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RESEARCH ARTICLE

GEN BANK NEW HOLOTYPE FOR *CORIOLOPSIS BYRSINA* PRK-1 WITH BLAST RECTANGLE TREE VIEW

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ABSTRACT

The Noble prize awarded PCR, an indispensable technique used in biological research for DNA sequencing, DNA-based phylogeny, and functional analysis of genes coupled with ribosomal deoxyribonucleic acid sequencing, polymerase chain reaction and deoxyribonucleic acid sequencing has played a pivotal role in the accurate identification of bacterial/fungal isolates and the discovery of novel bacteria with their 16S rDNA and fungi with their 18S rDNA sequencing in explosive laden soil. Ten different Bacterial isolates and three different Actinomycetes belongs to the genera *Acinetobacter, Bacillus, Enterobacter, Enterococcus, Staphylococcus, klebsiella, Aspergillus, Coriolopsis* were isolated and identified with their 16S and 18S rDNA sequences and deposited in the The GenBank Maryland USA and MycoBank Utrecht Netherlands (Specimen record 37316). All the isolates were named after the discoverer P Ravikumar, will be preserved in MTCC, India. Sanger dideoxy sequencing technology was employed and the number of base pairs, the base count of A, T, G and C was also studied. To fully utilise 16S/18S rDNA sequencing of bacteria and fungi in explosive laden soils and their bioremediation, the presence of *xpl*A and *xpl*B and other biodegrading gene/s were to be investigated. *Coriolopsis byrsina* PRK-1 18S ribosomal RNA gene, with the base count 222 a 169 c 246 g 244 t partial sequence with Accession KJ938683, Version KJ938683.1 (GI:675621792 bases 1-881) a novel strain present in the explosive laden soil of cracker industry is discussed here.

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INTRODUCTION

Coriolopsis is a genus of fungi in the Polyporaceae family. It was circumscribed by American mycologist William Alphonso Murrill in 1905 (Murrill, 1905). The genus is cosmopolitan, with most species in tropical areas (Ryvarden and Johansen, 1980). Morphological characterization of *C. byrsina* was studied by Kirk, 2013. Explosives are materials with high nitrogen and oxygen contents which on detonation expand to create a shock wave which exerts high pressures on the surroundings, causing an explosion and leaving toxic waste in the environment. The manufacturing, testing and use of explosive have resulted in severe contamination of both soils and groundwater (Brannon *et al*., 2005; Eisentraeger *et al*., 2007) thus necessitating their safe removal from the environment. The chemical properties and quantity of explosives waste determine their toxicity and persistence in the environment. The net result has been bioaccumulation and bio magnifications of these explosives waste in aquatic and terrestrial organisms. The incredible versatility inherited in microbes has rendered these explosives as a part of the biogeochemical cycle. Several microbes catalyse mineralization and/or nonspecific transformation of explosive waste either by aerobic or anaerobic processes. It is likely that on-going genetic adaptation, with the recruitment of silent sequences into functional catabolic routes and evolution of substrate range by mutations in structural genes, will further enhance the catabolic potential of bacteria toward explosives and ultimately contribute to cleansing the environment of these toxic and recalcitrant chemicals (Baljinder *et al*., 2012). Incineration of soil to rid it of explosives can result in the exposure of workers to high levels of toxins (Esteve-Núñez *et al*., 2001). Thus, bioremediation is considered both economically feasible and environmentally sound solution. Bioremediation is the use of organisms, such as microbes or plants, to degrade or detoxify hazardous materials on the contaminated sites. Over the years, many new biological methods of bioremediation for explosive contaminated soil have been developed (Lewis *et al*., 2004). Numerous factors can affect the biodegradation processes and depends on the nature of molecules to be degraded (e.g., molecule size, charge,

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number and position of functional groups, solubility and toxicity) as well as the environmental conditions.

MATERIALS AND METHODS

Study site and collection of sample

Valangaiman of Thiruvarur District of Tamilndu India (10°46′17.76″N 79°38′12.48″E) was selected as the sampling site for this study. Approximately 100gm of explosive laden soil from the cracker manufacturing unit and the grinding unit were collected form five different places and immediately placed into 500ml sterile air tight container, sealed to avoid contamination and transported to the laboratory for further processing.

Soil processing and isolation

3 gm of the soil samples were vigorously mixed with 3 ml of sterile distilled water and left for overnight.100 microliter of the upper surface soil liquid was then transferred into 5 ml Sabouraud Dextrose Agar GM063 with a sterile micro pipette and incubated at 36° C for 48 hrs. The plates incubated for two days were visually inspected daily until typical colonies formed. The colonies were purified by further subculture on Dextrose Broth GM033 (HIMEDIA) to confirm the purity and preserved at -20°C until further use.

Molecular confirmation

Identification with specific PCR

The colony morphologically identified *C.byrsina* was further identified by PCR procedures based on amplification of 18S rDNA gene. PCR was standardised with forward and reverse PCR primers and performed in a volume of 25 microliter, the reaction mixture containing 200 mM of each dNTP, 1.5µm MgCl2, 1xPCR buffer, 10 pmol of each primer, 1U of taq DNA polymerase and 10 ng DNA. The PCR cycle protocol consists of initial denaturation at 95°C for 6 min and 30 cycles of denaturation at 95°C for 1 min, primer specific annealing for 1 min and extension at 72°C for 2 min with the final extension at 72°C for 10 min. PCR products were electrophoresed on 1% agarose gel and visualised under UV.

18S rDNA analysis

18S rDNA sequencing was used to confirm PCR identified isolate and 18S rDNA sequence of the isolate was BLAST analysed (Gee *et al*., 2003).The PCR reaction mixture for the amplification of the 16S rDNA gene consisted of 200mM of each dNTP, 1.5 mM MgCl2, 1x PCR buffer, 10pmol of each primer, 1 U of Taq DNA polymerase and 10ng DNA. The reaction was made up to 25 microliter with sterile distilled water and the cycle consisted of initial denaturation at 95°C for 6 min and 30 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min with the final extension at 72°C for 10 min. PCR products were electrophoresed on 1.0% agarose gel and visualised under UV in a gel documentation system as above. Amplified 18S rDNA PCR products were sequenced by the dideoxy chain termination method (Sanger and Coulson, 1975 & Sanger *et al*., 1977) using Big dye Terminator v 3.1 sequencing kit and Big dye x Terminator Purification kit in an ABI 10 sequencer. The derived sequences were aligned using DNASTAR lasergene 9 Core Suit and BLAST analysis was with NCBI database.

RESULTS

From the soil samples sub cultured successfully one isolate was suspected and selected as possible *Coriolopsis byrsina* PRK-1 (Figure 1) based on characteristic colonial morphology, interestingly the environmental isolate was able to grow on of Sabouraud Dextrose Agar GM063 and in Sabouraud Dextrose Broth GM033 (HIMEDIA).

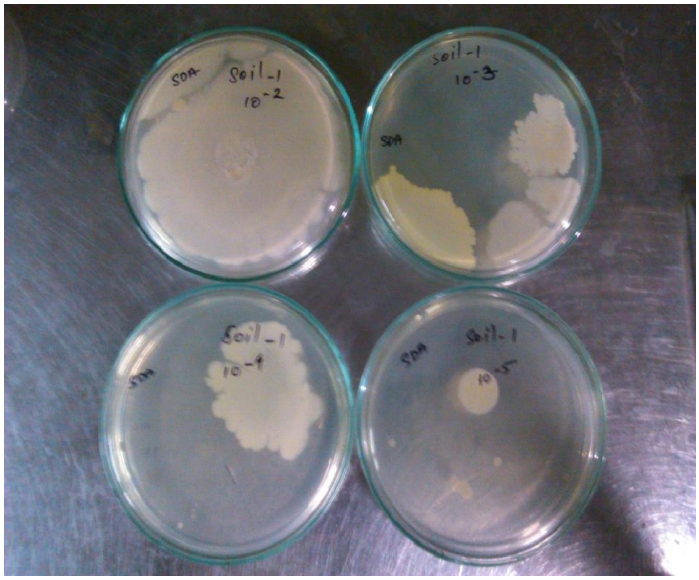

Figure 1. *Coriolopsis byrsina* on SDA

The one isolate confirmed by 18S rDNA sequencing was subjected to Sanger dideoxy sequencing. The FASTA of *Coriolopsis byrsina* strain PRK-1's 18S ribosomal RNA gene, partial sequence Molecule type nucleic acid Query Length 881 bp is depicted below:

>gi|675621792|gb|KJ938683.1| Coriolopsis byrsina strain PRK-1 18S ribosomal RNA gene, partial sequence

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CCTTACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGA

GTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGG
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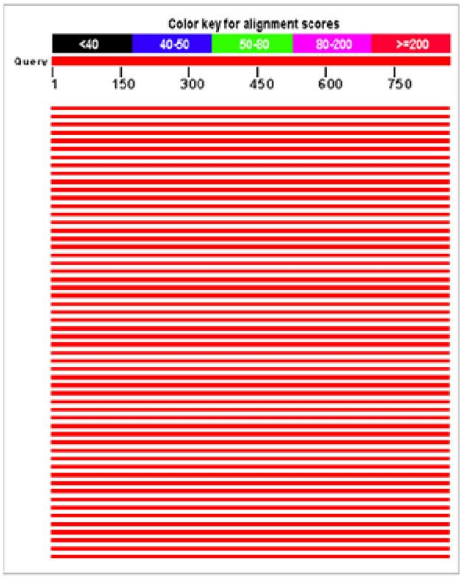
GACTCAACACGGGGAAACTCACCAGGTCCAGACATG
ACTAGGATTGACAGATTGATAGCTCTTTCATGATTTTA
TGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGT
GATTTGTCTGGTTAATTCCGATAACGAACGAGACCTT
AACCTGCTTAATAGCCAGGCCGGCTTTTGCTGGTCGC
CGGCTTCTTAGAGGGACTGTCTGCGTCTAGCAGACGG
AAGTTTGAGGCAATAACAGGTCATGTG

The regions of similarity between this sequence and other sequences, BLAST was used and the result is depicted below:
An overview of the database sequences aligned to the query sequence is shown. The score of each alignment is indicated by one of five different colours, which divides the range of scores into five groups. Multiple alignments on the same database sequence are connected by a striped line. Musing over an alignment shows the alignment definition and score in the box at the top.

NCBI/ BLAST/ blastn suite/ **Formatting Results - NE44EBW6015**



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Descriptions

Sequences producing significant alignments:

Sequences producing significant alignments:							
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Reading indexes 1-5

Cori olopsis byrsina strain PRK-1 18S ribosomal RNA gene, partial sequence
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Range 1: 1 to 881GenBankGraphics Next Match Previous Match First Match

Alignment statistics for match #1					
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And The GenBank deposite is as

Coriolopsis byrsina strain PRK-1 18S ribosomal RNA gene, partial sequence

GenBank: KJ938683.1
FASTA Graphics
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DEFINITION Coriolopsis byrsina strain PRK-1 18S ribosomal RNA gene, partial
sequence.
ACCESSION KJ938683
VERSION KJ938683.1 GI:675621792
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ORGANISM Coriolopsis byrsina
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Polyporales; Coriolopsis.
REFERENCE 1 (bases 1 to 881)
AUTHORS Ravikumar,P.
TITLE Direct Submission
JOURNAL Submitted (05-JUN-2014) Associate Professor of Botany, Government
Arts College (Autonomous), Coimbatore, Tamilnadu 641018, India
COMMENT ##Assembly-Data-START##
Sequencing Technology:: Sanger dideoxy sequencing
##Assembly-Data-END##

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121 gtctggctgg gtcttacctc ttggtgagcc ggcatgccct tcactgggtg tgtcggggaa
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Lineage Report
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. . . Fungi [fungi]
. . . . Agaricomycotina [basidiomycetes]
. . . . . Agaricomycetes [basidiomycetes]
. . . . . Agaricomycetes incertae sedis [basidiomycetes]
. . . . . Polyporales [basidiomycetes]
. . . . . Coriolaceae [basidiomycetes]
. . . . . Coriolopsis [basidiomycetes]
. . . . . Coriolopsis byrsina ------- 1628 4 hits [basidiomycetes] Coriolopsis byrsina strain PRK-1 18S ribosomal RNA gene, pa

and the Organism Report is

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1628 0.0

Taxonomy Report

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. . Opisthokonta ..... 101 hits 60 orgs
. . . Fungi ..... 100 hits 59 orgs
. . . .Agaricomycotina….90 hits 57 orgs [Dikarya; Basidiomycota]
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gb|KJ938683.1| (881 letters)

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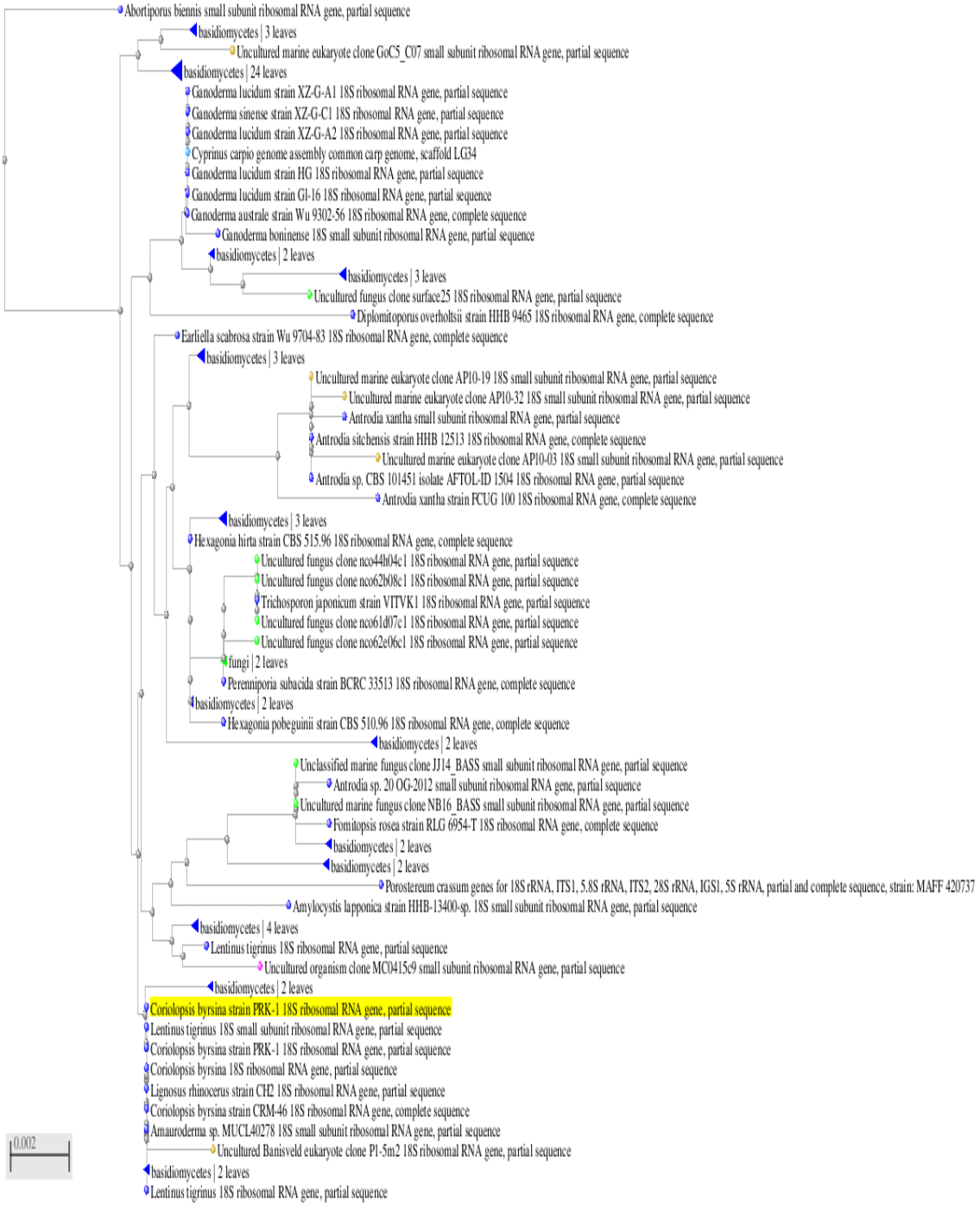
The BLAST RECTANGLE TREE VIEW of *Coriolopsis byrsina* PRK-1 is as



Conclusion

This research piece of work is the first report of the isolation and molecular confirmation of *Coriolopsis byrsina* strain PRK-1 from the explosive laden soil. This isolate was initially identified by conventional morphological methods and further confirmed by advanced molecular based methods of 18S rDNA sequencing and *Coriolopsis byrsina* strain PRK-1 specific PCR. The isolation of this important fungal strain from this part of India may with their *xpl*A, *xpl*B, other biodegrading gene/s and catabolic gene cassette sequences for the explosives should initiate further studies on the extent of environmental bioremediation.

Acknowledgement

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