The Open Access Journal of Science and Technology
Vol. 2 (2014), Article ID 101072, 7 pages
doi:10.11131/2014/101072

AgiAl Publishing House
http://www.agialpress.com/

Research Article

Bioinformatic Identification of Aldo-Keto Reductase from Newly Isolated *Arthrobacter Nicotianae* Strain PR and Its Phylogenetic Analysis among Soil Bacteria

Williard Mazhawidza, Yankuba Banda, and Narayanan Rajendran

Molecular Microbiology, Division of Mathematics and Sciences, Kentucky State University, Frankfort, KY-40601, USA

Corresponding Author: Narayanan Rajendran; email: narayanan.rajendran@kysu.edu

Received 28 December 2013; Accepted 26 June 2014

Academic Editor: Andrea Masotti

Abstract. Soil bacteria display their self-defense as an essential disposition to withstand unfavorable rhizobial conditions. They employ those mechanisms through catabolic and/or anabolic metabolisms such as detoxification of xenobiotis or biosynthesis of nutrients *de novo*. In this study, newly isolated soil bacterium *Arthrobacter nicotianae* strain PR was identified, characterized and bioinformatically examined to reveal its synthetase gene. The bacterial genomic DNA was probed in PCR with degenerate synthetase primers. The amplified PCR product was cloned and sequenced. The gene and protein sequence analysis was made using MEGA 4 software and BLASTX search of NCBI-linked protein databases. The conserved amino acid residues revealed the match to aldo-keto reductase (AKR). The multiple sequence-data alignments analysis confirmed the AKR, and multiple phylogeneitic data analysis confirmed its relationship with other bacteria. This enzyme, AKR, belongs to a growing oxidoreductase superfamily and metabolizes a wide range of substrates, including aliphatic aldehydes, monosaccharides, steroids, prostaglandins, and xenobiotics. Our phylogenetic study reveals that this protein is potentially vital in assisting bacteria withstand unfavorable soil environmental condition. Since AKR found in many bacteria and eukaryotes like mammals, amphibians etc, the phylogenetic relationship between our soil isolate and other bacteria was significant as it revealed a distant relationship with *A. aurescens*.

Keywords: Aldo-keto reductase, polymerase chain reaction, *arthrobacter nicotianae,* phylogenetics

1. Introduction

Soil bacteria play a major role in transforming inorganic constituents from one chemical form to another [1]. They are essential to the management and conservation of the soil. Their relationship with its niche also accords them the capability to defend themselves metabolically [2] in a hostile condition. Both prokaryotes, especially the soil bacteria, and a range of eukaryotes carry such metabolic enzyme Aldo-Keto Reductase genes commonly called AKR. Such defensive enzymatic mechanisms include (i) reductive metabolism of biogenic and xenobiotic materials to primary and secondary alcohols, as well as (ii) alternative biosynthetic metabolism for essential nutrient absorption [3].

Aldo-keto reductase (AKR) has numerous functions including the reduction of aldehydes and ketones, mediates such reductive process [4]. AKR belongs to a large superfamily of enzymes, which include xylose reductase and 2,5-diketo-D-gluconic acid reductase, and beta-keto ester reductase for prokaryotes [5]. Their basic structure is a beta-alpha-beta fold common nucleotide binding proteins with parallel beta-8/alpha-8-barrel that harbors a NADP binding motif [6]. In the present study, we made bioinformatic identification of aldo-keto reductase (AKR) in a newly isolated soil bacterium *Arthrobacter nicotianae* strain PR, after multiple PCR probing. The amplified PCR product, using degenerated synthetase primers, was cloned, sequenced and examined for phylogenetic relationship between our soil isolate with other bacteria in respect to AKR.

2. Materials and Methods

2.1. Isolation of soil bacteria. A modified technique was adopted based on the isolation approaches carried out by [7, 8]. One gram of sewage sludge soil from Versailles (KY, USA) wastewater treatment plant was added to 50 ml sterile PAF medium in a 300 ml flask. The PAF Medium contains (per liter) Peptone 10 g, Casein Hydrolysate 10 g, Anhydrous $MgSo_4$ 1.5 g, K_2HPO_4 1.5 g and Glycerol 10 ml. The flask was incubated with constant shaking at 220 rpm at 30ºC. After 24 hrs, 1 ml (0.5%) aliquot was removed from the growing culture and transferred into 50 ml of sterile PAF medium in a 300 ml flask. The flasks were incubated with constant shaking at 30ºC for another 24 hrs to avoid profound fungal growth. After 24 hours incubation, 1 ml (0.5%) aliquot was removed from the growing culture and transferred to 50 ml of sterile Nutrient Broth medium with 2 g/l NH_4Cl (as a N source) in a 300 ml flask. The flasks were incubated with constant shaking at 30ºC for 24 hrs. After 24 hrs, 1 ml (0.5%) aliquot was removed and serially diluted to obtain single bacterial colonies. This medium and the serial incubation procedure with 0.5% aliquot together reduce the number of unwanted growth of other bacterial and fungal colonies. Dilutions of the final bacterial culture were spread-plated on Nutrient agar plates using L-rod technique, and kept 30°C incubator for 48 hrs along with control plates.

2.2. Bacterial identification techniques. In order to confirm the identity of the isolated soil bacteria from a battery of samples, 16sRNA techniques and high resolution automated microbial identification techniques were used by a third party (Accugenix Inc. USA). Their culture collection with accession data on various bacterial species, the reference data bases for rRNA gene sequences, hybridization probes and computer-based molecular profiles gave a focal point for accurate identification and confirmed the species of the soil isolate.

2.3. DNA isolation and PCR method. The soil bacterial isolate was cultured at 30°C and the genomic DNA was isolated using DNA Bactozol kit (Molecular Research Center, OH, USA). In order to amplify the DNA, we used our conserved peptide synthetase primers and our standardized PCR reaction procedure as previously published [9]. The primers were synthesized at Integrated DNA technologies (Coralville, IA, USA). The PCR mixture was made with genomic DNA Premix Taq (Takara, cat #RR003) and the PCR reaction condition was set at 95ºC (5 min), 95ºC (1 min), 55ºC (1 min), and 72ºC (3 min) for 30 cycles.

2.4. Subcloning, DNA sequence and phylogenetic analysis. Following the manufacturer's protocol (Invitrogen, USA), amplified DNA was cloned using TA vector. Positive clones were screened using IPTG/X-Gal (Fermentas, USA) with Ampicillin (Sigma, USA). The positive colonies were then grown in LB broth culture together with Ampicillin. The plasmid isolation was carried out using Yield Plasmid Miniprep System (Promega, USA). The clones were later sequenced with T3 and T7 primers at the Center for Genetics and Molecular Medicine (CGeMM) DNA Core facility, University of Louisville (Kentucky, USA). The results were analyzed with NCBI-BLAST [10, 11] and MEGA4 software [12]. Homologous amino acid sequences were blasted against the NCBI databases (http://www.ncbi.nlm.nih.gov/BLAST/), and BOXSADE software was used to convert sequence alignment FASTA files to Microsoft Word files.

3. Results

Bacterial identification is difficult task due to lack of adequate taxonomic framework, requirement of pure cultivable cultures and need of proper identification systems. Hence both traditional and molecular techniques were employed to identify the species. First, the identity of our newly isolated soil bacteria from a battery of samples was initiated with Bergey's manual of determinative bacteriology [13] after the partial characterization of bacterial colonies. The molecular techniques such as 16sRNA and high resolution automated microbial identification were utilized.

Our partial cellular characterization of the soil isolates reveals that this newly isolated soil bacterium has pleomorphic growth pattern. They are coryneform, non-sporulating, Gram positive, obligate aerobes and exhibit a weak motility. Based on (i) multiple culture data (with accession data on various bacterial species), (ii) the reference data (bases for rRNA gene sequences), (iii) hybridization probing data, and (iv) computer-based molecular profiles, a focal point for accurate identification was made to confirm *Arthrobacter nicotiana*e.

In order to probe for potential synthetase genes of our new soil isolate, named *Arthrobacter nicotianae* strain PR, we employed our previously published conserved peptide synthetase primers, using PCR. Amplified PCR products

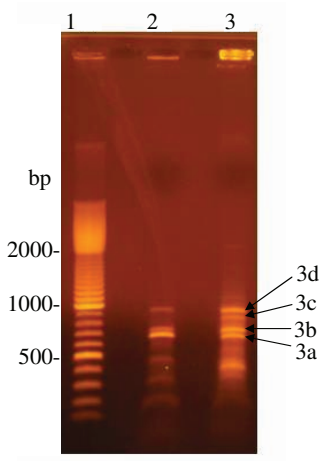

Figure 1: Electrophoretic resolution of PCR products. A8 and E2 primers were employed against the genomic DNA of newly isolated *A. nicotianae* strain PR with control DNA from bacterium *Exuguobacterium acetylicum* strain SN under conditions described in materials and methods. *Lane* 1 is DNA step ladder 100bp, *lane* 2: *Exiguobacterium acetylicum* strain SN and *lane* 3: *Arthrobacter nicotianae* strain PR. Shown are amplicons ranging from 700 to 1000 base pairs in length. The amplified DNA of *A. nicotianae* was cloned in TA vectors (Invitrogen) and sequenced. Significant matches of amplicon B were found in the NCBI-BLAST searches which reveal its identity as *Aldo-keto reductase* (AKR).

from the genomic DNA of *A. nicotianae* were revealed potential amplicons with length, ranging from 700 to 1000 base pairs (bp), as determined by agarose gel electrophoresis (Figure 1). The amplified DNA of *A. nicotianae,* as cloned in TA vectors (Invitrogen), yielded a well-readable sequence. Through a NCBI-BLAST search, a significant match from one of PCR products, amplicon B, was found to be aldo-keto reductase (AKR). Since several proteins had been identified and characterized as AKRs, and an additional number of genes were reported as encoding proteins related to this superfamily [4], the matching results with excising sequences were repeated for confirmation.

In order to ascertain the identity of the newly cloned aldo-keto reductase DNA matching results of BLASTX search were included in a multiple sequence alignment using CLUSTALW in MEGA 4 software (Figure 2). In comparison, the sequences exhibited conserved residues as well as amino acids homologous to the majority of the sequences. CLUSTALW also revealed the following conserved positions: G-16, LG-21 to 22, P-25, GxSExRIG-66 to 73, GxP-81 to 83, and TKxD-91 to 94. Other amino acids common to majority of the sequences include: LGLD-117 to 120 and LHDPE-127 to 131. We performed a phylogenic analysis of the matching bacteria in regards to AKR with MEGA 4 software. Contrary to our assumption, *A. nicotianae* strain PR did not cluster together with *A. aurescens* (Figure 3). In fact, it showed a phylogenetic distance from most the bacteria that were compared.

4. Discussion

Soil inhabiting bacteria need survival mechanisms to continue to exist in extreme conditions [1]. It includes synthesizing metabolic enzymes to detoxify threatening toxins as well as implementing metabolic pathways to enable the bacteria to biosynthesis important nutrients [14]. The enzyme aldo-keto reductase (AKRs) belongs to superfamily of approximately 120 enzymes, currently composed of 15 families [4]. Proteins of the AKR superfamily are monomeric (odl3)s-barrel proteins, about 320 amino acids in length, which bind NAD(P)(H) without a Rossmann-fold motif [15]. The AKR enzymes metabolize a range of substrates including aliphatic aldehydes, monosaccharides, steroids, prostaglandins, polycyclic aromatic hydrocarbons, isoflavinoid phytoalexins and related xenobiotics.

The AKRs represent NAD(P)(H)-dependent oxidoreductases [16] and found in a wide range of microorganisms, plants and animals. Earlier reports indicate that several microorganisms such as *Pseudomonas spp*., *Trichoderma viridae*, *Aspergillus oryzae* etc can degrade or involved in the detoxification of carbonyl group-containing xenobiotics and can metabolize many endogenous intermediate products, such as aldehydes, ketones, exogenous compounds like plant phytoallexins, toxins, and anthropogenic chemicals in the environment [3]. The role of AKR has been noticed earlier in bacteria like *Bacillus subtilis* and *Escherichia coli.* In these bacteria, AKR is known to reduce aldehydes and aldose substrates to alcohol [11, 12]. The enzyme exhibits a broad spectrum of activity with substrates such as glyoxal, dihydroxyacetone and DL-glyceeraldehyde, revealing the detoxification role in bacteria [12]. AKR-11C1 detoxifies 4-hydroxy-2, 3-trans-nonenal (HNE), a lipid preoxidation product in *Bacillis halonduran* [17].

Although many studies have been carried out on the degradation pathways in other bacteria, there is a lack of information on the phylogenetic relationship of AKR in position of *A. nicotianae*. In this study we revealed the presence of *aldo-keto reductase* (AKR) in *A. nicotianae* strain PR, and carried out the phylogenetic analysis. With the assistance of degenerate primers, we cloned a partial sequence of AKR, an enzyme responsible for the survival of the bacteria in harsh conditions. The presence of AKR in environmentally significant bacteria such as *Roseiflexus castenholzii*, similar to that in *A. nicotianae* strain PR, suggests a common metabolism played by the enzyme in both bacteria. It is noteworthy to mention that *R. castenholzii* is a thermophilic and photosynthetic bacterium that relies on lipid metabolism [18–20]. In general, lipid metabolisms in bacteria invariably produce toxic products, and the detoxification process becomes a critical metabolic need for the survival of the bacteria.

The anaerobic Gram positive bacterium, *Thermobaculum terrenum*, isolated from the Yellowstone National Park, has AKA and is closely related to several environmentally

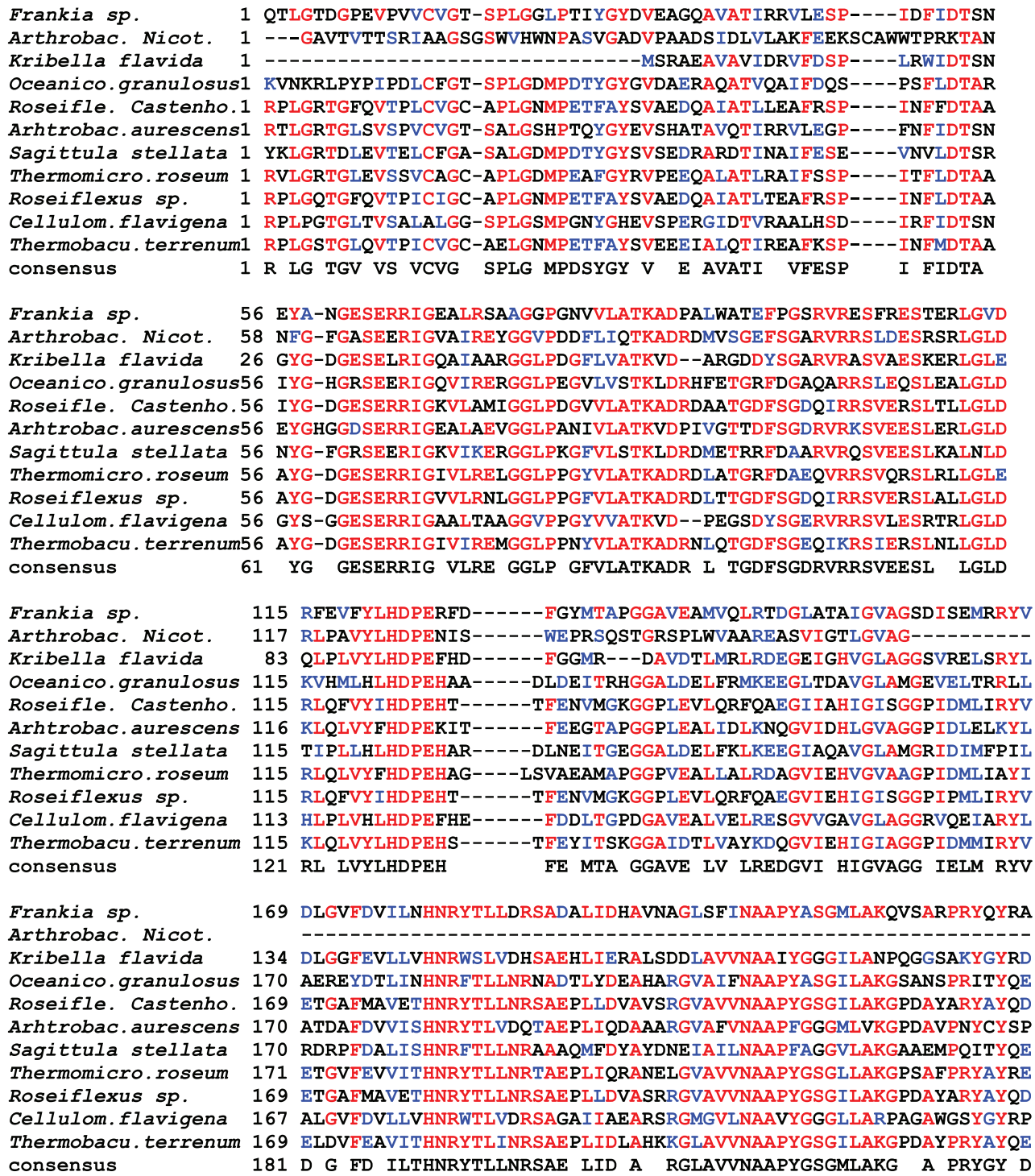

Figure 2: Multiple sequence alignment of Aldo-keto reductase reveals consensus amino acid residues. The homologous matches obtained from BLASTX search were aligned using CLUSTALW in MEGA 4 software. Shown are only 12 of many bacteria strains revealed by the NCBI blast search of microbe protein databases. Revealed conserved amino acids are indicated in the last line of the alignment block.

significant bacteria [21]. Since *A. nicotianae* strain PR is also a soil isolate, its phylogenetic similarity to *T. terrenum*, with respect to AKR may have been driven them in the same path of evolutionary development due to similar environmental conditions. Earlier, analyses of the rRNA genes have led the change in phyla of *T. roseum* from *Thermomicrobium* to *Chloroflexi*, a thermophilic phylum of bacteria [29]. It was perceived that glycosylation plays a critical role in

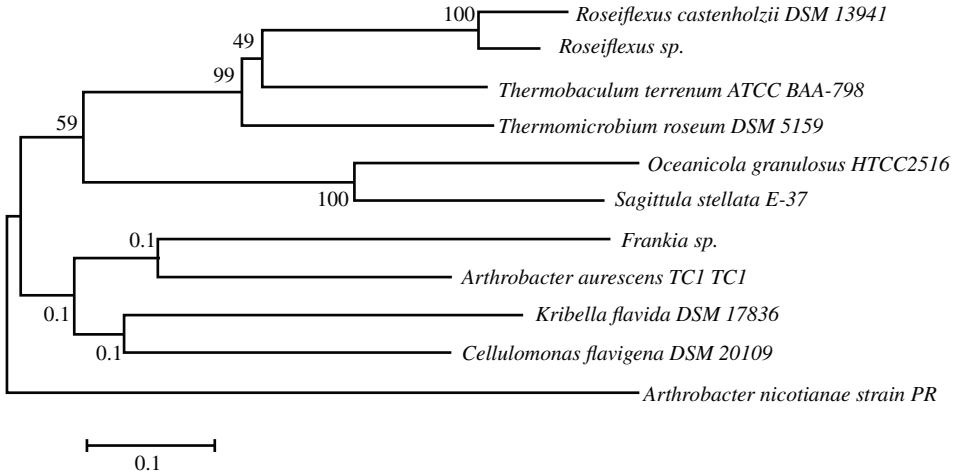

Figure 3: Aldo-keto reductase (AKR) phylogenetic tree constructed with matching sequences. The tree is unrooted and each node and length of arm represents sequence divergence estimated by the bootstrap values. Revealed by the comparison is the divergence of *A. nicotianae* strain PR from related bacteria.

the metabolism of the bacteria in order to detoxify the products of AGE (advanced glycosylation end) as reported by [22]. The matching of *A. nicotianae* strain PR to *T. rose* in glycosylation process may suggest the phylogenetic similarity of both soil isolates in detoxification pathways. It indicates that the presence of detoxification pathways in different groups of soil bacteria has functional similarity with environmental significance.

As reported earlier, marine bacteria like *Oceanicola granulosus* [22] and *Sagittula stellata* [23] require an extraordinary metabolic process in order to survive in harsh and salty conditions. Interestingly, these marine bacteria also have AKR. The presence of AKR in *A. nicotianae* strain PR suggests that this strain may have been exposed to harsh environment similar to that of marine conditions during its early phylogenetic diversification period. In some animals, the expression of AKR is induced by an exposure to aflatoxin [24]. Even though the metabolic triggering of superfamily of enzymes like AKR in those higher eukaryotes has the capability to detoxify such aflatoxin B-1 dialdehyde, its parallel metabolic process in prokaryotes are yet to be identified. However, whether the phylogenetic advancement towards the development of the AKR in eukaryotes is understandable, similar triggering metabolic process in prokaryotes such as *A. nicotianae* as influenced by the exposure to toxins such as aflatoxin, is a question that needs to be further investigated.

The genus *Frankia* is involved in nitrogen fixation in plants [25] and has a global distribution [26]. The habitation patterns of *Frankia spp.* such as the exposure to toxins in the soil and their ability to synthesize AKR enzymes to tackle the toxic is comparable with *A. nicotianae*. Similarly, the ability of *Cellulomonas* spp. to grow in temperatures ranging from 10^oC to 50^oC [27], indicates that the bacterium has a versatile metabolism to survive. This study reveals that *Cellulomonas* and *Kribella* are closer to *Arthrobacter aurescens*, which is a close relative of *Arhtrobacter nicotianae*. However, the phylogenetic analysis in relation to AKR revealed that *A. aurescens* is relatively distant from *A. nicotianae* strain PR, suggesting a difference in an environmental dynamic specificity. Our earlier study [28] revealed that environmental sustainability of soil bacteria, with potentially similar evolutionary development, was determined by the environmental factors including the need to neutralize intra cellular and/or extracellular toxins and synthesize essential carbon-based molecules. It could be a result of their adaptive mechanism for survival in an environment that requires the synthesis of protein/peptide molecules to detoxify the environmental toxins to release essential nutrients for their benefit.

5. Conclusion

In this study we demonstrated that the newly isolated bacterium *Arthrobacter nicotianae* strain PR harbors AKR, an important metabolic enzyme. AKR was found in a wide range of microorganisms, plants and animals and it can play a leading role to degrade carbonyl group-containing xenobiotics. Based on its phylogenetic relationship with other bacterial species, we can suggest that *A. nicotianae* strain PR has evolved with a detoxifying pathway in order to make this soil isolate an environmentally suitable and sustainable species in harsh and hostile soil conditions. The phylogenetic analysis showed that our *A. nicotianae* strain PR maintains a distant from those within the same genus. Hence the position and phylogenetic identification of the enzyme present in *A. nicotianae* strain PR has a significant bearing on its relationship with other soil bacteria. Revealing phylogenetic relationship of AKR in *A. nicotianae* strain PR as presented here with other soil bacteria has significance, because AKR belongs to a superfamily of detoxifying enzymes, it can play a major role in detoxification of toxic chemicals.

Acknowledgements

This study was part of a grant from the National Science Foundation, USA (NSF Grant # MCB-0639356) to Narayanan Rajendran.

References

[1] Narayanan Rajendran, The State of the Rhizoinhabitants in bridging the gap between plant productivity and persuasiveness during remediation, *Bacteria in Agronomy: Crop Productivity*, Springer, Germany, 463–496, (2013).

[2] Saraf Meenu, N. Tank, and N. Rajendran, Rhizomediation in Soil for Sustainable Agriculture, , **19**, I. K. International Publishing House Private Ltd, New Delhi, (India), 2008.

[3] H. Jiang, C. Yang, H. Qu, Z. Liu, Q. S. Fu, and C. Qiao, Cloning of a novel Aldo-Keto reductase gene from Klebsiella sp. strain F51-1-2 and its functional expression in Escherichia coli, *Applied and Environmental Microbiology*, **73**, no. 15, 4959–4965, (2007).

[4] J. M. Jez, T. G. Flynn, and T. M. Penning, A new nomenclature for the aldo-keto reductase superfamily, *Biochemical Pharmacology*, **54**, no. 6, 639–647, (1997).

[5] E. M. Ellis, Microbial aldo-keto reductases, *FEMS Microbiology Letters*, **216**, no. 2, 123–131, (2002).

[6] C. Binda, R. T. Bossi, S. Wakatsuki, S. Arzt, A. Coda, B. Curti, M. A. Vanoni, and A. Mattevi, Cross-talk and ammonia channeling between active centers in the unexpected domain arrangement of glutamate synthase, *Structure*, **8**, no. 12, 1299–1308, (2000).

[7] B. R. Glick, D. M. Karaturovic, and P. C. Newell, A novel procedure for rapid isolation of plant growth promoting pseudomonads, *Canadian Journal of Microbiology*, **41**, no. 6, 533–536, (1995).

[8] N. Rajendran, A. Rompf, M. A. Marahiel, and D. Jahn, Isolation and characterization of a transposon mutant of Pseudomonas fluorescens AU63 deficient in antifungal activity against Pythium ultimum, *Letters in Applied Microbiology*, **27**, no. 3, 152–157, (1998).

[9] N. Rajendran, R. V. Rajnarayanan, and D. R. Demuth, Molecular phylogenetic analysis of tryptophanyl-tRNA synthetase of Actinobacillus actinomycetemcomitans, *Zeitschrift fur Naturforschung - Section C Journal of Biosciences*, **63**, no. 5-6, 418–428, (2008).

[10] S. F. Altschul, T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman, Gapped BLAST and PSI-BLAST: A new generation of protein database search programs, *Nucleic Acids Research*, **25**, no. 17, 3389–3402, (1997).

[11] I. Korf, M. Yandell, and J. Bedell, BLAST, O'Reilly & Associates, Inc., Sebastopol, California (USA), 2003.

[12] J. Lei, Y. Zhou, L. Li, and X. Su, Structural and biochemical analyses of YvgN and YtbE from Bacillus subtilis, *Protein Science*, **18**, no. 8, 1792–1800, (2009).

[13] J. G. Holt, N. R. Krieg, P. H. Sneath, J. T. Staley, and S. T. Williams, Bergey's manual of determinative bacteriology, Williams and Wilkins, Baltimore, MD, 9th edition, 1994.

[14] N. Rajendran, T. Stachelhaus, and M. A. Marahiel, Multifunctional peptide synthetase required for non-ribosomal peptide synthesis, , **4**, Elsevier Science Ltd., Oxford, England, 1999.

[15] J.-M. Rondeau, F. Tete-Favier, A. Podjarny, J.-M. Reymann, P. Barth, J.-F. Biellmann, and D. Moras, Novel NADPH-binding domain revealed by the crystal structure of aldose reductase, *Nature*, **355**, no. 6359, 469–472, (1992).

[16] N. C. Bruce, D. L. Willey, A. F. W. Coulson, and J. Jeffery, Bacterial morphine dehydrogenase further defines a distinct superfamily of oxidoreductases with diverse functional activities, *Biochemical Journal*, **299**, no. 3, 805–811, (1994).

[17] T. Marquardt, D. Kostrewa, R. Balakrishnan, A. Gasperina, C. Kambach, A. Podjarny, F. K. Winkler, G. K. Balendiran, and X. Li, High-resolution crystal structure of AKR11C1 from Bacillus halodurans: An NADPH-dependent 4-hydroxy-2,3-trans-nonenal reductase, *Journal of Molecular Biology*, **354**, no. 2, 304–316, (2005).

[18] S. Hanada, S. Takaichi, K. Matsuura, and K. Nakamura, Roseiflexus castenholzii gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes, *International Journal of Systematic and Evolutionary Microbiology*, **52**, no. 1, 187–193, (2002).

[19] M. T. Van der Meer, S. Schouten, S. Hanada, E. C. Hopmans, J. S. Damsté, and D. M. Ward, Alkane-1,2-diol-based glycosides and fatty glycosides and wax esters in Roseiflexus castenholzii and hot spring microbial mats, *Archives of Microbiology*, **178**, no. 3, 229–237, (2002).

[20] M. T. J. Van Der Meer, S. Schouten, M. M. Bateson, U. Nübel, A. Wieland, M. Kühl, J. W. De Leeuw, J. S. S. Damsté, and D. M. Ward, Diel variations in carbon metabolism by green nonsulfur-like bacteria in alkaline siliceous hot spring microbial mats from Yellowstone National Park, *Applied and Environmental Microbiology*, **71**, no. 7, 3978–3986, (2005).

[21] L. M. Botero, K. B. Brown, S. Brumefield, M. Burr, R. W. Castenholz, M. Young, and T. R. McDermott, Thermobaculum terrenum gen. nov., sp. nov.: A non-phototrophic gram-positive thermophile representing an environmental clone group related to the Chloroflexi (green non-sulfur bacteria) and Thermomicrobia, *Archives of Microbiology*, **181**, no. 4, 269–277, (2004).

[22] D. Wu, J. Raymond, M. Wu, S. Chatterji, Q. Ren, J. E. Graham, D. A. Bryant, F. Robb, A. Colman, L. J. Tallon, J. H. Badger, R. Madupu, N. L. Ward, and J. A. Eisen, Complete genome sequence of the aerobic CO-oxidizing thermophile Thermomicrobium roseum, *PLoS ONE*, **4**, no. 1, p. e4207, (2009).

[23] J. M. Gonzalez, F. Mayer, M. A. Moran, R. E. Hodson, and W. B. Whitman, Sagittula stellata gen. nov., sp. nov., a lignin-transforming bacterium from a coastal environment, *International Journal of Systematic Bacteriology*, **47**, no. 3, 773–780, (1997).

[24] D. N. Johnson, P. A. Egner, G. OBrian, N. Glassbrook, B. D. Roebuck, T. R. Sutter, G. A. Payne, T. W. Kensler, and J. D. Groopman, Quantification of urinary aflatoxin B1 dialdehyde metabolites formed by aflatoxin aldehyde reductase using isotope dilution tandem mass spectrometry, *Chemical Research in Toxicology*, **21**, no. 3, 752–760, (2008).

[25] B. S. Mirza, A. Welsh, G. Rasul, J. P. Rieder, M. W. Paschke, and D. Hahn, Variation in Frankia populations of the Elaeagnus host infection group in nodules of six host plant species after inoculation with soil, *Microbial Ecology*, **58**, no. 2, 384–393, (2009).

[26] A. Welsh, B. S. Mirza, J. P. Rieder, M. W. Paschke, and D. Hahn, Diversity of frankiae in root nodules of Morella pensylvanica grown in soils from five continents, *Systematic and Applied Microbiology*, **32**, no. 3, 201–210, (2009).

[27] B. M. Beadle, W. A. Baase, D. B. Wilson, N. R. Gilkes, and B. K. Shoichet, Comparing the thermodynamic stabilities of a related thermophilic and mesophilic enzyme, *Biochemistry*, **38**, no. 8, 2570–2576, (1999).

[28] W. Mazhawidza, M. Somuah, and N. Rajendran, Molecular probing and phylogenetic analysis of glutamate synthase of arthrobacter nicotianae, *Internet Journal of Microbiology*, **7**, no. 2, (2010).

[29] J. Cho and S. J. Giovannoni, Oceanicola granulosus gen. nov., sp. nov. and Oceanicola batsensis sp. nov., poly-β-hydroxybutyrate-producing marine bacteria in the order 'Rhodobacterales', *International Journal of Systematic and Evolutionary Microbiology*, **54**, no. 4, 1129–1136, (2004).

[30] J. Ko, I. Kim, S. Yoo, B. Min, K. Kim, and C. Park, Conversion of methylglyoxal to acetol by Escherichia coli aldo-keto reductases, *Journal of Bacteriology*, **187**, no. 16, 5782–5789, (2005).

[31] S. Kumar, K. Tamura, and M. Nei, MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment, *Briefings in bioinformatics*, **5**, no. 2, 150–163, (2004).

[32] B. A. Neilan, E. Dittmann, L. Rouhiainen, R. A. Bass, V. Schaub, K. Sivonen, and T. Börner, Nonribosomal peptide synthesis and toxigenicity of cyanobacteria, *Journal of Bacteriology*, **181**, no. 13, 4089–4097, (1999).

[33] S. Z. Schade, S. L. Early, T. R. Williams, F. J. Kezdy, R. L. Heinrikson, C. E. Grimshaw, and C. C. Doughty, Sequence analysis of bovine lens aldose reductase, *Journal of Biological Chemistry*, **265**, no. 7, 3628–3635, (1990).