

Phylogenetic characterization of culturable bacteria and fungi associated with tarballs from Betul beach, Goa, India

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Abstract

Tarballs are semisolid blobs of crude oil, normally formed due to weathering of crude-oil in the sea after any kind of oil spills. Microorganisms are believed to thrive on hydrocarbon-rich tarballs and possibly assist in biodegradation. The taxonomy of ecologically and economically important tarball-associated microbes, however, needs improvement as DNA-based identification and phylogenetic characterization have been scarcely incorporated into it. In this study, bacteria and fungi associated with tarballs from touristic Betul beach in Goa, India were isolated, followed by phylogenetic analyses of 16S rRNA gene and the ITS sequence-data to decipher their clustering patterns with closely-related taxa. The gene-sequence analyses identified phylogenetically diverse 20 bacterial genera belonging to the phyla *Proteobacteria* (14), *Actinobacteria*(3), *Firmicutes*(2) and *Bacteroidetes* (1), and 8 fungal genera belonging to the classes *Eurotiomycetes* (6), *Sordariomycetes*(1) and *Leotiomycetes* (1) associated with the Betul tarball samples. Future studies employing a polyphasic approach, including multigene sequence-data, are needed for species-level identification of culturable tarball-associated microbes. This paper also discusses potentials of tarball-associated microbes to degrade hydrocarbons.

Keywords:Diversity, DNA barcoding, Microbes, Oil pollution, Tarball, Pathogens

Introduction

Tarballs are semisolid blobs of crude oil, normally formed due to weathering of crude oil in the sea after any kind of oil spills. They get advected long distance by ocean currents and waves to reach beaches, and cause pollution in coastal marine environment. Microbes originating either from seawater or beach-sand get attached to tarballs and possibly affect the biodegradation of hydrocarbon-rich tarballs. Tarballs act as a substratum for physical attachment of various microbes and the byproducts formed during their degradation possibly support microbial growth (Shinde et al. 2017).

The taxonomy of tarball-associated microbes, however, needs improvement as DNA-based identification and phylogenetic characterization have been scarcely incorporated into it (Bacosa et al. 2016, Nkem et al. 2016, Sanyal et al. 2016, Shinde et al. 2017, Barnes et al. 2018). To the best of our knowledge, Nkem et al. (2016) were the first to report 16S rRNA gene-sequencing of culturable tarball-associated bacteria. They characterized two bacterial species, *Acinetobacter baumannii* and *Cellulosimicrobium cellulans* associated with tarballs from Malaysia based on a polyphasic approach. Recently, tarball-associated bacteria were studied using a culture-independent method from Texas beaches following the 2014 Texas city “Y” spill (Bacosa et al. 2016) and provided insights into their unculturable diversity in the context of tarball chemistry.

In case of tarball-associated fungi, Sanyal et al. (2016) were the first to report phylogenetically diverse filamentous-fungi and yeasts, based on ITS sequence-data, from a touristically important Candolim beach in Goa. Recently, Barnes et al. (2018) reported two tarball-associated fungal species, *Fusarium equiseti* and *F. circinatum* from Betul beach, Goa and provided their multigene sequence-data. Nevertheless, information available on molecular diversity and DNA sequence-based identification of tarball-associated bacteria and fungi is scarce and patchy (Bacosa et al. 2016, Nkem et al. 2016, Sanyal et al. 2016, Shinde et al. 2017, Barnes et al. 2018), especially from tourist beaches of Goa state which has been facing tarball pollution since the 1970s (Nair et al. 1972, Suneel et al. 2013, 2016). This study, therefore, aimed to isolate both bacteria and fungi from tarballs collected from a touristic Betul beach in south Goa and characterize their phylogenetic relationships based on 16S rRNA gene (for bacteria) and the ITS sequence data (for fungi) analyses.

Material and methods

Sampling of tarballs

Betul is a coastal town located in the south Goa, India. The GPS coordinates for Betul are 15.144837°N 73.958244°E. The Betul beach was surveyed twice for tarball sampling, initially in October 2014 and subsequently in July 2016. Tarballs were handpicked randomly along a stretch on the beach wearing sterile nitrile gloves and collected in zip-lock bags. The bags were kept in ice box and carried to the laboratory. Samples were stored at 4 °C until further processing.

Isolation of bacteria

Tarball samples collected during October 2014 were processed for bacterial isolation using an isolation protocol, which was standardized in the lab after improving the procedure described by Tao et al. (2011). In the modified protocol, 1 g of tarball was weighed aseptically and washed with sterile sea water. The washed tarball samples were then added into a test tube containing 9 ml of sterile seawater. The sterile cotton swab was used to break tarballs and detach bacteria. The tubes were vortexed vigorously for 5–10 min. 1 ml of this was serially diluted further up to 10⁻⁵ dilution. 100 µl of each dilution was spread plated on Zobell Marine Agar (ZMA). The plates were then incubated at room temperature. After 48 hrs of incubation, the colony forming units (CFUs) were calculated.

Tarball samples collected during July 2016 were also processed for isolation of bacteria using the afore-mentioned procedure. To increase the recovery of diverse groups of bacteria, one more bacterial isolation protocol was standardized in the lab, after improving the isolation procedure described in Bayat et al. (2015). In this method, 1 g of the freshly collected tarball (from the July 2016 sample collection) was washed with sterile seawater to remove the particles adhered to it as much as possible and suspended it immediately in 100 ml of Zobell Marine Broth (ZMB) containing tarball (1g/ 100 ml) as a source of hydrocarbon to promote the growth of hydrocarbon-utilizing bacteria. As tarballs were insoluble in water, sterile cotton swab was used to break them, so that maximum possible number of bacteria could get into the culture medium. The medium was then incubated on a rotary shaker at 120 rpm and 35°C for 48 hrs. 1 ml of enriched medium was then transferred to ZMB containing tarball (1g/ 100 ml) and further incubated on a rotary shaker at 120 rpm and 35 °C for 48 hrs. Final transfer was made in 100 ml of Bushnell Hass medium containing tarball as only carbon source. After the incubation, 1 ml from the final medium was serially diluted in sterile sea-water and spread plated on ZMA. Morphologically diverse bacterial colonies were further isolated by streak plate method.

The bacterial cultures were selected based on their colony morphology and purified for further studies (Table 1). Colony morphology characters were noted down, and Gram staining was

performed for each isolate to confirm its purity. Purified bacterial cultures were maintained as 80% glycerol stocks and stored at -80 °C.

Isolation of fungi

Fungi were isolated from tarballs collected during July 2016 using the method described by Sanyal et al. (2016). Fungal colonies with unique culture morphology were selected for further characterization (Table 2) and their purified cultures were maintained at 4 °C.

DNA extraction and PCR amplification

DNA from both bacterial and fungal cultures was extracted using the Fungal and Bacterial DNA isolation kit (Zymo Research, USA) according to manufacturer's protocol. From fungal DNA, the ITS region was amplified by PCR using the primer-pair ITS1 and ITS4 (White et al. 1990), following the method detailed in Sanyal et al. (2016).

16S rRNA gene of bacteria was amplified by PCR using primer-pair 27F and 1492R (Piterina et al. 2010). The reaction for bacterial 16S rRNA gene amplification was performed in a 50 µl of volume, comprising 3 µl of template DNA (~50 ng/µl), 1 µl of each primer (20 pM) (Bioserve, India), 1 µl of dNTPs (10 mM) (Genaxy, India), 5 µl of Taq buffer A (10X with 15 mM MgCl₂) (GeNei, Bangalore), 1.5 µl of Taq polymerase (1U/µl) (Chromous Biotech, India), 37.5 µl of nuclease-free water (HiMedia, India). The reaction was carried out at 95 °C for 5 min (initial denaturation), followed by 35 cycles at 95 °C for 1 min (denaturation), 50 °C for 1 min (primer annealing), 72 °C for 2 min (elongation), and a final extension at 72 °C for 10 min.

PCR products were purified using QIAquick PCR purification kit (QIAGEN) following manufacturer's protocol. After purification, PCR products were sequenced by ABI 3130 XL DNA sequencer, using primers mentioned above, at the Biological Oceanography Division, CSIR-NIO, Goa.

Phylogenetic analysis

The raw sequences obtained from the forward and reverse primers were checked for quality in DNA Dragon (SequentiX, Germany). Consensus sequences were prepared in the same software using both forward and reverse sequences, wherever possible. Two different DNA sequence datasets were analyzed, one for 16S rRNA gene sequence data-set (for bacteria) and the ITS sequence data-set (for fungi), in MEGA 7 (Kumar et al. 2016) using the newly-generated sequences and reference sequences retrieved from NCBI-GenBank.

Results

Phylogeny of tarball-associated bacteria

A list of bacterial cultures isolated in this study, along with information on their Gram nature and GenBank accession numbers for 16S rRNA gene sequences is presented in Table 1. The CFUs of bacteria were found to be 8.3×10^6 per gram of tarball for the samples collected during October 2014, while the samples collected during July 2016 yielded 5.07×10^5 CFUs per gram of tarball. Eight bacterial isolates were retrieved by the dilution plate method from the October 2014 tarball samples (Table 1, prefixed with “MOB-“). Twenty-five and sixteen bacterial isolates were retrieved from the July 2016 tarball samples by the dilution plate method and the enrichment culture technique, respectively. Numbers and alphabets are used to differentiate between the cultures isolated by serial dilution method and those isolated by enrichment culture technique, respectively (Table 1). Bacteria belonging to *Alcanivorax*, *Bhargavaea*, *Nitratireductor*, *Oceanimonas* and *Tistrella* were exclusively recovered from the enrichment culture technique (Figure 1).

A Maximum Likelihood tree generated using the 16S rRNA gene sequence data-set in MEGA is shown in Figure 1. *Aquifex pyrophilus* (M83548) was the designated outgroup. The system output details generated by MEGA for the bacterial tree construction are presented in Supplementary Document 1. In the bacterial phylogenetic tree shown in Figure 1, 49 newly-generated sequences of tarball-associated bacteria from Betul beach clustered within four bacterial phyla [*Proteobacteria*: 38 (*Gammaproteobacteria*: 31, *Alphaproteobacteria*: 7), *Actinobacteria*: 6, *Firmicutes*: 4 and *Bacteroidetes*: 1].

In the *Gammaproteobacteria* clade (Figure 1), 11 bacterial isolates clustered within *Pseudomonas* clade. B_BETUL_19 and B_BETUL_7 clustered with *Pseudomonas* sp. (MF948939, a pyrene degrader), coralline algae-associated bacterium (MG551789) and uncultured bacterium clone isolated from a polluted aquatic environment (KC208430). B_BETUL_4 exhibited proximity with the type of *P. sihuiensis* (NR 148251). MOB_V2 clustered with *Pseudomonas* sp. from the soil of Liangshui river of Beijing (KT380612). B_BETUL_G and B_BETUL_11 grouped with uncultured bacterium clone (JX047093), *P. stutzeri* originated from soil of Xumen coral reef nature reserve (KT986148), the type of *P. stutzeri* (NR113652) and *Pseudomonas* sp. (KP730605, involved in Arsenite oxidation). B_BETUL_M clustered with *Pseudomonas* sp. (KF021702) of marine origin. B_BETUL_15, B_BETUL_13, B_BETUL_14 and BETUL_F formed a strongly-supported clade, with 97% bootstrap support, with the type of *P. aeruginosa* (LN681564); and *P. aeruginosa* from the estuary of Siak River (MG706126) and fish of Arabian Gulf region (KT005274). Three *Pseudomonas* isolates, B_BETUL_G, B_BETUL_M and B_BETUL_F, were recovered using the enrichment culture method.

Five bacterial isolates were found to cluster within *Marinobacter* clade. B_BETUL_23 and MOB_V5 exhibited proximity with the types of *M. alkaliphilus* (NR112223) and *M. halophilus* (NR137207) but this received poor statistical support. B_BETUL_27, MOB_V6 and B_BETUL_26 formed a strongly supported cluster (with 78% bootstrap support) with *M. hydrocarbonoclasticus* (NR074619, PAH degrader); and *Marinobacter* isolates from crude-oil contaminated surface water/ sponge (JX119040) and microalga (KU554471). MOB_V1 formed a strongly supported a clade with the type of *Shewanella algae* (NR114236), *S. upenei* from aquaculture farm water (KY176382) and uncultured *Shewanella* sp. from marine hot spring (JX047073). Similarly, B_BETUL_Q formed a clade with *Oceanimonas* sp. (MG456888, PAH degrader), uncultured bacterium clone from marine sediment from jetty (JQ257780) and the type of *O. doudoroffii* (NR114185). The *Oceanimonas* isolate B_BETUL_Q was recovered using the enrichment culture method.

In a major clade with strong bootstrap support, 6 bacterial isolates from the Betul tarball sample exhibited proximity with members *Vibrio*, *Pantoea*, *Klebsiella* and *Enterobacter*. B_BETUL_9 exhibited a strong clustering pattern with the type of *V. fluvialis* (NR114218), *Vibrio* sp. involved pyrene degradation (MF948987), *V. fluvialis* from shell-fish (KT163389) and *V. furnissii* from seawater (KU321330). B_BETUL_3 formed a strongly supported clade with the type of *Pantoea dispersa* (AB907780) and *Pantoea* sp. from straw (KU986664). B_BETUL_8 and B_BETUL_5 clustered with the type of *Klebsiella pneumoniae* subsp. *Rhinoscleromatis* (NR037084) and *K. pneumoniae* from river (KJ806340). *Enterobacter* was found to be paraphyletic, with one clade sister to *Klebsiella* and including B_BETUL_2, uncultured *Enterobacter* sp. from aerobic granular sludge (KX379247), *Enterobacter* sp. from mangrove sediment (GQ284538) and endophytic bacterium of *Spartina alterniflora* (KP757600). The second *Enterobacter* clade included B_BETUL_10, the type of *E. ludwigii* (NR042349) and bacterium isolated from borewell (MG650114).

B_BETUL_O, B_BETUL_H, B_BETUL_I and B_BETUL_P, recovered solely in the culture enrichment method, formed a well-supported with the type of *Alcanivorax xenomutans* (NR133958), *A. dieselolei* from sea lettuce (KJ849833) and deep-sea sediment (LT673801), and uncultured *Alcanivorax* sp. from Gujarat coastal water (LC269892). Similarly, B_BETUL_1, B_BETUL_24 and B_BETUL_22 clustered with the type of *Pseudoxanthomonas indica* (NR116019), *P. mexicana* (AB246768, chemosynthetic resin degrader), enrichment culture clone from soil sample (JN867356), *Pseudoxanthomonas* isolate from oil-contaminated soil (MF 405126), uncultured bacterium clone from PAH contaminated soil (HQ218747) and *P. indica* capable of degrading alachlor (KX682025). Of the 7 newly-generated sequences clustered within the *Alphaproteobacteria* (Figure 1), *Nitratireductor* was represented by four isolates, while *Marispirillum*, *Pelagibaca* and *Tistrella*

included one isolate each. B_BETUL_C formed a well-supported clade with the type of *Tistrella mobilis* (NR114036), *T. mobilis* from water of South China sea (HQ425697), uncultured bacterium clone from water sample from oil well (KC442816), *T. mobilis* isolate involved in PAH degradation (EU440998) and *Tistrella* sp. from oil field ooze (EU306603). *Tistrella* isolate B_BETUL_C was retrieved using the enrichment culture method. Similarly, MOB_V7 formed a well-supported clade with the type of *Marispirillum indicum* (NR044545), phenanthrene degrading *Marispirillum* isolate (MG386659) and uncultured *Marispirillum* sp. from oil well-water (KF733613). MOB_V8 grouped with the type of *Pelagibaca abyssi* (NR148263), *P. abyssi* of marine origin (JX878396) and bacterium originated from hydrothermal vent (AF254109). B_BETUL_N, B_BETUL_A, B_BETUL_D and B_BETUL_R, recovered solely using the enrichment culture method, formed a well-supported clade within the *Alphaproteobacteria*. B_BETUL_R clustered with the type of *Nitratireductor aquimarinus* (NR117929), *Nitratireductor* isolate associated with *Synechococcus* sp. (KY770364), uncultured bacterium clone from seawater (KY774825), and *Nitratireductor* sp. from shallow sea hydrothermal system (KX989302). B_BETUL_A and B_BETUL_D grouped with *N. aquibiodomus* from OMZ water column (KY616455).

Phylum *Bacteroidetes* was the least represented with one isolate in the genus *Owenweeksia*. MOB_V4 clustered with the type of *Owenweeksia hongkongensis* (NR040990) with 99% bootstrap support. Among the *Actinobacteria*, *Cellulomonas* and *Brevibacterium* were represented by three and two isolates, respectively, while *Jonesia* included only one isolate. MOB_V9 formed a strongly supported cluster with the type of *Jonesia denitrificans* (NR074806) and *Jonesia* sp. involved in biowaste degradation (AB098584). B_BETUL_21(1) and B_BETUL_21(2) formed a clade with the type of *Brevibacterium oceani* (NR 042458), *B. epidermidis* of marine origin (HF968437), *Brevibacterium* sp. from sediment of Atlantic ocean (KJ534269) and Actinobacterium from snail body (GQ222440). Similarly, B_BETUL_28, B_BETUL_29 and B_BETUL_30 clustered, with a strong bootstrap support, with the type of *Cellulomonas denverensis* (NR042936), *C. hominis* from crude-oil sample (KP282788), *Cellulomonas* spp. of marine origin (KC335136 and KU560419) and bacterium from Chromium (VI) reducing enrichment culture (AY617099). Among the *Firmicutes*, *Bhargavaea* was represented by three isolates, while *Bacillus* included only one isolate. B_BETUL_6 formed a well-supported clade with the type of *Bacillus wiedmannii* (NR152692) and *B. cereus* from fish sample (MG725733). B_BETUL_K, B_BETUL_L and B_BETUL_J, recovered solely in the culture enrichment method, formed a strongly supported clade with the type of *Bhargavaea beijingensis* (NR117988), *B. beijingensis* involved in arsenic oxidation (KT596919) and *B. beijingensis* from Viking spacecraft Teflon ribbon (KT720202).

Phylogeny of tarball-associated fungi

A list of the fungal cultures included in this study, along with information on the GenBank accession numbers for ITS sequences is presented in Table 2. Colony morphology of select tarball-associated fungal cultures is presented in Supplementary Figures 1 and 2. A Maximum Likelihood tree generated using the ITS sequence dataset in MEGA is shown in Figure 2. *Issatchenkia* clade was the designated outgroup. The system output details generated by MEGA for the fungal tree construction is presented in Supplementary Document 1.

In the fungal phylogenetic tree shown in Figure 2, 18 newly generated sequences of tarball-associated fungi from Betul clustered within three classes of phylum *Ascomycota* [*Eurotiomycetes*: 15, *Sordariomycetes*: 2, and *Leotiomycetes*: 1]. Among the *Eurotiomycetes*, *Monascus* was represented by four isolates, *Penicillium* and *Talaromyces* included three isolates each, *Aspergillus* and *Paecilomyces* had two isolates each and *Byssochlamys* included only one isolate. F_BETUL_F1 and F_BETUL_G clustered within the *Aspergillus* clade. The former grouped with the types of *Aspergillus tubingensis* (NR131293), *A. awamori* (NR077143) and *A. niger* (NR111348); *A. tubingensis* from Lunar/ Mars analog system (KX664307); and two *A. niger* isolates (KT921201, crude-oil degrader; KT899788, from crude-oil polluted soil). The latter clustered with the type of *A. terreus* (NR131276); and three *A. terreus* isolates (KT9211197, crude-oil sludge degrader; JQ400189, oil-degrader; KY053122, from petroleum contaminated soil). F_BETUL_I2, F_BETUL_A1 and F_BETUL_M formed a moderately supported cluster with the types of *Penicillium coffeae* (NR121312), *P. indicum* (NR121311) and *P. chermesinum* (NR121310); *P. indicum* from marine soil (KM277999), *P. coffeae* from beach soil water (LC057404) and *P. chermesinum* from beach soil (KJ767051). F_BETUL_J1, F_BETUL_J2 and F_BETUL_O clustered with the types of *Talaromyces ricevillensis* (KX657343) and *T. loliensis* (NR 103680); and *T. radicus* (AY256855, phosphate solubilizer). F_BETUL_B1 and F_BETUL_P grouped with the type of *Paecilomyces formosus* (NR149329) and *P. formosus* (KX668142, oil-degrader). F_BETUL_E1 formed a moderately supported clade with the type of *Byssochlamys lagunculariae* (NR145144), *Byssochlamys* sp. (KU556515, denitrifier) and the type of *P. dactylethromorphus* (NR145144). F_BETUL_U, F_BETUL_K2, F_BETUL_I1 and F_BETUL_L clustered with *Monascus pallens* (GU733328: Xerophilic), in the proximity of *M. pallens* from river sediment (KY635849).

In the *Leotiomycetes* clade, *Xylogone* was represented by one isolate. F_BETUL_H formed a well-supported clade with the type of *Xylogone sphaerospora* (NR145360) and three nontype isolates of *X. sphaerospora*, including pathogen of cultivated mushrooms (GQ272626), wood decaying fungus (KU194337) and isolate from crop field soil (KY316386). Among the *Sordariomycetes*, *Trichoderma* included two isolates, F_BETUL_S1 and F_BETUL_R, which grouped with the types of *T. longibrachiatum* (NR 120298) and *T. bissettii* (NR 134442); and *T. longibrachiatum* isolates from

oil-contaminated sites (KP296146 and KU935695). The phylogenetic tree also included the ITS sequences of tarball-associated fungi from Candolim, Goa (*Candida*, *Alternaria*, *Biploaris*/*Curvularia*, *Cladosporium* and *Issatchenkia*) (Sanyal et al. 2016) and that of *Fusarium* species associated with tarballs from Betul beach, south Goa (Barnes et al. 2018).

Discussion

Tarball-associated bacteria

Tarballs are oily residues and therefore difficult to dissolve in water. Two bacterial isolation protocols used in this study have been standardized after improving the known methods from the literature to retrieve phylogenetically diverse bacteria. *Nitratireductor* and *Alcanivorax* species were predominantly isolated in the enrichment culture technique, followed by species of *Bhargavaea*, *Oceanimonas* and *Tistrella*. *Pseudomonas* species, however, were recovered in both the isolation methods (Table 1). The number of bacterial CFUs recovered in our study (8.3×10^6 CFUs per gram of tarball for the October 2014 samples and 5.07×10^5 CFUs per gram of tarball for the July 2016 samples) is in agreement with previous findings. Itah and Essien (2005) reported 3.00×10^4 – 3.18×10^6 CFUs of heterotrophic bacteria per gram of tarball, while Tao et al. (2011) reported the numbers in the range of 5.1×10^6 – 8.3×10^6 CFUs per gram of tarball.

Tarballs and tarball-like substrates are known to support phylogenetically diverse bacteria from 31 identified genera (Supplementary Table 1). Among them, only *Alcanivorax*, *Bacillus*, *Pseudomonas*, *Psychrobacter* and *Vibrio* have been reported from multiple locations. Of the 20 bacterial genera identified to be present in Betul tarball samples in this study, 16 genera are new records for tarball-associated bacteria, while *Alcanivorax*, *Bacillus*, *Pseudomonas* and *Vibrio* have been reported earlier from other sampling location/s (Supplementary Table 1). Some species of *Enterobacter*, *Klebsiella*, *Pantoea*, *Pseudomonas* and *Vibrio* exist as pathogens (Delétoile et al. 2009), and their association with tarballs will have implications in human and animal health issues (Tao et al. 2011).

The 16S rRNA gene-based tree (Figure 1) provides indications about ecology and potential abilities to degrade xenobiotics of tarball-associated bacteria reported in this study. These indications can be utilized to devise an effective screening strategy for tarball-associated microbes. For example, *Pseudomonas* species are known to be present in marine habitats such as from Coralline algae (MG551789), marine hot spring (JX047093), soil of coral reef nature reserve (KT986148), *Spartina alterniflora* (endophyte) (KP757672) and fish of Arabian Gulf region (KT005274). They are also known from oil polluted environments such as soil (HM461889) and have a history of involvement in PAH degradation (KF052990), specifically pyrene degradation (MF948939), and Arsenite oxidation (KP730605). Similar observation and analysis for other hydrocarbonoclastic bacteria such

as members of *Alcanivorax*, *Marinobacter*, *Nitratireductor*, *Tistrella* (Hassanshahian et al. 2012, Gao et al. 2015, Rahman et al. 2002) can provide hints about their hydrocarbon-degradation history and potentials. Most hydrocarbonoclastic bacteria are reported to have *nifH* gene and supposed to be diazotrophic in nature (Dashti et al. 2015). This feature can help in dealing with the issue of nitrogen availability, which is a major growth factor for bacteria and its scarcity limits biodegradation process in oceans (Das and Chandran 2011).

Tarball-associated fungi

Fungal diversity associated with tarballs has already been reviewed in our previous study (Sanyal et al. 2016). Tarballs (and tarball-like substrates) are known to support phylogenetically diverse 31 fungal genera (Supplementary Table 1). Of the 33 reported fungal genera, 15 genera are known to be associated with tarball samples from Goan beaches. This study summarizes 14 fungal genera, identified based on DNA sequence-data, from tarballs collected from Goa (Figure 2, Sanyal et al. 2016, Barnes et al. 2018). Among the known tarball-associated fungal genera, *Aspergillus*, *Paecilomyces*, *Penicillium*, *Talaromyces* and *Trichoderma* have been extensively studied for their hydrocarbon-degrading potential (Dawoodi et al. 2015) and Barnes et al. (2018) have summarized recent studies on bioremediation potentials of hydrocarbon-degrading fungi. Some species of *Trichoderma* and *Candida* are potential human pathogens (Sanyal et al. 2016) and further studies are required to assess the risks of tarballs as carriers of human and animal pathogens in the coastal environment.

Future perspectives

This study reveals the presence of phylogenetically diverse 28 microbial genera (20 bacterial genera and 8 fungal genera), characterized based on gene sequence-data, in the tarball samples collected from Betul beach in south Goa, India. The DNA datasets (i.e. 16S rRNA gene and the ITS) generated in this study will strengthen future studies on DNA sequence-based identification and phylogenetic characterization of tarball-associated microbes. Future studies employing a polyphasic approach for species-level identification of tarball-associated bacteria and fungi are required, as the monophasic approach based solely on morphology or single gene sequence-dataset is prone to errors. It is imperative that in future studies the uncultivable microbial fraction is also studied along with culturable tarball-associated microbes, especially to obtain insights into the diversity of anaerobic bacteria and fungi in tarballs.

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Legends

Table 1: List of tarball-associated bacteria included in this study, along with details of their cultural morphology and 16S rRNA gene sequence accession numbers

Table 2: List of tarball-associated fungi included in this study, along with details of their ITS sequence accession numbers

Figure 1: Phylogenetic diversity of tarball-associated bacteria from Betul beach, Goa, India based on 16S rRNA gene sequence analysis. The newly-generated sequences from this study are highlighted in red italics. The sequences from type materials are represented in bold.

Figure 2: Phylogenetic diversity of tarball-associated fungi from Betul beach, Goa, India based on ITS sequence analysis. The newly-generated sequences from this study are highlighted in red italics. The sequences from type materials are represented in bold.

Supplementary Table 1: Updated list of bacteria and fungi associated with tarballs and tarball-like substrates from the reported global studies

Supplementary Document 1: System output details generated by MEGA for the bacterial and fungal tree constructions

Supplementary Figure 1: Culture morphology of select tarball-associated fungi from Betul, Goa: **A)** *Aspergillus* sp. F_BETUL_F1, **B)** *Aspergillus* sp. F_BETUL_G2, **C)** *Monascus* sp. F_BETUL_I1, **D)** *Monascus* sp. F_BETUL_K2, **E)** *Byssochlamys* sp. F_BETUL_E1 and **F)** *Paecilomyces* sp. F_BETUL_P

Supplementary Figure 2: Culture morphology of select tarball-associated fungi from Betul, Goa: **A)** *Penicillium* sp. F_BETUL_A1, **B)** *Penicillium* sp. F_BETUL_M, **C)** *Talaromyces* sp. F_BETUL_J1, **D)** *Talaromyces* sp. F_BETUL_O, **E)** *Trichoderma* sp. F_BETUL_S1, and **F)** *Xylogone* sp. F_BETUL_H

Table 1

Sl. no.	Taxon name	Isolate number	Culture morphology	GenBank acc. no.
1	<i>Alcanivorax</i> sp.	B_BETUL_O	Gram negative, Short rods	MG664223
2	<i>Alcanivorax</i> sp.	B_BETUL_I	Gram negative, Short rods	MG664222
3	<i>Alcanivorax</i> sp.	B_BETUL_P	Gram negative, Coccobacilli	MG664224
4	<i>Alcanivorax</i> sp.	B_BETUL_H	Gram negative, Coccobacilli	MG664221
5	<i>Bacillus</i> sp.	B_BETUL_6	Gram positive, Short rods	MG664225
6	<i>Bhargavaea</i> sp.	B_BETUL_K	Data unavailable	MG664227
7	<i>Bhargavaea</i> sp.	B_BETUL_L	Gram positive, Short rods	MG664228
8	<i>Bhargavaea</i> sp.	B_BETUL_J	Gram negative, Rods	MG664226
9	<i>Brevibacterium</i> sp.	B_BETUL_21(1)	Data unavailable	MG664229

10	<i>Brevibacterium</i> sp.	B_BETUL_21(2)	Data unavailable	MG664230
11	<i>Cellulomonas</i> sp.	B_BETUL_28	Gram negative, Short rods	MG664231
12	<i>Cellulomonas</i> sp.	B_BETUL_30	Gram negative, Short rods	MG664233
13	<i>Cellulomonas</i> sp.	B_BETUL_29	Gram negative, Short rods	MG664232
14	<i>Enterobacter</i> sp.	B_BETUL_2	Gram negative, Coccobacilli	MG664235
15	<i>Enterobacter</i> sp.	B_BETUL_10	Gram negative, Coccobacilli	MG664234
16	<i>Jonesia</i> sp.	MOB_V9	Data unavailable	MG797567
17	<i>Klebsiella</i> sp.	B_BETUL_8	Gram negative, Cocci	MG664237
18	<i>Klebsiella</i> sp.	B_BETUL_5	Gram negative, Cocci	MG664236

19	<i>Marinobacter</i> sp.	B_BETUL_23	Gram negative, Short rods	MG664238
20	<i>Marinobacter</i> sp.	B_BETUL_27	Data unavailable	MG664240
21	<i>Marinobacter</i> sp.	B_BETUL_26	Gram negative, Rods	MG664239
22	<i>Marinobacter</i> sp.	MOB_V5	Gram negative, Rods	MG797563
23	<i>Marinobacter</i> sp.	MOB_V6	Gram negative, Rods	MG797564
24	<i>Marispirillum</i> sp.	MOB_V7	Gram negative, Rods	MG797565
25	<i>Nitratireductor</i> sp.	B_BETUL_R	Gram negative, Short rods	MG664244
26	<i>Nitratireductor</i> sp.	B_BETUL_N	Gram negative, Short rods	MG664243
27	<i>Nitratireductor</i> sp.	B_BETUL_D	Gram negative, Rods	MG664242
28	<i>Nitratireductor</i> sp.	B_BETUL_A	Gram negative, Thin short rods	MG664241

29	<i>Oceanimonas</i> sp.	B_BETUL_Q	Gram negative, Coccobacilli	MG664245
30	<i>Owenweeksia</i> sp.	MOB_V4	Data unavailable	MG797562
31	<i>Pantoea</i> sp.	B_BETUL_3	Gram negative, Coccobacilli	MG664246
32	<i>Pelagibaca</i> sp.	MOB_V8	Gram negative, Coccobacilli	MG797566
33	<i>Pseudomonas</i> sp.	B_BETUL_4	Gram negative, Thin short rods	MG664252
34	<i>Pseudomonas</i> sp.	B_BETUL_19	Gram negative, Short rods	MG664251
35	<i>Pseudomonas</i> sp.	B_BETUL_7	Gram negative, Short rods	MG664253
36	<i>Pseudomonas</i> sp.	B_BETUL_M	Gram negative, Short rods	MG664256
37	<i>Pseudomonas</i> sp.	B_BETUL_G	Gram negative, Coccobacilli	MG664255

38	<i>Pseudomonas</i> sp.	B_BETUL_11	Data unavailable	MG664247
39	<i>Pseudomonas</i> sp.	B_BETUL_F	Gram negative, Short rods	MG664254
40	<i>Pseudomonas</i> sp.	B_BETUL_14	Gram negative, Short rods	MG664249
41	<i>Pseudomonas</i> sp.	B_BETUL_15	Gram negative, Coccobacilli	MG664250
42	<i>Pseudomonas</i> sp.	B_BETUL_13	Gram negative, Short rods	MG664248
43	<i>Pseudomonas</i> sp.	MOB_V2	Gram negative, Rods	MG797561
44	<i>Pseudoxanthomonas</i> sp.	B_BETUL_1	Gram negative, Rods	MG664257
45	<i>Pseudoxanthomonas</i> sp.	B_BETUL_22	Gram negative, Rods	MG664258
46	<i>Pseudoxanthomonas</i> sp.	B_BETUL_24	Gram negative, Short rods	MG664259

47	<i>Shewanella</i> sp.	MOB_V1	Gram negative, Rods	MG797560
48	<i>Tistrella</i> sp.	B_BETUL_C	Gram negative, Rods	MG664260
49	<i>Vibrio</i> sp.	B_BETUL_9	Gram negative, Short rods	MG664261

Table 2

Sl. no.	Taxon name	Isolate no.	GenBank acc. no.
1	<i>Aspergillus</i> sp.	F_BETUL_F1	MG664262
2	<i>Aspergillus</i> sp.	F_BETUL_G2	MG664263
3	<i>Byssochlamys</i> sp.	F_BETUL_E1	MG664272
4	<i>Monascus</i> sp.	F_BETUL_L	MG664269
5	<i>Monascus</i> sp.	F_BETUL_U	MG664270

6	<i>Monascus</i> sp.	F_BETUL_I1	MG664267
7	<i>Monascus</i> sp.	F_BETUL_K2	MG664268
8	<i>Paecilomyces</i> sp.	F_BETUL_P	MG664264
9	<i>Paecilomyces</i> sp.	F_BETUL_B1	MG664271
10	<i>Penicillium</i> sp.	F_BETUL_I2	MG664274
11	<i>Penicillium</i> sp.	F_BETUL_M	MG664275
12	<i>Penicillium</i> sp.	F_BETUL_A1	MG664273
13	<i>Talaromyces</i> sp.	F_BETUL_J1	MG664276
14	<i>Talaromyces</i> sp.	F_BETUL_J2	MG664277
15	<i>Talaromyces</i> sp.	F_BETUL_O	MG664278
16	<i>Trichoderma</i> sp.	F_BETUL_R	MG664265
17	<i>Trichoderma</i> sp.	F_BETUL_S1	MG664266
18	<i>Xylogone</i> sp.	F_BETUL_H	MG664279

Supplementary Table 1

Taxon name	Group	Sampling location	Reference
<i>Acinetobacter baumannii</i>	Bacteria	Rhu Sepuluh beach, Terengganu, Malaysia	Nkem et al. (2016)
<i>Actinobacteria</i> member	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Alcanivorax</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Alcanivorax</i> sp.	Bacteria	Galveston Island, USA	Bacosa et al. (2016)
<i>Alcanivorax</i> sp.	Bacteria	Mustang Island, USA	Bacosa et al. (2016)
<i>Alphaproteobacteria</i> member	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Bacillus</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Bacillus 'submarinus'</i>	Bacteria	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Bacteroidetes</i> member	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Bhargavaea</i> sp.	Bacteria	Betul beach, Goa, India	This study

<i>Brevibacterium</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Cellulomonas</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Cellulosimicrobium cellulans</i>	Bacteria	Rhu Sepuluh beach, Terengganu, Malaysia	Nkem et al. (2016)
<i>Chloroflexi</i> sp.	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Chromobacterium violaceum</i>	Bacteria	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Corynebacterium glutamicum</i>	Bacteria	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Cyanobacteria</i> member	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Enterobacter</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Escherichia coli</i>	Bacteria	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Firmicutes</i> member	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)

<i>Flavobacteriales</i> member	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Gammaproteobacteria</i> member	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Jonesia</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Klebsiella</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Marinobacter</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Marispirillum</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Nitratireductor</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Nocardia marina</i>	Bacteria	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Oceanimonas</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Oceanospirillales</i> member	Bacteria	Mustang Island, USA	Bacosa et al. (2016)
<i>Owenweeksia</i> sp.	Bacteria	Betul beach, Goa, India	This study

<i>Pannonibacter phragmitetus</i>	Bacteria	The weathered crude oil from Australia	Petra et al. (2012)
<i>Pantoea</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Pelagibaca</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Planctomycetes</i> member	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Proteobacteria</i> members	Bacteria	Sea surface oil mounds from the northern Gulf of Mexico	Liu and Liu (2013)
<i>Pseudoalteromonas</i> sp.	Bacteria	Mustang Island, USA	Bacosa et al. (2016)
<i>Pseudomonas aeruginosa</i>	Bacteria	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Pseudomonas</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Pseudomonas</i> sp.	Bacteria	The weathered crude oil from Australia	Petra et al. (2012)
<i>Pseudoxanthomonas</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Psychrobacter</i> sp.	Bacteria	Galveston Island, USA	Bacosa et al. (2016)

<i>Psychrobacter</i> sp.	Bacteria	Mustang Island, USA	Bacosa et al. (2016)
<i>Rhodobacteraceae</i> member	Bacteria	The weathered crude oil from Australia	Petra et al. (2012)
<i>Shewanella</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Thalassospira</i> member	Bacteria	The weathered crude oil from Australia	Petra et al. (2012)
<i>Tistrella</i> sp.	Bacteria	Betul beach, Goa, India	This study
Unclassifiedbacteria	Bacteria	Galveston Island, USA	Bacosa et al. (2016)
Unclassifiedbacteria	Bacteria	Mustang Island, USA	Bacosa et al. (2016)
<i>Vibrio parahaemolyticus</i>	Bacteria	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Vibrio</i> sp.	Bacteria	Betul beach, Goa, India	This study
<i>Vibrio vulnificus</i>	Bacteria	Beaches of Alabama and Mississippi, USA	Tao et al. (2011)
<i>Xanthobacter autotrophicus</i>	Bacteria	The weathered crude oil from Australia	Petra et al. (2012)
<i>Acremonium kiliense</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)

(= <i>Sarocladium kiliense</i>)			
<i>Acremonium</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Acremonium strictum</i> (= <i>Sarocladium strictum</i>)	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Alternaria alternata</i>	Fungi	Beaches of Oman	Elshafie et al. (2007)
<i>Alternaria</i> sp.	Fungi	Candolim beach, Goa, India	Sanyal et al. (2016)
<i>Alternaria</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus chevalieri</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus ficuum</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus fumigatus</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus heteromorphus</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus nidulans</i>	Fungi	Beaches of Oman	Elshafie et al. (2007)

<i>Aspergillus niger</i>	Fungi	Beaches of Oman	Elshafie et al. (2007)
<i>Aspergillus niger</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus ochraceus</i>	Fungi	Beaches of Oman	Elshafie et al. (2007)
<i>Aspergillus repens</i> (= <i>Aspergillus pseudoglaucus</i>)	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus ruber</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus</i> sp.	Fungi	Betul beach, Goa, India	This study
<i>Aspergillus</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus sydowii</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus terreus</i>	Fungi	Akshi coastal area, Maharashtra, India	Lotfinasabasi et al. (2012)
<i>Aspergillus terreus</i>	Fungi	Beaches of Oman	Elshafie et al. (2007)

<i>Aspergillus terreus</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus ustus</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Aspergillus versicolor</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Beauveria</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Byssochlamys</i> sp.	Fungi	Betul beach, Goa, India	This study
<i>Candida marina</i> (= <i>Bandonia marina</i>)	Fungi	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Candida</i> sp.	Fungi	Candolim beach, Goa, India	Sanyal et al. (2016)
<i>Cercosporidium</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Chaetomium globosum</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Chaetomium murorum</i> (= <i>Botryotrichum murorum</i>)	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)

<i>Chaetomium</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Chaetomium spirale</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Cladosporium cladosporioides</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Cladosporium cladosporoides</i>	Fungi	Beaches of Oman	Elshafie et al. (2007)
<i>Cladosporium resinae</i> (= <i>Amorphotheca resinae</i>)	Fungi	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Cladosporium resinae</i> (= <i>Amorphotheca resinae</i>)	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Cladosporium</i> sp.	Fungi	Candolim beach, Goa, India	Sanyal et al. (2016)
<i>Cryptococcus albidus</i> (= <i>Naganishia albida</i>)	Fungi	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Cunninghamella elegans</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Curvularia/ Bipolaris</i> clade	Fungi	Candolim beach, Goa, India	Sanyal et al. (2016)

<i>Epicoccum purpurascens</i> (= <i>Epicoccum nigrum</i>)	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Fusarium circinatum</i>	Fungi	Betul beach in Goa, India	Barnes et al. (2018)
<i>Fusarium equiseti</i>	Fungi	Betul beach in Goa, India	Barnes et al. (2018)
<i>Fusarium</i> sp.	Fungi	Undesignated beach, Goa, India	Nair and Lokabharathi (1977)
<i>Graphium</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Humicola fuscoatra</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Issatchenkia</i> clade	Fungi	Candolim beach, Goa, India	Sanyal et al. (2016)
<i>Lophotrichus ampullus</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Microascus cinereus</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Micrococcus varians</i> (= <i>Kocuria varians</i>)	Fungi	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)

<i>Monascus</i> sp.	Fungi	Betul beach, Goa, India	This study
<i>Mucor</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Paecilomyces</i> sp.	Fungi	Beaches of Oman	Elshafie et al. (2007)
<i>Paecilomyces</i> sp.	Fungi	Betul beach, Goa, India	This study
<i>Penicillium chrysogenum</i>	Fungi	Beaches of Oman	Elshafie et al. (2007)
<i>Penicillium chrysogenum</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Penicillium citrinum</i>	Fungi	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Penicillium citrinum</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Penicillium herquei</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Penicillium javanicum</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)

<i>Penicillium oxalicum</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Penicillium</i> sp.	Fungi	Beaches of Oman	Elshafie et al. (2007)
<i>Penicillium</i> sp.	Fungi	Betul beach, Goa, India	This study
<i>Penicillium</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Rhizopus</i> sp.	Fungi	Akshi coastal area, Maharashtra, India	Lotfinasabasi et al. (2012)
<i>Rhizopus stolonifer</i>	Fungi	Beaches of Oman	Elshafie et al. (2007)
<i>Rhodotorula</i> sp.	Fungi	Calangute beach, Goa, India	Nair et al. (1972)
<i>Rhodotorula</i> sp.	Fungi	Ratnagiri beach, Maharashtra, India	Nair et al. (1972)
<i>Saccharomyces 'estuari'</i>	Fungi	Ibeno beach, the Bight of Bonny, Nigeria	Itah and Essien (2005)
<i>Scopulariopsis brevicaulis</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Sordaria fimicola</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)

		Pacific Oceans	al. (1988)
<i>Sordaria</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Syncephalastrum racemosum</i>	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Talaromyces</i> sp.	Fungi	Betul beach, Goa, India	This study
<i>Tetracoccusporium</i> sp.	Fungi	North Atlantic and the north Pacific Oceans	Snellman et al. (1988)
<i>Trichoderma</i> sp.	Fungi	Betul beach, Goa, India	This study
Unidentified fungi	Fungi	Calangute beach, Goa, India	Nair et al. (1972)
<i>Xylogone</i> sp.	Fungi	Betul beach, Goa, India	This study

Supplementary Document 1

The system output details for the bacterial tree construction (Figure 1)

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [1]. The tree with the highest log likelihood (-18455.3429) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A

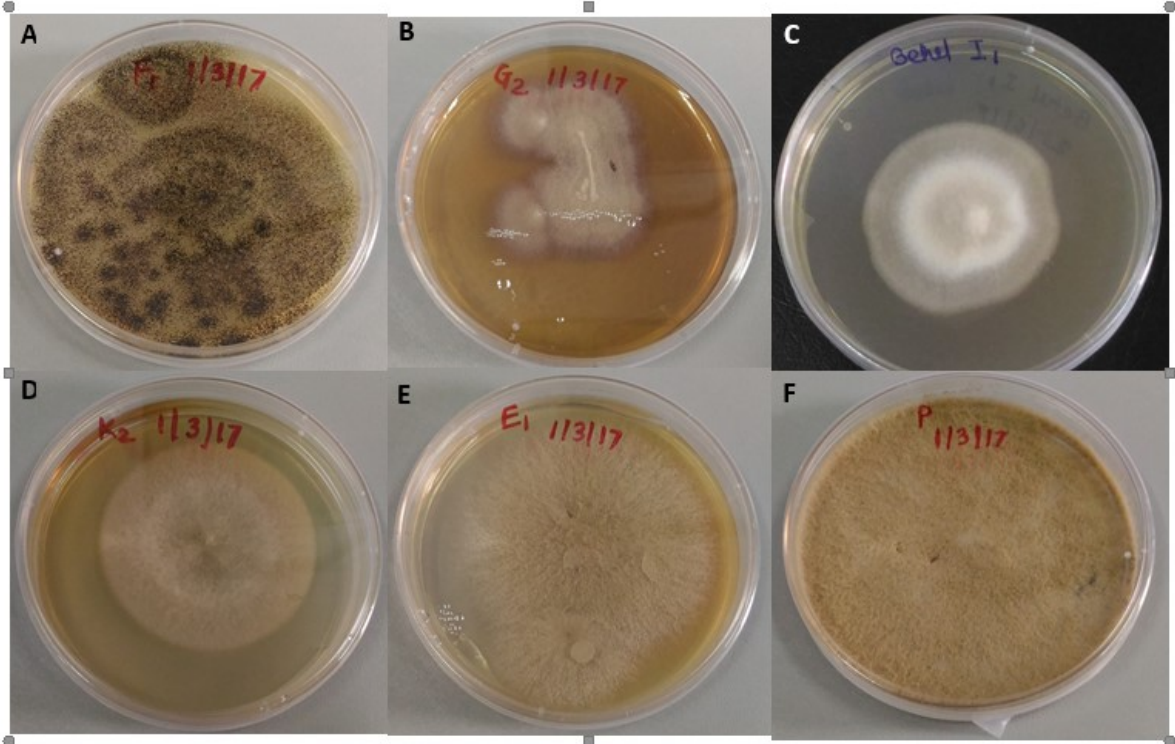
discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4337)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 138 nucleotide sequences. There were a total of 1546 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [2].

The system output details for the fungal tree construction (Figure 2)

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model [1]. The tree with the highest log likelihood (-6119.2333) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.8491)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 73 nucleotide sequences. There were a total of 641 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [2].

1. Kimura M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111-120.
2. Kumar S., Stecher G., and Tamura K. (2015). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* (submitted).

Supplementary Figure 1



Supplementary Figure 2

