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ORIGINAL PAPER

# Identification of culturable and originally non-culturable endophytic bacteria isolated from shoot tip cultures of banana cv. Grand Naine

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**Abstract** In this article we describe the identification of endophytic bacteria belonging to three groups isolated from shoot tip cultures of banana cv. Grand Naine in a recent study (Thomas et al. 2008) based on partial 16S rRNA gene sequence homology analysis. The first group included banana stocks that displayed obvious colony growth on MS based tissue culture medium during the first in vitro passage. The second group constituted stocks that were tissue index-negative for cultivable bacteria initially but turned index-positive after a few to several (4–8) in vitro passages while the third group formed one sub-stock that turned index-positive after about 18 passages. The organisms belonged to about 20 different genera comprising of $\alpha$, $\beta$, $\gamma$-proteobacteria, Gram-positive firmicutes and actinobacteria. Visibly expressing easily cultured organisms during the first in vitro passage included *Enterobacter*, *Klebsiella*, *Ochrobactrum*, *Pantoea*, *Staphylococcus* and *Bacillus* spp. Organisms of second group that were not detected or non-culturable originally constituted *Brevundimonas*, *Methylobacterium*, *Alcaligenes*, *Ralstonia*, *Pseudomonas*, *Corynebacterium*, *Microbacterium*, *Staphylococcus*, *Oceanobacillus* and *Bacillus* spp. while the third group that turned cultivable after extended in vitro culturing included mostly non-filamentous actinobacteria (*Brachybacterium*, *Brevibacterium*, *Kocuria* and *Tetrasphaera* spp.). The identification results suggested that the endophytes of second and third groups were not strictly obligate or fastidious microbes but those surviving in viable but-non-culturable (VBNC) state and displaying gradual activation to cultivable form during continuous tissue culturing. Several of the organisms isolated are known as beneficial ones in agriculture while some organisms have possible implications in human health. The use of tissue cultures for isolating uncommon endophytes is discussed.

Supply of live bacterial cultures or genetic material for research purpose is subject to their revival from glycerol stocks (as some of the organisms showed poor tolerance) and the requestor obtaining written permission from the Director General, Indian Council of Agricultural Research, New Delhi-110001.

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**Keywords** Actinobacteria · Environmental microbiology · Fastidious bacteria · Microbial contamination · Microbial biodiversity · Microbial ecology · *Musa* sp. · Plant tissue culture · Viable but non-culturable (VBNC)

**Abbreviations**

| | |
|---|---|
| BIM | Bacteriological indexing medium |
| NA | Nutrient agar |
| OC | Original culture |
| TCM | Tissue culture medium |
| TSA | Trypticasein soy agar |
| VA | Viss et al. (1991) agar |
| VBNC | Viable but non-culturable |

## Introduction

Microbial contamination is a major problem hindering tissue culture applications. There is a general tendency to attribute microbial contamination in tissue cultures to ineffective explant disinfection at culture initiation or inefficient sterile practices during culture handling. Our recent observations with banana and papaya shoot-tip cultures employing a combination of bacterial 16S rRNA gene-based molecular technique, light microscopy and cultivation-based approaches have shown the ubiquitous presence of endophytic bacteria in field shoots as well as apparently clean in vitro cultures across different genotypes either in culturable and/or non-culturable form (Thomas et al. 2007a, b, 2008). With bananas, only a small portion of field shoot tips showed visibly expressing or cultivable endophytes at culture initiation. The remaining apparently clean stocks harbored fastidious or viable but-non-culturable (VBNC) bacteria as brought out through microscopic/molecular observations and indexing of tissue segments or plating of tissue homogenate on different bacteriological media. These stocks displayed gradual activation of the originally non-culturable organisms to cultivation over subculture passages rendering almost all the apparently clean banana stocks as quiescent bacteria-harboring after a few to several (2–10) passages. A few stocks that stayed consistently index-negative however turned index-positive upon recurrent in vitro culturing over 2 years. Such covert bacteria harboring stocks showed substantial levels of colonization to the tune of $10^5$–$10^7$ colony forming units $g^{-1}$ tissue of cv. Grand Naine. This study has also brought out the quiescent survival of endophytic bacteria in unsuspecting suspension cultures and highlighted the implications of such association on micropropagation, in vitro gene banks and molecular profiling, and in tissue culture related issues like habituation and somaclonal variation (Thomas et al. 2008).

During the course of above study with banana 'Grand Naine', we have isolated bacteria belonging to three groups namely organisms that emerged as obvious contaminants during the first passage, those detected in apparently clean stocks after a few passages and those emerged after prolonged in vitro culturing (Thomas et al. 2008). This study was taken up with the objective of establishing the identity of organisms isolated in the above study.

## Materials and methods

### Bacteriological media and isolation of organisms

Bacteriological media used included nutrient agar (NA), Viss et al. (1991) 523 agar medium (VA) and trypticasein soy agar (TSA) in single-use 10-cm plates (Hi-Media Laboratories, Mumbai, India). Unless mentioned differently, NA or nutrient broth (NB) was used for all bacterial isolation and further culturing works. The nutrient plates used in culture indexing were pre-incubated at 37°C for 2–3 days to ensure their freedom from all accidental contaminants while that meant for dilution plating were prepared the same day. The plates were sealed hermetically in sterile polypropylene bags both before and after indexing/plating.

Thirty-seven bacterial isolates belonging to three groups that were retrieved from shoot-tip cultures of 'Grand Naine' described in the earlier experiment (Thomas et al. 2008) were taken up in this study. The first group included seven clones isolated after serial dilution and plating of bacterial growth that emanated from visibly contaminated stocks on tissue culture medium (TCM) at culture initiation (one from January 2004 batch and six from January 2005 set) (Table 1). The second group included 21 isolates retrieved from the bacteriological medium during tissue indexing of visibly clean stocks after 4–8 in vitro passages (Table 2). The third group included nine isolates that were detected during tissue

**Table 1** Endophytic bacteria isolated from banana 'Grand Naine' shoot tip cultures during the first in vitro passage as obvious contaminants

| Stock culture | Isolate ID | NCBI accession no. | Closest NCBI species match[a] | | | Phylogeny | Gram reaction |
|---|---|---|---|---|---|---|---|
| | | | Organism | Identity (%) | Accession no. | | |
| Jan-04 OC10 | GN04-10 | DQ872436 | *Enterobacter cloacae* | 99 | DQ988523 | γ-proteobacteria | −ve |
| Jan-05 OC03 | GN05-3a | DQ872453 | *Klebsiella variicola* | 99 | AJ783916 | γ-proteobacteria | −ve |
| Jan-05 OC03 | GN05-3b | DQ872454 | *Ochrobactrum intermedium* | 99 | AM409326 | α-proteobacteria | −ve |
| Jan-05 OC05 | GN05-5a | DQ872455 | *Pantoea agglomerans* | 99 | AY924376 | γ-proteobacteria | −ve |
| Jan-05 OC06 | GN05-6a | DQ872456 | *Bacillus subtilis* | 99 | AY775778 | Firmicutes | +ve |
| Jan-05 OC09 | GN05-9 | DQ872457 | *Klebsiella variicola* | 99 | AJ783916 | γ-proteobacteria | −ve |
| Jan-05 OC12 | GN05-12 | DQ872458 | *Staphylococcus epidermidis* | 99 | AY752920 | Firmicutes | +ve |

[a] *E*-value in all the instances was zero

**Table 2** Endophytic bacteria isolated from banana 'Grand Naine' shoot tip cultures after a few to several in vitro passages as covert contaminants

| Stock culture | Isolate(s) ID | NCBI accession no.(s) | Closest NCBI species/genus match[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | Organism | Identity (%) | Accession no. | Phylogeny | Gram reaction |
| Jan-04 OC06[(p#5)] | GN04-6a, 6b | DQ872437, 38 | *Brevundimonas* sp. | 99 | DQ341416 | α-proteobacteria | −ve |
| | GN04-6c | DQ872439 | *Oceanobacillus picturae* | 99 | AM237397 | Firmicutes | +ve |
| Jan-04 OC07[(p#5)] | GN04-7a | DQ872440 | *Alcaligenes faecalis,* | 99 | AY866407 | β-proteobacteria | −ve |
| | GN04-7b | DQ872441 | *Ralstonia mannitolilytica* | 99 | AY043378 | β-proteobacteria | −ve |
| Jan-04 OC08[(p#5)] | GN04-8a, 8b, 8c | DQ872442, 43, 44 | *Brevundimonas* sp. | 99 | DQ676936 | α-proteobacteria | −ve |
| Jan-04 OC09[(p#5)] | GN04-9a | DQ872445 | *Staphylococcus epidermidis* | 100 | AY030342 | Firmicutes | +ve |
| | GN04-9b | DQ872446 | *Alcaligenes faecalis* | 99 | AY866407 | β-proteobacteria | −ve |
| | GN04-9c, 9e, 9f, 9h | DQ872447, 49, 50, 51 | *Bacillus fusiformis* | 100 | AM292996 | Firmicutes | +ve |
| | GN04-9d | DQ872448 | *Corynebacterium amycolatum* | 99 | AY831726 | Actinobacteria | +ve |
| | GN04-9i | DQ872452 | *Pseudomonas stutzeri* | 100 | AJ288151 | γ-proteobacteria | −ve |
| Jan-05 OC11[(p#10)] | GN05-11a | DQ872459 | *Microbacterium testaceum* | 99 | AF474325 | Actinobacteria | +ve |
| | GN05-11b | DQ872460 | *Staphylococcus arlettae* | 100 | AB009933 | Firmicutes | +ve |
| | GN05-11c | DQ872461 | *Bacillus neonatiensis* | 99 | AY904032 | Firmicutes | +ve |
| | GN05-11d | DQ872462 | *Methylobacterium hispanicum* | 99 | AJ785570 | α-proteobacteria | −ve |
| | GN05-11e | DQ872463 | *Bacillus pumilus* | 100 | AF526907 | Firmicutes | +ve |

[a] *E*-value in all instances was zero

[p#] = passage number in which isolation was undertaken

**Table 3** Endophytic bacteria isolated as covert contaminants from banana 'Grand Naine' shoot tip culture Jan.2004-OC11.4 sub-stock that were activated to cultivation after prolonged in vitro culturing

| Stock culture | Isolate ID | NCBI accession no. | Closest NCBI species/genus match[a] | | | Phylogeny | Gram reaction |
|---|---|---|---|---|---|---|---|
| | | | Organism | Seq. identity (%) | Accession no. | | |
| Passage 19; Jan-04 OC11.4 | GN0406-11.4.1 | DQ890503 | *Brachybacterium* sp. | 98 | AJ415376 | Actinobacteria | −ve |
| | GN0406-11.4.2 | DQ890504 | *Brevibacterium* sp. | 99 | AY577816 | Actinobacteria | +ve |
| Passage 20; Jan-04 OC11.4 | GN0406-11.4ps.a | DQ890507 | *Brachybacterium* sp. | 99 | AJ415376 | Actinobacteria | −ve |
| Shoot part | GN0406-11.4ps.a | DQ890508 | *Brachybacterium paraconglomeratum* | 99 | AJ415377 | Actinobacteria | +ve |
| | GN0406-11.ps.c | DQ890509 | *Kocuria rosea* | 100 | DQ060382 | Actinobacteria | +ve |
| | GN0406-11.4ps.d | DQ890510 | *Brevibacterium* sp. | 100 | AB210943 | Actinobacteria | +ve |
| | GN0406-11.4.ps.e | DQ890511 | *Tetrasphaera* sp. | 99 | AF408982 | Actinobacteria | +ve |
| Corm part | GN0406-11.4cm.a | DQ890512 | *Brachybacterium* sp. | 99 | AJ415376 | Actinobacteria | −ve |
| | GN0406-11.4.cm.b | DQ890513 | *Staphylococcus epidermidis* | 99 | AY030342 | Firmicutes | +ve |

[a] *E*-value in all instances was zero

indexing or homogenate-plating of one sub-stock from January 2004 batch (OC January 2004-11.4) (Table 3) that remained consistently index-negative but turned index-positive after about 20 passages spanning over 2 years, detected on VA and TSA but not on NA (Thomas et al. 2008).

## Bacterial identification

Stringent laboratory sterile practices were followed during the course of in vitro culturing as well as bacterial isolation to rule out the accidental introduction of contaminants (Thomas et al. 2008). The identity of the organisms was determined based on partial (600–900 bp) 16S rRNA gene sequence analysis. Single colonies were selected after two to three rounds of dilution plating followed by one round of streaking on NA or TSA. DNA was extracted from overnight broth culture (2 ml) using a microbial DNA extraction kit (MOBIO Laboratories, Inc., Solano Beach, CA) or as per the CTAB protocol of Ausubel et al. (2005). 16S rDNA was amplified in 40 µl polymerase chain reaction (PCR) using 200 ng DNA (as determined using a ND1000 Spectrophotometer; Nanodrop Technologies, Inc., Wilmington, DE), 50 µM dNTPs and 1.0 unit of *Taq* DNA polymerase (Genie, Bangalore, India) and 20 pmol each of bacterial primers 27F (5′-AGAGTT TGATCCTGGCTCAG-3′) and 1492R-Y (5′-GGYT ACCTTGTTACGACTT-3′; Y = C/T) (Thomas et al. 2008). Thermocycling conditions consisted of initial one denaturation step of 94°C for 5 min followed by 32 amplification cycles of 94°C for 30 s, 55°C for 40 s, 72°C for 40 s followed by a final extension at 72°C for 5 min using an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany).

PCR amplification was ascertained by running 6 µl samples in a 1% agarose gel. The remaining samples were purified using a PCR purification kit (Axygen Private Ltd., New Delhi) and the 16S rRNA gene was single-end sequenced using 27F at M/s Macrogen Inc., Seoul, Korea (http://www.macrogen.com) or at MWG Biotech, Bangalore, India (http://www.mwgdna.com). Similarity of partial 16S rRNA gene nucleotide sequences with known sequences in the NCBI Genbank database (http://www.ncbi.nlm.nih.gov/) was determined using BLAST version 2.2.15 (Altschul et al. 1997). The organism was assigned to a species if the sequence was ≥99% similar to a valid species sequence deposited with NCBI Genbank at the time of analysis (December 2006) as per Drancourt et al. (2000), or to genus if the species identity was not conclusive but the similarity was ≥97%. A dendrogram based on the neighbor joining

method was created using ClustalW (1.75) package at the site http://www.bioinfo.cnio.es/treeapp/clustal-form.

Microscopy of microbial cultures

Colony observations and staining of pure cultures were carried out as per Cappuccino and Sherman (1996). Gram reaction was assessed as per Suslow et al. (1982) using 3% KOH. The slides were observed under oil immersion (1000×) using a Leica DM2000 microscope (Thomas et al. 2007b, 2008).

Accession number of nucleotide sequences

The partial 16S rRNA gene sequence data generated in this study have been deposited with the NCBI Genbank under the accession numbers DQ872436-DQ872463 in respect of the isolates retrieved during the first in vitro passage or detected after 4–10 in vitro passages, and DQ890503-DQ890513 for isolates that were detected after 2 years of continuous culturing.

## Results and discussion

The endophytes that emerged as obvious contaminants during the first in vitro cycle of cultured shoot tips belonged to the genera of *Enterobacter*, *Klebsiella*, *Ochrobactrum*, *Bacillus*, *Pantoea* or *Staphylococcus*, falling under three bacterial subdivisions as per Bergy's Manual of Systematic Bacteriology (2005) namely α- and γ-proteobacteria and firmicutes. Generally one and occasionally two organisms (e.g. January2005-OC03) were isolated from a single stock soon after its detection as a contaminated culture (Table 1).

The organisms isolated from stocks that showed bacterial activation after a few to several passages belonged to α-proteobacteria (*Brevundimonas*, *Methylobacterium*), β-proteobacteria (*Alcaligenes*, *Ralstonia*), γ-proteobacteria (*Pseudomonas*), actinobacteria (*Corynebacterium*, *Microbacterium*), spore-forming firmicutes (*Oceanobacillus picturae*, *Bacillus fusiformis*, *Bacillus neonatiensis*, *Bacillus pumilus*) and non-spore forming firmicutes (*Staphylococcus epidermidis* and *Staphylococcus arlettae*) (Table 2). The organisms derived from the sub-stock January 2004-OC11.4 during 19th passage that had remained index-negative for almost 2 years included *Brachybacterium* and *Brevibacterium* spp., both non-filamentous actinobacteria. Dilution plating of the tissue homogenate from this sub-stock during the 20th passage yielded five diverse colony types on TSA from the shoot part which included *Brevibacterium*, *Kocuria*, *Tetrasphaera* spp. and *Brachybacterium* sp. (2×) and two from corm (*Brachybacterium* and *Staphylococcus* spp.) (Table 3). Although the tissue homogenate did not show any colony development on NA upon plating, once activated on TSA, the above isolates showed the ability to grow on NA. However, TSA continued to be more suitable based on the extent of colony growth.

The endophytic organisms isolated in this study displayed considerable diversity including Gram-negative α, β, γ-proteobacteria, Gram-positive low G + C spore-forming firmicutes, non-spore forming firmicutes and Gram-positive and Gram-negative actinobacteria, distributed under 20 genera and 24 species. The organisms isolated in the three groups tended to cluster together under the respective group (Fig. 1). No common organism was detected with

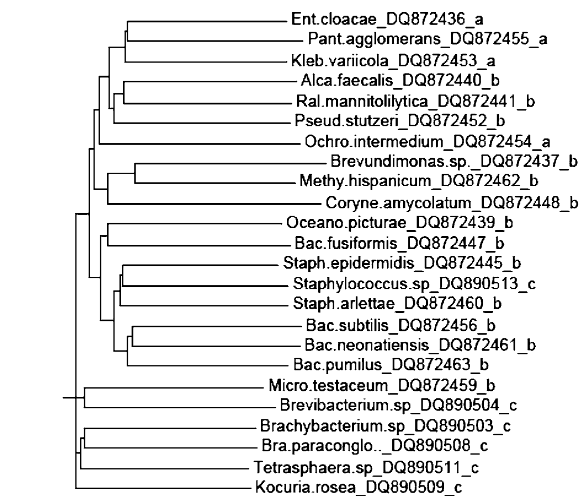


**Fig. 1** Dendrogram using Clustal W (1.75) program based on the partial 16S rDNA sequence data of different endophytic bacteria isolated from in vitro cultured corm tips of banana 'Grand Naine'. The name of the organism is followed by the NCBI accession no. and a, b, c represent the organisms retrieved as easily cultivable ones during the first in vitro passage, those activated after 4–10 in vitro passages and that emerged in a part of the stocks after 2 years of continuous in vitro culturing, respectively. Scale bar represents 0.01 substitutions per nucleotide position

different stocks taken up in this study. The isolates retrieved during the first in vitro passage as easily cultivable contaminants included more of Gram-negative organisms (75%) endorsing the earlier reports with endophytes (Bell et al. 1995; Reiter et al. 2002; Thomas et al. 2007a) while those present initially as fastidious or VBNC organisms but turned cultivable subsequently displayed a Gram-positive predominance (53 and 67% in group two and three, respectively). It was noteworthy that 90% of the belatedly activated organisms (after 2 years) belonged to the sub-division of actinobacteria, to which spore-forming Gram-positive filamentous actinomycetes belong. The isolates in this study did not show filamentous growth on nutrient media, and one organism (*Brachybacterium* sp.) appeared clearly Gram-negative (Table 3).

Earlier studies at INIBAP Musa Germplasm Centre cited covert bacterial association in only a small proportion of stocks (4.5–6%) which was detected during culture screening through explant base streaking on nutrient medium (Van den Houwe et al. 1998; Van den Houwe and Swennen 2000; Strosse et al. 2004). Majority of the organisms in the above studies (60–65%) belonged to Gram-positive spore forming *Bacillus* spp. (*B. circulans*, *B. pumilus, B. sphaericus, B. subtilis* and *B. stearothermophilus*) which have supposedly accumulated in the cultures as heat resistant spores over time. The rest included Gram-positive *Mycobacterium* sp. (35%) or Gram-negative *Acinetobacter* sp. (Van den Houwe et al. 1998; Van den Houwe and Swennen 2000). The deviant observations at INIBAP could be contributed by the absence of detailed tissue screening, the low culture incubation temperatures followed (16°C) or that the organisms were yet to be activated to cultivation.

A wide range of Gram-positive and Gram-negative bacteria including *Bacillus*, *Enterobacter*, *Pseudomonas*, *Staphylococcus*, *Xanthomonas*, *Corynebacterium*, *Micrococcus*, *Brevibacillus*, *Microbacterium*, *Pantoea*, *Brevundimonas*, *Sphingomonas*, *Agrobacterium*, *Methylobacterium* spp. have been reported in tissue cultures of various plant species (Leifert and Cassells 2001; Herman 2004; Thomas 2004a, b, 2006; Kulkarni et al. 2007; Thomas et al. 2007a). Habiba et al. (2002) isolated four Gram-positive (*Cellulomonas uda*, *C. flavigena*, *Corynebacterium paurometabolum* and *Bacillus megaterium*) and three Gram-negative isolates (*Klebsiella*, *Erwinia* and *Pseudomonas* spp.) from table banana cultures. The present study with banana has identified some uncommon endophytic organisms such as *Ochrobactrum intermedium*, *Alcaligenes faecalis*, *Ralstonia mannitolilytica*, *Oceanobacillus picturae*, *Bacillus neonatiensis*, *Brachybacterium*, *Brevibacterium*, *Kocuria rosea*, *Tetrasphaera* spp. etc.

The identification results have indicated that the group two and group three organisms in this study (*Brevundimonas*, *Methylobacterium*, *Alcaligenes*, *Ralstonia*, *Pseudomonas*, *Corynebacterium*, *Microbacterium*, *Staphylococcus*, *Oceanobacillus*, *Bacillus*, *Brachybacterium*, *Brevibacterium*, *Kocuria*, *Tetrasphaera* spp.) did not really belong to the obligate or fastidious type that has complex or undeciphered growth requirements. On the other hand, it unraveled the capability of these organisms to enter the VBNC state as endophytes and their gradual activation to cultivable form during continuous in vitro culturing. Many bacteria are known to enter the VBNC state in response to changes in environmental conditions or stress, and cells can remain so for long periods (Xu et al. 1982; Colwell et al. 1985; Alexander et al. 1999) and the same has been reported with endophytic bacteria (Reiter et al. 2002; Reiter and Sessitsch 2006). Several factors are known to induce the organisms to VBNC state and this phenomenon in Gram-negative bacteria is akin to sporulation in Gram-positive bacteria. The factor(s) that contributed to the activation of VBNCs to cultivation have not been understood (Thomas et al. 2008). Change in medium pH from acidic to alkaline, release of tissue breakdown products, gradual inoculum build up with time, exposure of internal tissue during recurrent subculturing etc. appeared some possibilities (Thomas 2004a, b; Thomas et al. 2006). Basaglia et al. (2007) attained the resuscitation of VBNC cells of *Sinorhizobium meliloti* through exposure to a mix of antibiotics. Some of the antibiotic-supplied watermelon stocks in our earlier studies showed obvious growth on the TCM suggesting that controlling of certain bacteria by one antibiotic led to the emergence of other bacteria (Thomas 2004b; Thomas et al. 2006). In papaya, supply of tissue constituents resulted in significant growth enhancement in the endophytic isolates, *Microbacterium*, *Pantoea*, *Enterobacter*, *Brevundimonas*, *Sphingomonas*, *Methylobacterium*,

*Agrobacterium* and *Bacillus* spp. (Thomas et al. 2007a).

The delayed detection of bacteria in the cultures *prima facie* suggested the possibility of lateral introduction. However, hermetic sealing of culture vessels together with stringent sterility checks, microscopic detection of bacterial cells including spores in the tissue sap at culture initiation (Thomas et al. 2008), similar observations with different stocks and batches and the fact that most of the organisms isolated in this study were not common laboratory contaminants discounted such a possibility. When chanced upon organisms like *S. epidermidis*, which could be associated with human skin, we undertook mock inoculations using several nutrient plates, ruling out the possibility of lateral introduction.

There is an emerging interest in endophytes as agents in plant growth promotion and stress alleviation (Azevedo et al. 2000; Hallmann 2001; Thomas et al. 2007a) and several of the organisms isolated in this study have been reported as potential beneficial organisms (Hallmann 2001). *Streptomycete* antagonists of fusarium wilt in *Musa* have been isolated from banana roots (Cao et al. 2005). Growth promotion in rice by *Enterobacter cloacae* (Mehnaz et al. 2001), bioconrol effect by *Pantoea agglomerans* (Nunes et al. 2002) are some other documented examples. Actinomycetes are known to promote plant growth and are involved in biocontrol (Conn and Franco 2004). On the other hand, some of the organisms isolated in this study have possible implications in human health as they showed high sequence identity (99–100%) to organisms associated with diseases or infections, such as *Ralstonia mannitolilytica* isolated from respiratory secretions of cystic fibrosis patients (Coenye et al. 2002), *Staphylococcus epidermidis* from chronic wounds (Frank et al. 2005), *Corynebacterium amycolatum* from a urinary tract infected patient (Goldenberger et al. 2002) or *Bacillus neonatiensis* from a patient with neonatal sepsis (Ko et al. 2005). Human pathogens have been isolated as endophytes in other studies as well, which included dreaded *Salmonella* and *Nocaridia* spp. (Rosenblueth and Martínez-Romero 2006). Several bacteria isolated here are often reported from environmental samples, which is a reflection of the poor knowledge about the endophytes rather than the possibility of their lateral entry as contaminants. It is quite likely that the endophytes reach the soil, sewage, sludge, untreated water etc. at the end of the life cycle of the host or through plant debris and get isolated frequently from environmental samples. One species may be present in diverse environments but the strains could be different. For instance, three morphotypes of *Curtobacterium citreum* that we isolated from chrysanthemum showed the highest similarity to an isolate from deep sea (Panicker et al. 2007). 16S rRNA gene based identification cannot discriminate between different strains.

This study also demonstrates the utility of tissue culture system in harnessing some less common organisms or novel endophytes by bringing the VBNC cells to cultivation and making them amenable for future exploitation. The fact that a part of these organisms would perpetuate as endophytes with the new suckers while the rest returns to the soil at the culmination of normal life span of banana plants (12–15 months) and become a part of soil microbial community adds credence for these observations to soil and environmental microbiology and offers a new vista to study microbe–plant cyclic association.

In summary, endophytic bacteria isolated from in vitro cultures of banana belonged about 24 species falling under 20 genera. The study has identified several endophytic organisms with potential ability to survive in VBNC state. Such organisms turned cultivable after recurrent in vitro culturing of stock plants displaying a Gram-positive predominance, including actinobacteria and spore-forming *Bacillus* spp. The organisms that were easily cultivable and expressed as visible contaminants during the first in vitro passage included common endophytes with a Gram-negative predominance. The isolated organisms included those reportedly useful in plant growth promotion or biocontrol but also the ones with implications in human health. The study thus demonstrates the utility of tissue culture system in isolating uncommon endophytes and to explore into endophytic microbiology.

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