds to comparison of the presence or absence of different bacterial taxa under the different biomes. Green triangles, purple circle, and red hexagon represent soil, water, and marine organisms association from (a) bacteria and (b) Archaea communities, respectively. The percentage of variation explained by the plotted principal coordinates is indicated on the axes. A total of the 6,540 and 1,893 16S rRNA gene sequences were aligned for the bacteria and Archaea, respectively. Same region of 16S rRNA genes, the V1-V2 region, was chosen, and sequences shorter than 300 bp were removed from the analysis. Archaeon from soil was not included. Bottom: Taxonomic classification of 16S rRNA sequences based on classifier tool provided by Ribosomal Database Project. (c) Contribution of main bacterial phyla. (d) Contribution of Archaeon phyla

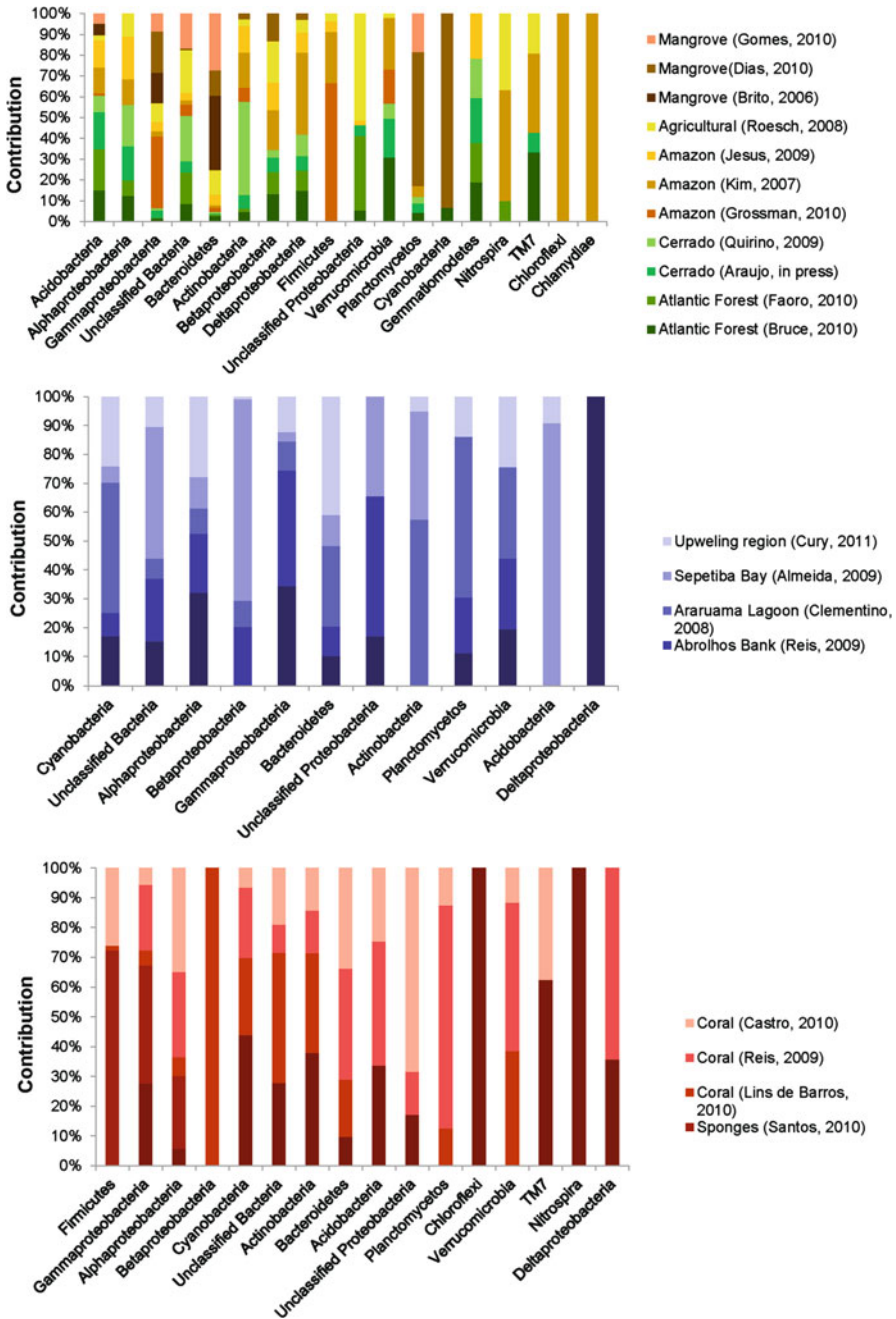


Fig. 13.3 A detailed view of the data presented in Fig. 13.2c

M. hispida. The prevalence of this bacterial group in the genus *Favia Siderastrea* and reefs in the Caribbean and Red Sea is related to the black band disease (Barneah et al. 2007; Sekar et al. 2006). The most commonly found genera in the *M. hispida* were *Azospirillum*, *Fabibacter*, *Blastochloris*, *Stella*, *Vibrio*, *Flavobacterium*, *Ochrobactrum*, *Terasakiella*, *Alkalibacter*, *Azospirillum*, *Propionibacterium*, *Arcobacter*, and *Paenibacillus* (de Castro et al. 2010). An enrichment of *Bacteroidetes* was observed in diseased *M. braziliensis* compared to healthy corals and water (de Castro et al. 2010). The microbiota of healthy corals and surrounding seawater had different composition. In addition, a comprehensive study on the microbial diversity of the three *Mussismilia* species by means of pyrosequencing of V6 intergenic 16S rRNA region disclosed a core microbiota for each *Mussismilia* species (Fernando et al. 2011). This core microbiome may represent true endosymbionts of *Mussismilia* that have coevolved in close relationship with this holobiont. (Fernando et al. 2011). These data reinforce the model of phase shift of the microbial community in healthy and diseased coral holobionts (Mao-Jones et al. 2010). Recent studies also showed that most archaeal communities associated with *M. hispida* appear to comprise new species of the phylum *Crenarchaeota*, indicating the need of microbial isolation and further taxonomic studies (Lins-de-Barros et al. 2010; Wegley et al. 2007).

Microbial Diversity in Urban Coastal Areas

Guanabara Bay is one of the most famous bays in Brazil and is located in the state of Rio de Janeiro. This bay covers part of the metropolitan region of Rio de Janeiro state that is characterized by a zone of intense urban and industrial activity with clear impacts in the bays' microbiota (Clementino et al. 2007). The main (a) biotic parameters that shape these microbial communities include phosphorus, nitrogen, and salinity (Gregoracci et al. 2012) and are not completely understood. Apparently, oil spill and sewage influence the bacterial and archaeal community structures, with the dominance of potential indicator microorganisms (Vieira et al. 2007; Vieira et al. 2008). In the Sepetiba Bay (located nearby Rio de Janeiro city), there was a reduced microbial diversity possibly due to the intense industrial activity, sewage, and metal contamination (e.g., zinc). The main groups belonged to acidophilic *Acidocella*, *Acidosphaera*, and *Acidiphilium*. Most *Archaea* belong to the phylum *Crenarchaeota*, related to the genera *Sulfolobus* and *Metallosphaera* (Almeida et al. 2009). The Araruama Lagoon is the largest hypersaline lagoon in the world and is connected to the Atlantic Ocean through the Channel Itajuru. This lagoon is nearby Guanabara Bay. The first study on the microbial community diversity of Araruama Lagoon determined the main taxonomic groups and possible spatial patterns related to salt concentration. The genera *Methanomicrobia* and *Methanothermococcus* (*Archaea Euryarchaeota*) were found at lower salt concentration areas (Clementino et al. 2008). On the other hand, the genera *Haloarcula* (*Archaea*) and *Salinibacter* (*bacteria*) were dominant in hypersaline areas (saturated with salt). The bacterial community of the pond water showed a dominance of sequences related to

Gammaproteobacteria, *Actinobacteria*, and *Synechococcus* (Clementino et al. 2008). These microbes may be involved in nitrogen and carbon fixation in this lagoon. The hypersaline environment of Araruama Lagoon showed influence over the multicellular magnetotactic prokaryote *Candidatus Magnetoglobus multicellularis* (Martins et al. 2009). It is a Gram-negative, phylogenetically related to sulfate-reducing *Deltaproteobacteria* first described in Araruama Lagoon (Abreu et al. 2007). It is characterized to be able to form flagellated bacterial cell aggregates, organized in a sphere shape, that swim in either helical or straight trajectories. The composition and abundance of its magnetosomes and the fact that the magnetosomes can be digested by their ciliate predators suggest a role for *Ca. M. multicellularis* in the biogeochemical cycles of iron and sulfur.

Brazilian Biomes Have Specific Microbiotas

The analysis of bacterial and archaeal 16S rRNA gene sequences from different studies allowed a clear differentiation between terrestrial and aquatic biomes (Fig. 13.2a, b). Sequences appeared to group in the principal component analysis according to biome (Fig. 13.2a). The first group consisted of three libraries from soil samples (Faoro et al. 2010; Bruce et al. 2010; Araujo J 2999). The second cluster consisted of four libraries from seawater (Reis et al. 2009; de Castro et al. 2010; Clementino et al. 2008; Cury et al. 2011). The third group comprised four libraries of microorganisms associated with marine animals (Reis et al. 2009; de Castro et al. 2010; Lins-de-Barros et al. 2010; Hardoim et al. 2009). The most evident differences between terrestrial and seawater environments related to the fact that *Acidobacteria*, *Actinobacteria*, and *Firmicutes* were more common in soils, whereas *Cyanobacteria* and *Bacteroidetes* were more abundant in water (Figs. 13.2c, d and 13.3). A high number of 16S rRNA sequences (about 10% in soil and 20% in water) were not classified using the classifier tool (Ribosomal Database Project II), with a confidence level of 80%. These sequences were used for a phylogenetic reconstruction in order to reveal the closest phylogenetic neighbors. Unclassified sequences from soil samples were more heterogeneous than sequences from the water (Figs. 13.4 and 13.5). It was also observed groups containing sequences from different studies (Figs. 13.4 and 13.5). These groups deserve further taxonomic investigation as they represent potentially new phyla or classes.

Discovery of New Enzymes by Metagenomics

Mussismilia metagenomic DNA and Atlantic Forest soil metagenomic DNA were obtained and approximately 10 kb fragments generated by partial digestion and randomly cloning in the pcf430 plasmid (Bruce et al. in prep). Using EPI300 *E. coli*

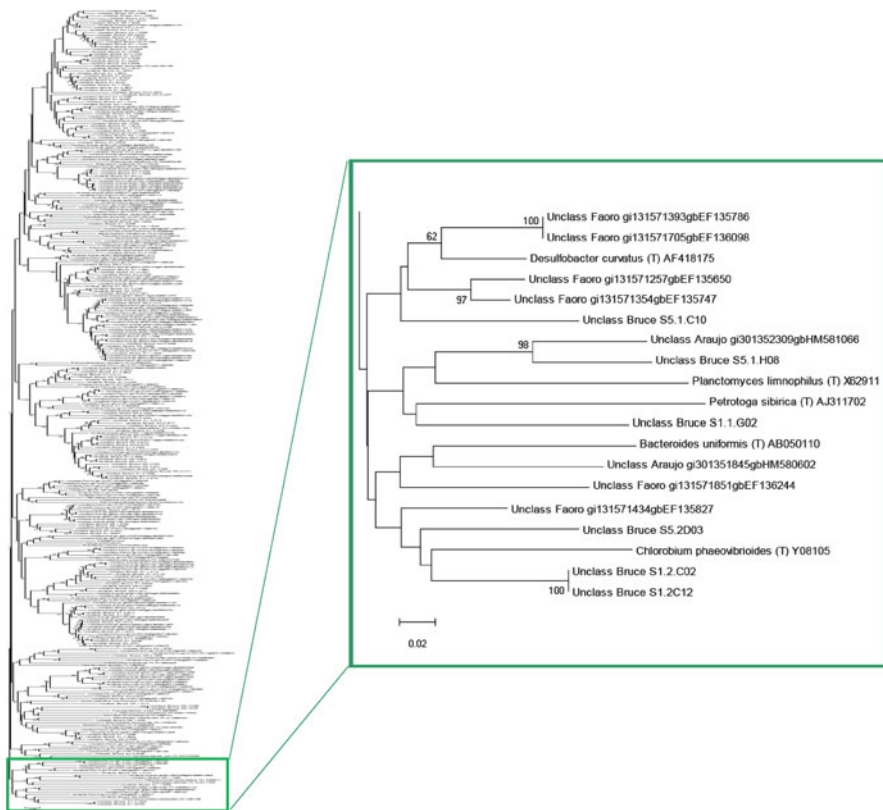


Fig. 13.4 16S rRNA gene phylogenetic tree of unclassified sequences recovered from soil sequences compared with type sequences obtained from public database (RDP). All sequences were aligned using MUSCLE software. Phylogenetic analyses were performed with the software MEGA, using the neighbor-joining model. Bootstrap values are shown for those branches that had >50% support in a bootstrap analysis of 1,000 replicates. Green line corresponds to example of singleton sequences with high evolutionary distance in comparison with type strain 16S rRNA gene sequences to demonstrate heterogeneity of soil diversity

cells, a large metagenomic library was established containing approx. 70,000 clones. In one attempt to find enzymatic activities, clones were screened for cellulolytic activity in minimal medium supplemented with carboxymethyl cellulose (CMC), a soluble derivative of cellulose. The complete degradation of cellulose requires a complex formed by enzymes endoglucanases, β -glycosidases, and exoglucanases (Lynd et al. 2002). The activity was assessed by the occurrence of degradation halos revealed by Congo red reagent capable of joining the β -glucose 1,4 links (Teather and Wood 1982). The formation of discrete halos was observed around some clones that were further selected for enzymatic essays. Clones at the stationary phase (approximately 5 h) had high endo- and exoglucanase (toll- β , 1-4, and total pulp) activities. This first attempt showed the tremendous potential of marine holobionts and the Atlantic Forest soil for the discovery of bioactive molecules to be used in the industry.

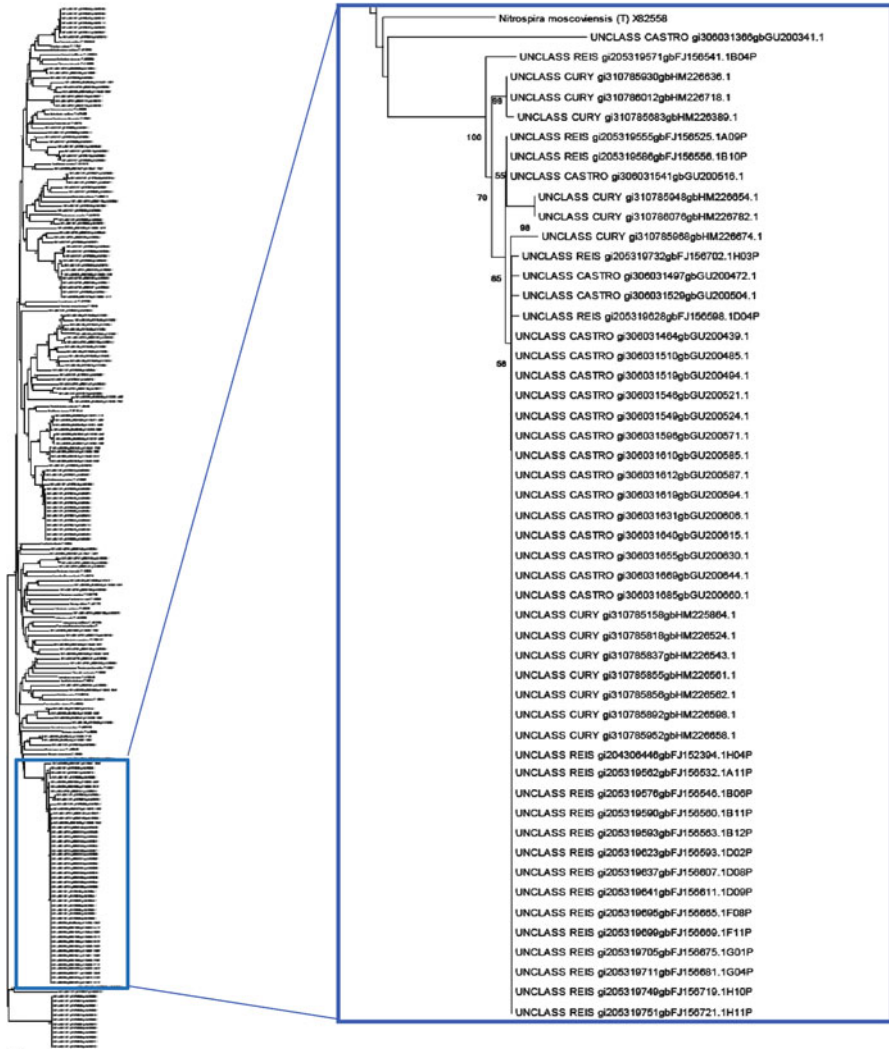


Fig. 13.5 16S rRNA gene phylogenetic tree of unclassified sequences recovered from water sequences compared with type sequences obtained from public database (RDP). All sequences were aligned using MUSCLE software. Phylogenetic analyses were performed with the software MEGA, using the neighbor-joining model. Bootstrap values are shown for those branches that had >50% support in a bootstrap analysis of 1,000 replicates. Blue line corresponds to an OTU with very similar sequences (>97% of similarity of rRNA 16S genes) found by different authors in aquatic environments. Observe several other large novel phylogenetic branches in the tree, representing potential new taxa

Conclusions and Perspectives

The huge microbial diversity of Brazilian biomes represents an untapped reservoir of new genes and metabolisms that play pivotal roles in environmental health. In addition, the microbial diversity represents a vast reservoir for biotechnological discovery and applications. Metagenomics-based discovery and massive isolation of target microorganisms may be suitable approaches to use this vast biodiversity. Identifying the culturable biodiversity by genomic taxonomy is also now a feasible endeavor. Further, microbial diversity studies are deploying second (454 – Roche, Illumina, SOLiD – Life)- and third (ion torrent – Life)-generation sequencing technology to uncover the biodiversity in the marine environment. In one recent study, we analyzed the metagenomic diversity of the Abrolhos reef bank. This analysis allowed us to determine that marine protected areas have a higher microbial diversity and homeostasis than unprotected (pouched) surrounding areas (Bruce et al. 2011). Marine-protected areas promote high coral cover and fish biomass. In another study, we are mapping the metagenomic diversity of the Brazilian oceanic islands (São Pedro and São Paulo, Trindade and Martin Vaz) and deep sea in the talude of Abrolhos reef bank. The studies are integrated in order to establish an overview of the metagenomic diversity in the whole South Atlantic Ocean. Clearly, the time is exciting and challenging for new biodiversity discoveries in Brazil.

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