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Microbial Characterization and Phylogenetic Analysis of a Mesophilic Chemolithotrophic Bacterium Isolated from Fresh Iron Mine Overburden Spoil

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Abstract

Iron mining activities generate huge amount of mine spoil dumped in the form of overburden, which represents nutrient deficient situation inhospitable for the existence of flora and fauna. The deterioration in soil health because of the mining activities is a matter of environmental concern for which it deserves priority for its reclamation. The investigation about the microbial community structure and its characterization provide greater understanding about the microbial activities and their contribution towards ecosystem functioning. In the present study, a mesophilic bacterium was isolated from fresh iron mine overburden spoil and identified through 16S rDNA sequence analysis. Microbial characterization revealed that the isolated bacterium was gram negative, obligatory and facultative chemolithotroph. The bacterium exhibited optimal growth at 37^{o}C in culture medium with pH 5, which suggested that the bacterium was mesophilic and acidophilic in nature. The growth response of the bacterium revealed relatively higher growth rate in heterotrophic condition as compared to chemolithotrohic culture condition. Molecular phylogenetic analysis based on 16S rDNA sequence homology suggested that the isolated bacterium exhibited closer resemblance with *Ralstonia solanacearum* NC_003296.1 (100% sequence identity), which posses wide geographic distribution, large host range and the exceptional capacity to adapt in many different environments.

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Introduction

Mining activity causes drastic perturbations in properties and processes of soil profile leading to degradation and decline in soil quality (Lal, 1997). Pit scarred landscape with huge dumps of mine spoils in the form of overburden usually presents the common scenario in the opencast iron mine area (Juwarkar et al., 2004). Fresh iron mine spoil refers to a mixture of many impurities such as pyrite (FeS_2), chalcopyrite ($CuFeS_2$), pyrrhotite (FeS), arsenopyrite (FeAsS), galena (PbS), sphalerite (ZnS), pentlantite (FeNiS) and cobaltite (CoS) (Natarajan, 2008), which create a larger risks and hazards that jeopardize the ecosystems (Ghose and Sen, 1997, 1998). Due to the extremities in conditions like high temperature, acidic or alkaline pH and at high calibers of salt (Mendo et al., 2004; Walczak and Donderski, 2004; Awais et al., 2008; Muhammad et al., 2009), it allows the existence of some specific microbes such as proteobacteria and acidobacteria which subsequently allow the colonization of other microorganism to induce the soil productivity.

Increased exploration and exploitation of resources in the iron mine leading to anthropogenic disturbances causes extinction of species in the unprecedented rate thereby

creating a negative impacts on ecosystem functioning, which may lead to the discovery of new species that bring changes in soil microbiological and ecological properties (Druschel et al., 2004). Members of proteobacteria and acidobacteria are the most abundant soil bacteria (Janssen, 2006). Proteobacteria lineage is more diverse and stable than acidobacteria lineage, suggesting that the acidobacteria are more susceptible to variation in soil properties and to the disturbing factors (Youssef and Elshahed, 2009). However, proteobacteria can adapt to the extremities of fresh iron mine overburden spoil by creating their own microenvironment, as they encompass an enormous level of morphological, physiological and metabolic diversity and are of great importance to global carbon, nitrogen and sulfur cycling (Kersters et al., 2006). The most abundant and diverse class within the proteobacteria was alphaproteobacteria, followed by the beta, delta, gamma and epsilon proteobacteria distinguished by small differences in their rRNA sequences. Further, they are divided on the basis of their response to different temperatures such as mesophiles (20 to 40^{o}C), moderate thermophiles (40 to 60^{o}C) and extreme thermophiles (above 60^{o}C).

The microbes relevant to proteobacteria group (Smith, 1896; Yabuuchi et al., 1995) function as plant growth-promoting rhizobacteria (Kang et al., 2002). These bacterial strains are free living or root associated bacteria that can increase the plant growth and productivity through the production of phytohormones (Glick, 1995; Chanway, 1997; Bottini et al., 2004), antimicrobial compounds such as antibiotics and siderophores that solubilize and sequester iron (Emmert and Handelsman, 1999; Compant et al., 2005). Further, it elicit plant defensive mechanism against foliar and soil borne pathogens, hence reduce the incidence of plant diseases (Weller, 1988; Achari and Ramesh, 2014). In addition, the bacterium contributes towards the development of biofilm through various mechanisms (Mansfield et al., 2012), able to dissimilate nitrates and to degrade xenobiotic compounds (Bossis et al., 2000). Besides, soil microorganisms are sensitive to environmental perturbations that alter the microbial community composition and activities thereby influence the ecosystem stability (Smith and Padendick, 1993). Therefore, microbial characterization is very essential for a broader understanding of soil health.

Microbiological constraints of mine spoil have been the subject of microbiological research with the assessment of microbial community profiling to maintain the soil environment has become common place with high-throughput techniques such as 16S rRNA (or rDNA) gene amplicon sequencing. Therefore, the microbial communities have been profiled using PCR amplification of rRNA genes, with the goal of understanding their ecological function (Pastrik et al., 2002). Comparisons of rRNA sequences defined the main lineages in the evolution of microorganisms as rRNAs are integral elements of the protein synthesizing apparatus, the basic components of which are present in all primary kingdoms, and are among the most highly conserved cellular molecules. Because of the similarity exist in rDNA gene sequence even in distantly related microorganisms; the sequences can be precisely aligned helpful in making differences among the microbes. Besides, the rDNA molecules have long been recognized for their utility as molecular chronometers (Woese, 1987). However, the larger rDNA molecules contain many domains with independent rates of sequence change related to their structural and functional attributes (Kent and Triplett, 2002). Thus, the study of genes that encode the 16S rDNA and PCR technique have been extensively used to determine taxonomy, phylogeny (evolutionary relationships) and to estimate rates of species divergence among bacteria isolated from different sources with an understanding of their role in soil health (Mahdy et al., 2012).

Keeping in view, the present study was designed for microbial characterization of the bacterium isolated from fresh iron mine overburden spoil. In addition, the detailed molecular phylogenetic analysis and taxonomic placements of 16S rDNA gene sequencing by specific PCR amplification can be shifted more towards computing more diligent and accurate estimates of species richness and evenness, identification of novel bacteria phyla (Elshahed et al., 2008) and computational comparisons of communities between different soil types due to its good discriminatory power and excellent ease of interpretation and performance.

Materials and methods

Study site

The present study was carried out in the Thakurani iron mining area in Noamundi (geographical location: 85° 28' 02.61" east longitude and 22° 8' 33.93" north latitude), maintained by M/s. Sri Padam Kumar Jain sponge mines Private Ltd. located in the revenue district of West Singhbhum, Jharkhand, India. The study site is surrounded by a number of new, old and abandoned mine

of iron ore overburden. The district experiences semi-arid climate with annual average rainfall estimated to be 1250.43mm as compared to the state average of 1340mm. The mean annual temperature and humidity is around 19.67°C and 20% respectively. The study site is situated away from the mean sea level of about 581m altitude.

Mine spoil sampling

Sampling was done from the fresh iron mine overburden in accordance with the methodology described by Parkinson et al., 1971. The fresh iron mine overburden was divided into 5 blocks, and from each block five mine spoil samples were collected randomly from (0-15) cm soil depth by digging pits (15 x 15 x 15) cm^3 size referred as 'sub-samples'. The sub-samples were mixed thoroughly to from one composite sample, which was sieved (0.2 mm mesh) and stored at 4°C for further analysis.

Isolation of bacterium

The bacterium was isolated from fresh iron mine overburden spoil through standard agar plate method (Hallberg and Lindstrom, 1994; Johnson, 1995). Citrate broth was used for isolation, cultivation and maintenance of the bacterium with following medium composition: $(NH_4)_2SO_4$: 0.5g, $NaNO_3$: 0.5g, $MgSO_4$: 0.5g, K_2HPO_4: 0.5g, Fe $(NH_4)_3(C_6H_5O_7)_2$: 10g, $CaCl_2$: 0.2g, Agar: 15g per liter of double distilled water and pH adjusted to 6.5 with 1N HCL. About 100µl bacterial culture was streaked on citrate agar and subjected to incubation at 37°C for 24 hrs. Isolated colonies were randomly taken and these steps were repeated in order to obtain pure culture of the bacterium.

Microbial characterization

Gram stain response of the bacterium isolated from fresh iron mine overburden spoil was performed through differential straining of the heat fixed smear of the bacterial culture with crystal violet followed by the addition of 1/2 drops of saffranin stain and observed under the microscope.

Optimum pH required for growth of the isolated bacterium was determined through serial dilution technique (10^8 folds). For the purpose, about 100µl diluted culture was spread individually onto the already solidified citrate agar medium maintained at different pH and incubated at 37ºC for 24 hrs. The optimal pH range required for bacterial growth was estimated through the enumeration of bacterial CFU.

Thermal death time of the isolated bacterium was determined by inoculating 100µl of bacterial culture in 5ml of citrate broth and subjected to incubation at 60ºC for different time interval. After incubation, the bacterial culture was streaked onto the citrate agar individually and incubated at 37ºC for 24 hrs for development of colonies. Gradual reduction in bacterial CFU revealed the time dependence on the temperature induced effect on the bacterium.

Antimicrobial sensitivity test of the bacterium was performed through disc diffusion technique following Kirby-Bauer's method (Madigan and Martinko, 2006) using different antibiotics such as amikacin, amoxycillin, azithromycin, cefotaxime, ciprofloxacin, erythromycin, gentamycin, kanamycin, levofloxacin, norfloxacin, ofloxacin, rifampicin, roxythromycin, streptomycin and tetracycline having concentration (0.5mg/ml) in triplicates and incubated at 37ºC for 24 hrs. The diameter of the zone of inhibition was measured. Growth analysis of bacterium was performed using citrate broth in chemolithotrophic (without glucose) as well as heterotrophic culture condition (with 10g/l glucose) individually, and was incubated at 37ºC for different time intervals. The growth response of the bacterium was determined by taking absorbance at 640 nm against control.

Genomic DNA isolation

Isolation of genomic DNA was performed using the genomic DNA isolation kit (Chromous bacterial genomic DNA Spin-50). About 100 mg bacterial pellet was suspended with 750 µl of 1X suspension buffer followed by addition of 5µl of RNaseA with intermittent mixing and kept at 65ºC for 10min. Then, 1ml of lysis buffer was added, mixed gently and kept at 65ºC for 15min followed by centrifugation at 13000g at room temperature. The supernatant was collected, loaded on to the spin column (600µl each time) and centrifuged at 13000g for 1 min at room temperature. The supernatant was discarded. About 500µl of 1X wash buffer was added to column and centrifuged at 13000g for 3 min. The spin column was placed in a fresh 1.5ml vial followed by addition of 50µl of warm elution buffer (kept at 65ºC) and centrifuged at 13000g for 1min. The eluted DNA was resolved by 1% agarose gel to estimate the quality and quantity of template DNA used for PCR amplification.

Gel elution of PCR products

Amplified PCR product was cut from the gel and kept in a 2ml microcentrifuge tube. About 3 volumes of gel extraction buffer was added to 1 volume of gel and incubated at 55°C for 10 min with intermittent mixing. Then, 1 volume of isopropanol was mixed with the gel extracted solution, loaded on to the spin column (600µl each time) and centrifuged at 13000g for 1 min at room temperature. Then, 500µl of wash buffer was added and centrifuged at 13000g for 3 min at room temperature. The content of the collection tube was discarded. The spin column was placed in a fresh 1.5ml microcentrifuge tube with 15µl of elution buffer and centrifuged at 13000g for 1 min at room temperature. The purified DNA was collected and stored at 4ºC for further analysis.

16S rDNA sequencing and analysis

The 16S rDNA fragment was subjected to sequencing based on the chain termination reaction (Imhoff et al., 2013) using 'BigDye terminator sequencing Ready Reaction kit' (version 3.1) (PE Applied Biosystems) through the automated ABI 3500 XL genetic analyzer using both the primers (Forward: 5'-AGAGTTTGATCMTGGCTCAG-3' and Reverse: 5'-TACGGYTACCTTGTTACGACTT-3'). The sequencing mixture (10µl) included BigDye terminator ready reaction mix: 4µl; template (100ng/ul): 1µl; primer (10pmol/λ): 2µl; Milli Q water: 3µl. The PCR conditions includes 25 cycles with initial denaturation at 96°C for 1 min, denaturation at 96°C for 10 sec, hybridization at 50°C for 5 sec followed by the final elongation at 60°C for 4 min.

The 16S rDNA sequence was aligned using the 'JustBio online bioinformatics tool' (http://www.justbio.com) and assembled into a contiguous chain. Besides, the 16S rDNA sequence of the isolated bacterium was subjected to homology search using 'BLAST' at NCBI (Altschul et al., 1997). The representative sequences were retrieved from the databases and subjected to multiple sequence alignment using CLUSTAL W. The computed alignment was analyzed for chimera using QIIME (version 1.5) (http://www.qiime.org). The final sequence of 16S rDNA was deposited in the GenBank using BankIt submission tool. Based on the 16S rDNA sequence homology, the evolutionary distances were computed through MEGA (version-6.0) program (Tamura et al., 2013) with *p*-distance using neighbor-joining method (Saitou and Nei, 1987). Further, the bootstrap values were calculated from 1000 replications to represent the evolutionary history of the microbial taxa (Felsenstein, 1985) using MEGA (Version 6.0).

Results and discussion

Microbial characterization

The bacterium derived energy for its grow and proliferate utilizing the nutrients supplemented with citrate broth and hence was used for isolation and maintenance of the bacterium. The isolated colonies of the bacterium appeared on the citrate agar plates were observed to be (1-2) mm in diameter, smooth, circular, brown in colour, convex and greater opacity in their sizes. Gram stain response revealed that the isolated bacterium was rod shaped gram negative bacteria. Based on the variation in CFU counts of the bacterium grown under culture conditions with different pH, the study suggested that the bacterium exhibited relatively higher growth rate at pH 5. Besides, the isolated bacterium exhibited a decline trend with respect to their CFU counts upto 1½ hrs, but no colony was observed at 2 hrs of incubation at 60ºC. It is evident from the study that the thermal death time of the bacterium was found to be 2 hrs at 60ºC and was suggested to be mesophilic, which cannot be susceptible at moderately higher temperature for a longer time.

Antibiotics sensitivity test revealed clear circular zone of inhibition against different antibiotics, which indicated their potency against the isolated bacterium (Fig. 1). The study suggested that the bacterium was found to be resistant against gentamycin, kanamycin and roxythromycin, where as sensitive towards the antibiotics such as amikacin, amoxicillin, azithromycin, cefotaxime, ciprofloxacin, erythromycin, levofloxacin, norfloxacin, ofloxacin, rifampicin, streptomycin and tetracycline.

Growth analysis

The growth response of the isolated bacterium was performed in citrate broth with chemolithotrophic (without glucose) as well as heterotrophic (with 10g/l glucose) culture condition at 37ºC (Fig. 2). The lag phase continued upto 5th hr of incubation and thereafter the exponential phase continued till 48th hr of incubation in the chemolithotrophic culture condition. Similarly, the lag phase continued upto 45min of incubation in heterotrophic culture condition. The exponential phase started after 45min and continued till 43rd hr of incubation in heterotrophic culture condition (Fig. 2).

The study clearly revealed variation in growth rate of the bacterium with respect to the different culture conditions. It is evident from the analysis that the specific growth rate (μ) of the bacterium in heterotrophic condition (0.046 hr^{-1}) was comparatively higher than chemolithotrophic culture condition (0.041 hr^{-1}). Relatively less growth rate of the bacterium in chemolithotrophic culture condition may be explained on the basis of slow energy yielding activities attributed to the nutrient deficient situation of fresh iron mine overburden spoil. Besides, the study revealed the potency of the bacterium to shift from the chemolithotrophic to heterotrophic condition supplemented with extraneous organic carbon.

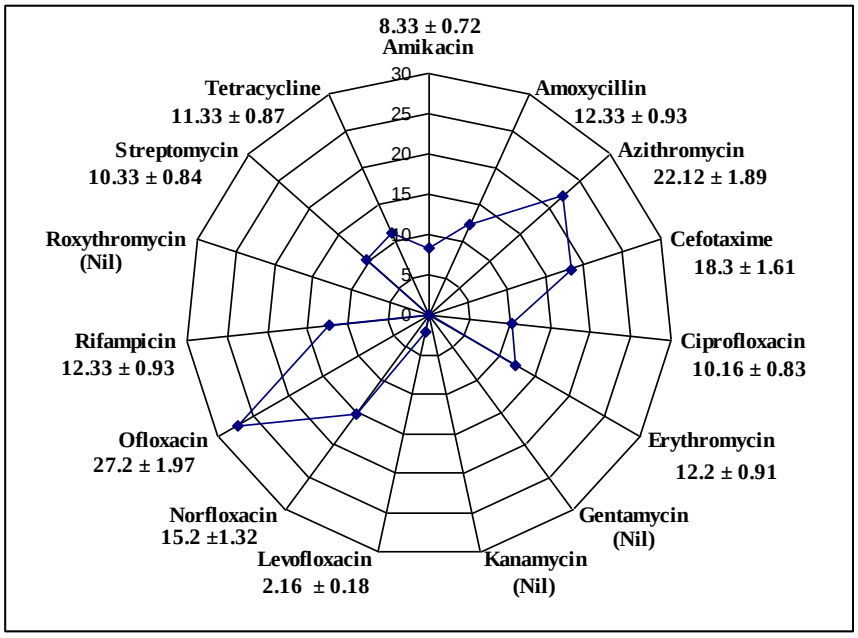


Fig. 1: Antibiotics sensitivity test of the isolated bacterium. The diameter of zone of inhibition is expressed in (mm ± SD); n= 3.

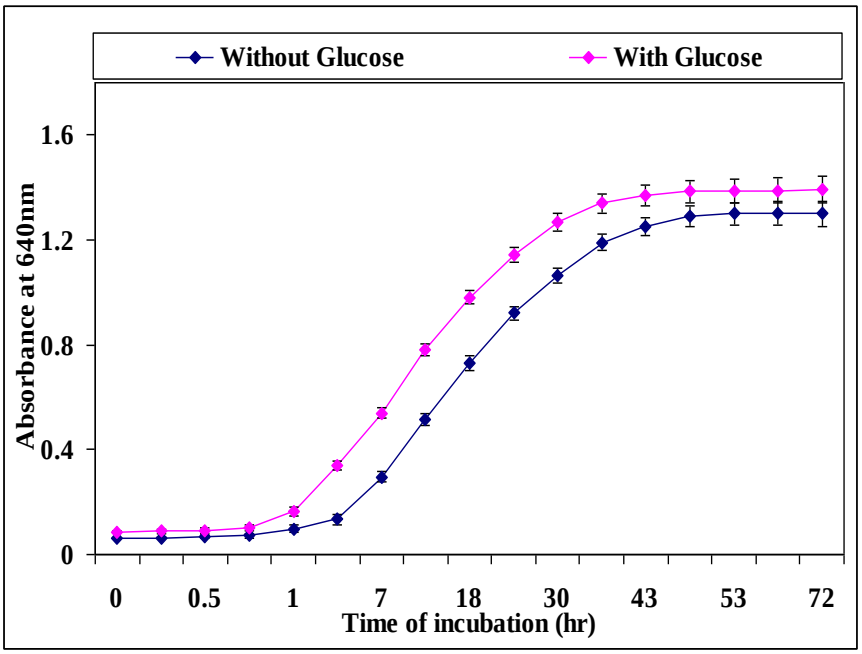


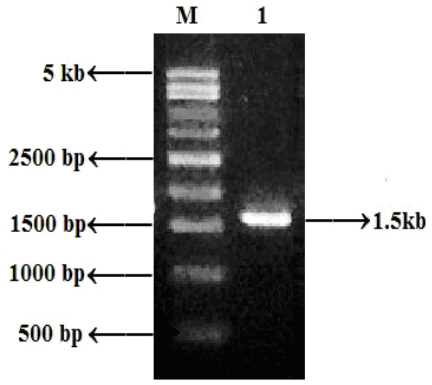
Fig. 2: Growth curve of the bacterium in chemolithotrophic (without glucose) and heterotrophic (with glucose) culture conditions at different hour of incubation at 37°C.

PCR amplification

The identification of the bacterium becomes a challenging mission for the microbial ecologists to unravel microbial diversity. PCR amplification of 16S rDNA with forward: 5'-AGAGTTTGATCMTGGCTCAG-3' and reverse: 5'-TACGGYTACCTTGTTACGACTT-3' primers generated the amplicon size of ~1.5 Kb in 1.5% agarose gel (Fig. 3).

Fig. 3: PCR amplification of 16S rDNA of the bacterial isolate revealed the amplicon size of ~1.5Kb (Lane M: marker DNA; Lane 1: amplicon size of ~1.5Kb).

Blast analysis and sequence homology



The 16S rDNA sequence of the isolated bacterium was subjected to homology search using BLAST. Highest degree of sequence homology exhibited by the 16S rDNA sequence was represented (Table 1). The study suggested that the 16S rDNA sequence of the isolated bacterium shared 100% sequence identity with the 16S rDNA of *Ralstonia solanacearum* GMI1000 (NC_003296.1) and 99% sequence identity with 16S rDNA of *Ralstonia pickettii* 12J (NC_010678.1). Besides, it exhibited 97% sequence identity with the 16S rDNA sequence of *Herbaspirillum seropedicae* SmR1 (NC_014323.1) and *Collimonas fungivorans* Ter331 (NC_015856.1) respectively (Table 3). In addition, it shared 96% sequence identity with *Burkholderia rhizoxinica* HKI 454 (NC_014722.1), *Chromobacterium violaceum* ATCC 12472 (NC_005085.1); 95% sequence identity with the 16S rDNA sequence of *Burkholderia glumae* BGR1 (NC_012724.2), *Burkholderia phymatum* STM815 (NC_010622.1). Further, it showed 94% sequence identity with the 16S rDNA sequence of *Cupriavidus metallidurans* CH34 (NC_007973.1), *Burkholderia xenovorans* LB400 (NC_007952.1), *Basilea psittacipulmonis* DSM 24701 (NZ_CP009238.1), *Nitrosospira multiformis* ATCC 25196 (NC_007614.1), *Basilea psittacipulmonis* DSM 24701 (NZ_CP009238.1), *Burkholderia phenoliruptrix* BR3459a (NC_018695.1) and 93% sequence identity with the 16S rDNA sequence of *Dechlorosoma suillum* PS (NC_016616.1), *Castellaniella defragrans* 65Phen (NZ_HG916765.1) and *Azoarcus aromaticum* EbN1 (NC_006513.1) respectively (Table 1).

Table 1. 16S rDNA sequence homology of the query sequence (Accession no. KU500376) with closely related sequences from databases using BLAST analysis.

Microbial strains	NCBI (Accession No.)	Query coverage	E-value	Identity
Ralstonia solanacearum GMI1000	NC_003296.1	99%	2e-179	100%
Ralstonia pickettii 12J	NC_010678.1	99%	1e-172	99%
Herbaspirillum seropedicae SmR1	NC_014323.1	85%	7e-160	97%
Collimonas fungivorans Ter331	NC_015856.1	85%	7e-160	97%
Burkholderia rhizoxinica HKI 454	NC_014722.1	99%	1e-157	96%
Chromobacterium violaceum ATCC 12472	NC_005085.1	85%	9e-154	96%
Burkholderia glumae BGR1	NC_012724.2	97%	1e-152	95%
Burkholderia phymatum STM815	NC_010622.1	96%	3e-148	95%
Cupriavidus metallidurans CH34	NC_007973.1	99%	1e-147	94%
Burkholderia xenovorans LB400	NC_007952.1	96%	1e-146	94%
Ralstonia eutropha H16	NC_008314.1	99%	1e-142	94%
Nitrosospira multiformis ATCC 25196	NC_007614.1	85%	2e-140	94%
Basilea psittacipulmonis DSM 24701	NZ_CP009238.1	99%	2e-141	94%
Burkholderia phenoliruptrix BR3459a	NC_018695.1	99%	7e-145	94%
Dechlorosoma suillum PS	NC_016616.1	84%	3e-138	93%
Castellaniella defragrans 65Phen	NZ_HG916765.1	99%	4e-137	93%
Azoarcus aromaticum EbN1	NC_006513.1	84%	4e-137	93%

Molecular phylogenetic analysis

The 16S rDNA sequence obtained from the isolated bacterium was subjected to phylogenetic analysis conducted in MEGA 6.0 (Tamura et al., 2013). The evolutionary history was inferred using Neighbor Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). Evolutionary distance was computed using the *p*-distance using NJ method (Nei and Kumar, 2000) reflecting the units of the number of base substitutions per site. The analysis involved 18 nucleotide sequences. The dendrogram is drawn to scale with branch length in the same unit as the evolutionary distance used to infer phylogeny (Fig. 4). All positions containing gaps and missing data were eliminated. There were a total of 310 positions in the final datasets.

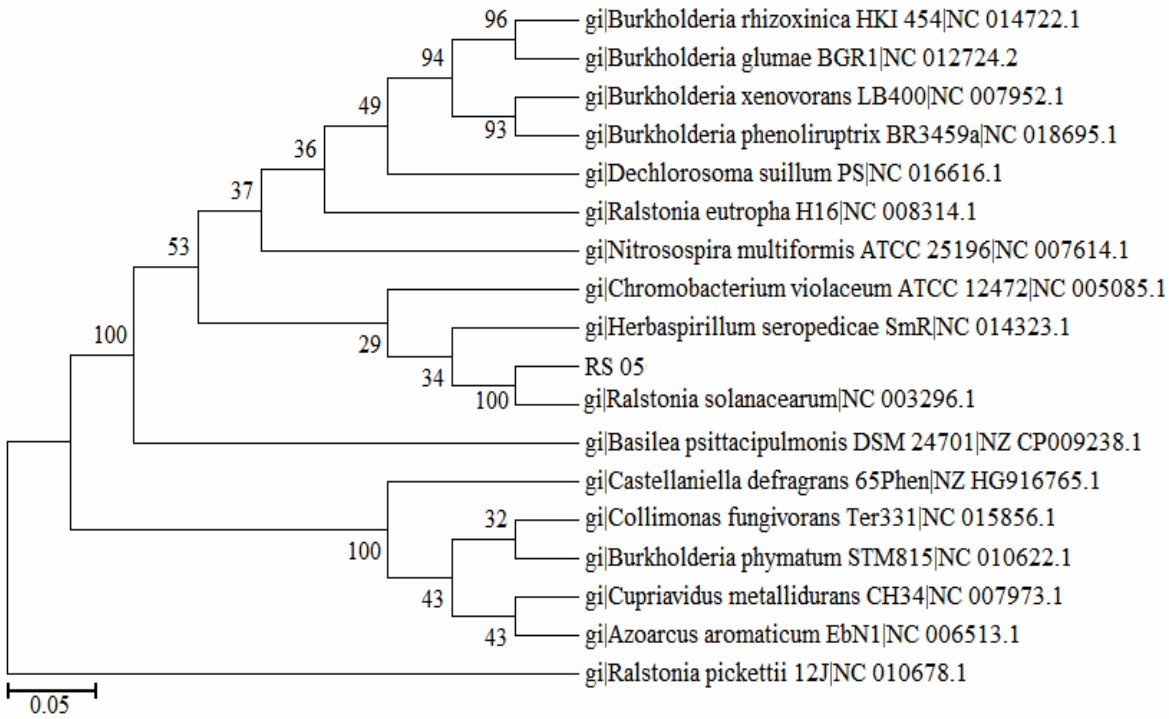


Fig. 4: Dendrogram based on NJ method showing the relatedness between 16S rDNA sequence of the bacterium and its closest relative sequences retrieved from databases.

Conclusion

Molecular phylogenetic analysis based on 16S rDNA sequence homology suggested that the isolated bacterium cultured from fresh iron mine overburden spoil exhibited close resemblance with *Ralstonia solanacearum* NC_003296.1 (100% sequence identity). The genus *Ralstonia* is within the β-subdivision of the proteobacteria and includes five species, *R. picketii, R. insidiosa, R. mannitolilytica, R. syzygii* and *R. solanacearum* (Yabuuchi et al., 1995). Its wide geographic distribution, large host range and the exceptional capacity to adapt in many different environments made it more important for the soil environment. Further, the sequence variability of rDNAs and the advent of PCR made rDNA even more accessible for sequencing providing a unique opportunity for in-depth phylogenetic analysis to highlight the breadth of diversity within various major bacterial phyla encountered in soil. The 16S rDNA sequence of the bacterium was submitted to NCBI GeneBank (Accession no. KU500376).

Conflict of interest statement

Authors declare that they have no conflict of interest.

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References

Achari, G.A., Ramesh, R., 2014. Diversity, biocontrol and plant growth promoting abilities of xylem residing bacteria from Solanaceous crops. Int. J. Microbiol. 1-14.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389-3402.

Awais, M., Pervez, A., Qayyum, S., Saleem, M., 2008. Effects of glucose, incubation period and pH on the production of peptide antibiotics by *B. pumilus*. African J. Microbiol. Res. 2, 114-119.

Bossis, E., Lemanceau, P., Latour, X., Gardan, L., 2000. The taxonomy of *P. fluorescens* and *P. putida*: current status and need for revision. Agronomie. 20, 51-63.

Bottini, R., Cassan, F., Piccoli, P., 2004. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl. Microbiol. Biotechnol. 65, 497-503.

Chanway, C.P., 1997. Introduction of tree roots with plant growth promoting soil bacteria: An emerging technology for reforestation. Forest Sci. 43, 99-112.

Compant, S., Nowak, B.J. Clement, C., Barka, E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71, 4951-4959.

Druschel, G.K., Baker, B.J., Gihring, T.M., Banfield, J.F., 2004. Acid mine drainage biogeochemistry at Iron Mountain, California. Geochem. Trans. 5(2), 13-32.

Elshahed, M.S., Youssef, N.H., Spain, A.M et al. 2008. Novelty and uniqueness patterns of rare members of the soil biosphere. Appl. Environ. Microbiol. 74, 5422-5428.

Emmert, E.A., Handelsman, J., 1999. Biocontrol of plant disease: A (Gram-) positive perspective. FEMS Microbiol. Lett. 171, 1-9.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evol. 39, 783-791.

Ghose, M.K., Sen, P.K., 1997. Recovery of usable ore fines from iron ore tailing and their environmental management a base study. Land Contam. Reclam. 7(2), 143-149.

Ghose, M.K., Sen, P.K., 1998. Disposal of tailings from iron ore beneficiation plants and their environmental impacts. J. Institute of Public Health Engineers. 3, 5-12.

Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41, 109-117.

Hallberg, K.B., Lindstrom, E.B., 1994. Characterization of *Thiobacillus caldus* sp. nov., a moderately thermophilic acidophile. Microbiol. 140, 3451-3456.

Imhoff, J.F., Sahling, H., Suling, J., Kath, T., 2003. 16S rDNA-based phylogeny of sulphur-oxidising bacterial endosymbionts in marine bivalves from cold-seep habitats. Marine Ecology Progress Series. 249, 39-51.

Janssen, P.H., 2006. Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes. Appl. Environ. Biol. 72(3), 1719-1728.

Johnson, D.B., 1995. Mineral cycling by microorganisms: iron bacteria. In: Microbial diversity and ecosystem function. (Eds.: Allsopp, D., Colwell, R.R., Hawksworth, D.L.). CAB International, University Press, Cambridge, UK. pp.137-159.

Juwarkar, A. A., Jambulkar, H.P., Singh, S.K., 2004. Appropriate strategies for reclamation and revegetation of coal mine spoil dumps. In: Proceedings of National Seminar on Environmental Engineering with Special Emphasis on Mining Environment. pp.1-9.

Kang, Y., Liu, H., Genin, S., Schell, M.A., Denny, T.P., 2002. *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. Mol. Microbiol. 2, 427-437.

Kent, A.D., Triplett, E.W., 2002. Microbial communities and their interactions in soil and rhizosphere ecosystems. Ann. Rev. Microbiol. 56, 211-236.

Kersters, K., De, V.P., Gillis, M., Swings, J., Vandamme, P., Stackebrandt, E., 2006. Introduction to the Proteobacteria. The Prokaryotes, 3rd Edn. 5, 3-37.

Lal R., 1997. Soil quality and sustainability. In: Methods of Assessment of Soil Degradation (Eds.: Lal, R., Blum, W.E.H., Valentin, C., Stewart, B.A.). CRC Press, Boca Raton, FL. pp.17-30.

Madigan, M.T., Martinko, J.M., 2006. Brock Biology of Microorganisms. 11th Edn. Pearson Education, Upper Saddle River, New Jersey.

Mahdy, A.M.M., El-Habbaa, G.M., Mohamed, F.G., Badr, A.A., 2012. Virulence of *Ralstonia solanacearum* the causal of potato brown rot disease under Egyptian conditions. Ann. Agric. Sci. (Moshtohor). 50(1), 59-67.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A. et al. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. 13, 614-629.

Martin, C., French, E.R., 1978. Some characteristics of isolates of *Pseudomonas solanacearum* affecting potatoes and other Solanaceae in the amazan basin. Abstract Proceedings of American Phytopathology. pp.138-139.

Mendo, S., Faustino, N.A., Sarmento, A.C., Amado, F., Moir, A.J., 2004. Purification and characterization of a new peptide antibiotic produced by a thermotolerant *Bacillus licheniformis* strain. Biotechnol. 26, 115-119.

Mohammad, M., Dar, A., Soomro, M.T., Tariq, M., Latif, M. 2009. Antioxidants/antioxidative agents and superoxide: An electrochemical monitoring device. Int. J. Gene. Mol. Biol. 1, 105-114.

Natarajan, K. A., 2008. Microbial aspects of acid mine drainage and its bioremediation. Trans. Nonferrous Metals Soc. China. 18, 1352-1360.

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Parkinson, D., Gray, T.R.G., Williams, S.T., 1971. Methods to study ecology of soil microorganisms. IBP Handbook No. 19, Blackwell Scientific Publ. Oxford. 116p.

Pastrik, K.H., Elphinstone, J.G., Pukall, R., 2002. Sequence analysis and detection of *R. solanacearum* by multiplex PCR amplification of 16s -23s ribosomal intergenic spacer regions with internal positive control. Eur. J. Plant Pathol. 108, 831-842.

Saitou, N., Nei, M., 1987. The neighbor joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406-425.

Smith L. J., Papendick, R. I., 1993. Soil organic matter dynamics and crop residue management. In: Soil Microbial Ecology (Ed.: Metting, B.). Marcel Dekker, New York. pp. 65–94.

Smith, E.F., 1896. A bacterial disease of tomato, pepper, eggplant and Irish potato (*Bacillus solanacearum* nov. sp.). United States Department of Agriculture: Division of Vegetable Physiology and Pathology, Bulletin. 12, 1-28.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725-2729.

Teuben, A., Roelofsma, T.A.P.J., 1990. Dynamic interactions between functional groups of soil arthropods and microorganisms during decomposition of coniferous litter in microcosm experiments. Biol. Fertil. Soils. 9, 145-151.

Walczak, M., donderski, W., 2004. Antibiotic sensitivity of neustonic bacteria in lake Jeziorak Mały. Pol. J. Environ. Stud. 13(4), 429-434.

Weller, D.M., 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria, Annu. Rev. Phytopathol. 26, 379-407.

Woese, C., 1987. Bacterial evolution. Microbiol. Rev. 51, 221-71.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H., Nishiuchi, Y., 1995. Transfer of two *Burkholderia* and on *Alcaligenes* species to *Ralstonia* gen. nov. Proposal of *R, picketii* (Ralston, Palleroni and Doudoroff, 1973) Comb. nov. *R. solanacearum* (Smith, 1896) and *R. eutropha* (Davis, 1969) Comb. nov. Microbiol. Immunol. 39(11), 897-904.

Youssef, N.H., Elshahed, M.S., 2009. Diversity rankings among bacterial lineages in soil. ISME J. 3, 305-313.

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