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RESEARCH ARTICLE OPEN ACCESS

Arthrobacter Strains from Industrial Polluted Soil and its Oxidative Potential of Choline Oxidase Gene

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Abstract

Betaine is a trimethylglycine, serves as osmoregulator to prevent dehydration and plasmolysis under adverse hyperosmotic environments. Choline oxidase gene from *Arthrobacter* sp. catalyzes two step oxidation reaction of choline to betaine followed by betaine accumulation in cells which in turn help them to survive and thrive in harsh environmental condition. To identify potential choline oxidase gene source, a gram stain positive, rod shaped, catalase and oxidase positive, motile, aerobic bacterial strains designated as *HYJE003* and *HYJE005* was isolated based on the colony morphology, biochemical and molecular characterization from the industrially polluted soil samples of Hyderabad, India. Optimum growth of the isolated strains was observed at 32°C on nutrient agar media and was found that both the strains were capable of utilizing variety of sugars as carbon source. The 16S rRNA gene sequence analysis revealed that strain *HYJE003* was closely related to *Arthrobacter globiformis* with pairwise sequence similarities of 99.85%, 99.63%, 98.76% and 98.12% respectively. The strain *HYJE005* was closely related to *Arthrobacter phenanthrenivorans* with pairwise sequence similarities of 99.93%, 99.47%, 99.25% and 98.11% respectively. Choline oxidase gene potential of the isolates was studied by feeding the cultures with choline chloride and converted betaine was assessed by the formation of betaine reineckate. Findings revealed that the isolated strain *Arthrobacter globiformis-HYJE003* has four times higher conversion rate of choline chloride to betaine than the strain *Arthrobacter phenanthrenivorans HYJE005*.

Keywords: *Arthrobacter*, BLAST, Pleomorphic, Reineckate, Betaine, Snapping division.

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INTRODUCTION

Soil bacteria are considered as economical source of useful biocatalysts in numerous industrial processes. *Arthrobacter* are, a typical soil bacterium widely distributed in diverse environmental conditions. This community gained special interest when compared to other soil microorganisms due to their ability to perform key metabolic functions such as: recycling of elements, recycling of wastes, detoxification of hazardous chemicals[1], including heavy metal and organic pollutants[2]. All *Arthrobacter* species are pleomorphic, since they are rod shaped during exponential growth phase and cocci during their stationary phase[3]. Choline oxidase of *Arthrobacter* family is the only enzyme that catalyzes the two-step oxidation reaction of choline to betaine[4]. Betaine acts as osmoprotectant and plays an instrumental role in the growth of *Arthrobacter* in very harsh environmental conditions. The enzyme choline oxidase has high importance because, betaine is one of the limited numbers of compatible solutes that can accumulate in cell cytoplasm at very high concentration in adverse hyperosmotic environments[5,6] to prevent dehydration and plasmolysis.

Betaine is an organic base present in fruits, vegetables, grains and seafood, thus betaine rich diet potentially favors human health. Betaine is a product well known to improve meat characteristics, weight gain, growth, carcass characteristics and feed conversion ratio in domestic animals[7]. Betaine allows a good water retention in meat and poultry, it increases breast meat yield, reduces abdominal fat and reduction of backfat thickness in swine[8,9]. As betaine is a methyl donor, it reduces the amount of methionine/cysteine from deamination and allows higher protein synthesis[9]. It modulates the histone and DNA methylation for epigenetic gene expression[10]. Various studies have demonstrated that betaine play critical role in embryonic and fetal development[11]. Considering several prospective and retrospective studies on betaine, in 2011 European Food Safety Authority has authorized a health claim of betaine on homocysteine metabolism.

Natural betaine is currently extracted and purified from beetroot, sugar beet and its derivatives[12]. Due to high demand, betaine is chemically produced in the form of betaine hydrochloride. Recent comparative studies between natural betaine and betaine hydrochloride clearly illustrates that the chemically synthesized betaine hydrochloride is not effective as the natural betaine[13]. In view from the above findings, the current study is aimed at isolation, identification and characterization of *Arthrobacter* strains from bacterial consortium of industrially polluted soil to evaluate their choline oxidase gene potential of choline to betaine oxidation.

MATERIALS AND METHODS

Strain isolation and purifications

Five soil samples were collected from Jeedimetla industrial area, a well-known heavy metal polluted site in Hyderabad, India[14]. Samples were aseptically sampled and grinded in lab by using a sterile mortar. From the homogenized soil samples, one gram each weighed and distributed in five respective sterile flasks with 99 ml of sterile saline solution (0.85% of NaCl). After incubation at 25°C for 15 minutes at 200rpm, each sample was diluted to tenfold and from fourth to fifth dilution series 0.1ml of inoculum was added to the surface of 1X, 0.5X, 0.25X and 0.1X strength SCDA (Soya bean Casein Digest Agar) culture medium and later incubated at 30°C for 2days. Based on the cultural condition, colony morphology and microscopic characteristics two *Arthrobacter* strains were isolated from the culture plate of 0.1X SCDA with 10^{-4} dilution plating. Further pure cultures were established with the isolates by two level streak plating on nutrient agar media.

Biochemical and molecular analysis

Isolated pure cultures were subjected to biochemical analysis such as catalase, oxidase, urease, nitrate reduction and indole test[15]. To evaluate the sugar utilization profile of the isolated *Arthrobacter* strains, Hi-Carbohydrate kit from Hi-Media was used as per manufacture protocol. Later mobility test, gram staining and endospore staining were performed[16]. The genomic DNA from the isolated strains was extracted from CATB DNA extraction method[17]. Spectrophotometer and agarose gel electrophoresis was used to quantify the extracted DNA quality[18]. By using 27F-AGAGTTTGATCCTGGCTCAG and 1492R-GGTTACCTTGTTACGACTT[18,19] primers nearly full length 16S rRNA gene was amplified

from isolated strains. The PCR solution consist of 2X Go Taq Green mix 10µl, primers of 0.5µl each from 10µM stock, genomic DNA 1µl (10ng) and ddH2O was added to make up the final volume to 20µl. PCR condition of 94°C for 5 minutes and then 94°C for 20 seconds, 60°C for 30 second, 72°C for 1 minutes for 35 cycles followed by final extension of 72°C for 5 minutes. The amplified PCR products was verified by agarose gel electrophoresis, followed by PCR clean up sent for sequencing. Sequencing was performed using Applied Biosystems DNA sequencer, the 16S rRNA sequences were BLAST analyzed (National Centre for Biotechnology Information) to determine the closest available database match[20].

Choline to betaine conversion of isolates

Identified strains were further evaluated for their choline oxidase gene potential to convert betaine from choline chloride by feeding the cultures with 100 mM of choline chloride in the nutrient broth. Cells were harvested from the overnight grown culture, lysed by sonication and centrifuged at high speed of 18000 rpm to remove any cell debris. To the 10 ml of supernatant, 1 ml of 25% trisodium phosphate solution and 10 ml of ammonium reineckate (2%) reagent was added and incubated in ice bath for one hour. Centrifuged at 18000rpm, carefully supernatant was transferred into fresh 15ml centrifuge tube set aside in an ice bath for one hour after addition of 1ml hydrochloric acid (3N). Then centrifuged for twenty minutes at 18000rpm and the clear supernatant was discarded, betaine reineckate pellets were air dried and dissolved in 10ml of acetone water (75:25) mixture. Optical density of the solution was recorded at 525 nm in a spectrophotometer and the amount of the betaine conversion was calculated by a similarly treated betaine standard curve[21] (6.25, 12.5, 25, 50, and 100 mg/L).

The PCR was performed to isolate and confirm the choline oxidase gene from the isolated *Arthrobacter globiformis HYJE003* strain. The choline oxidase gene was amplified by using oligonucleotide primers of COX-F_ AATTGAATTCGATGCACATCGACAACATCGAG and COX-R_ AGCTAAGCTTAGGCGAGGGCCGCGCTCA. The PCR was carried with 5% DMSO, with the cycling condition of initial denaturation of one minute at 94°C, followed by 35 cycles of one of minute of cyclic denaturation at 94°C, 30 second at 50°C annealing, cyclic extension of 2 minute at 72°C and a final extension at 72°C for 10 minutes. 10 ng of template DNA, 2U of Pfu Taq DNA polymerase was used and the amplified product was agarose gel purified by QIAGEN kit following manufacture protocol. The amplified choline oxidase gene was then sequenced to confirm the gene sequence.

Table 1. Colony morphology of isolated strains

Strain Name	Colonial Morphology								
	Shape	Margins	Elevation	Colour	Consistency	Form	Opacity	Structure	Size in mm
HYJE 003	Circular	Smooth	Convex	white	Membranous	Spreading	Opaque	Filamentous	2 mm
HYJE 005	Circular	Smooth	Convex	white	Membranous	Spreading	Opaque	Filamentous	3 mm

Table 2. Biochemical result of the isolated strains

Test	Strain HYJE003	Strain HYJE005
Grams Satin	Positive	Positive
Crystal Blue Satin	No Spores	No Spores
Motility Test	Negative	Negative
Catalase Test	Positive	Positive
Oxidase Test	Positive	Negative
Indole Test	Positive	Positive
Nitrate Reduction Test	Positive	Positive
Urease Test	Positive	Positive
Gelatin Hydrolysis Test	Positive	Positive
Bile Esculin	Negative	Negative

RESULTS AND DISCUSSION

Form the 0.1X strength SCDA media plating with 10^{-4} dilution (Fig. 1 and Fig. 2) two

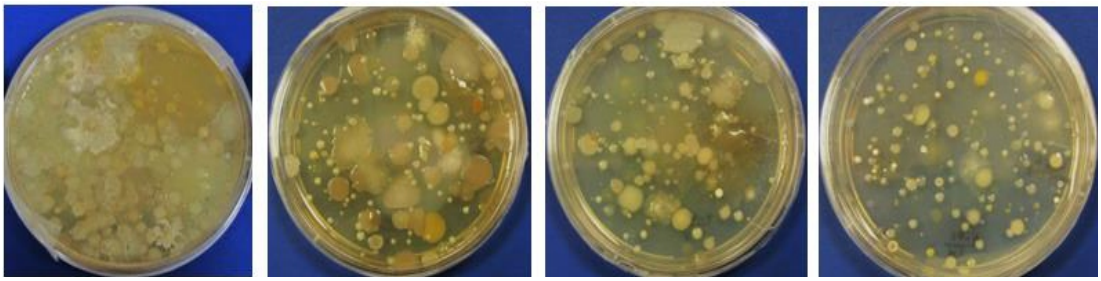

Fig. 1. Dilution plating result on SCDA media

Fig. 2. Pure colony of the isolated on nutrient agar media (1-Strain *HYJE003* and 2-Strain *HYJE005*)

strains (*HYJE003* and *HYJE005*) were isolated based on colony morphology and snapping cell division[3,22] (Fig. 3). *Arthrobacter* colonies on nutrient agar media have no distinctive pigmentation with undulate colony morphology appeared as smooth, circular with convex elevation, entire margin and nonsporulating (Tabel 1 and Fig. 2). In the early stage, cultures had rod shaped bacteria where as in older culture the bacteria were coccoid[23]. Microscopic observation reviled the size of the rod cells ranged between 0.5 to 0.8 by 1.5 to 2.0µ meter and the late stage coccoid cells were 0.5 to 0.8µ meter. The isolates showed growth in neutral to slightly alkaline pH and optimal growth was observed at 32°C temperature.

Both the isolated strains were recorded positive for grams staining, catalase test, indole test, urease test, nitrate reduction and gelatin hydrolysis assays (Table 2). On the other hand, both culture types produced negative result for crystal blue stain, motility test and bile esculin assays[4] (Table 2). But strain *HYJE003* produced

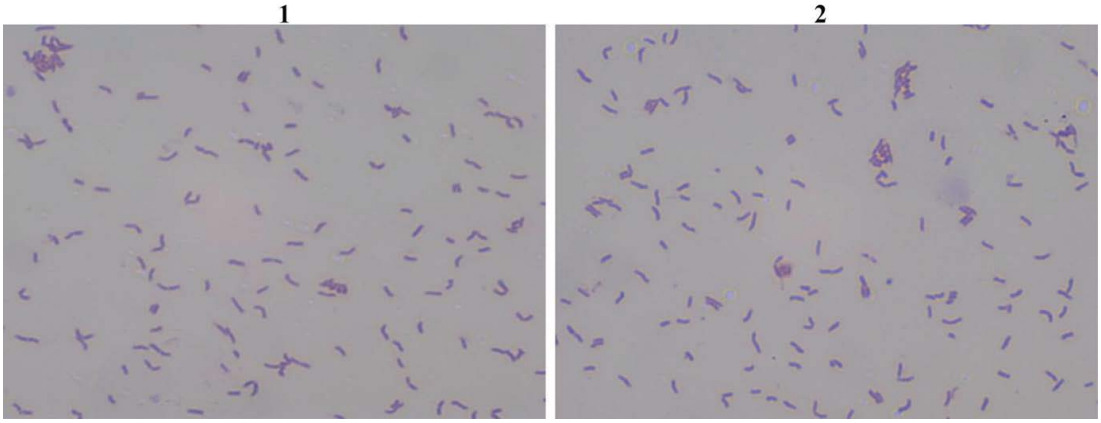

Fig. 3. Grams staining of the isolate to demonstrate snipping cell division (1-Strain *HYJE003* and 2-Strain *HYJE005*)

Table 3. Hi-Carbohydrate kit result for isolated strains

Name	Sugar Name	HYJE003	HYJE005
Part-A	Lactose	Positive	Negative
	Xylose	Positive	Positive
	Maltose	Positive	Positive
	Fructose	Positive	Positive
	Dextrose	Positive	Positive
	Galactose	Negative	Negative
	Raffinose	Negative	Positive
	Trehalose	Positive	Negative
	Sucrose	Positive	Positive
	Melibiose	Positive	Positive
	L-Arabinose	Positive	Negative
	Mannose	Positive	Positive
Part-B	Inulin	Positive	Negative
	Sodium gluconate	Positive	Positive
	Glycerol	Positive	Negative
	Salicin	Positive	Negative
	Dulcitol	Positive	Negative
	Inositol	Negative	Positive
	Sorbitol	Positive	Positive
	Mannitol	Positive	Positive
	Adonitol	Positive	Positive
	Arabitol	Positive	Positive
	Erythritol	Positive	Positive
	Alpha-Methyl-D-glucoside	Negative	Negative
Part-C	Rhamnose	Negative	Negative
	Cellobiose	Negative	Negative
	Melezitose	Positive	Positive
	Methyl-D-Mannoside	Positive	Positive
	Xylitol	Positive	Negative
	ONPG	Positive	Positive
	Esculin	Positive	Positive
	D-Arabinose	Positive	Negative
	Citrate	Positive	Positive
	Malonate	Positive	Positive
	Sorbose	Positive	Positive

positive result for oxidase test and on the other hand strain *HYJE005* produced negative result and the nitrate reduction test was also scored positive in the current study[4]. The sugar utilization result (Table 3, Fig. 4 and 5) reveals isolated strains were able to grow in a vast variety of sugar/carbohydrate source and this ability of *Arthrobacter* genus make them widely distributed and abundant in soils of harsh environmental conditions. Almost full length of 16S rRNA gene was amplified from the extracted DNA (Fig. 6 and 7) and read length of 1350 bp of high-quality sequences was obtained.

Fig. 4. Hi-Carbohydrate kit sugar utilization result for the isolated strain HYJE003.

Fig. 5. Hi-Carbohydrate kit sugar utilization result for the isolated strain HYJE005.

Table 4. Spectrophotometer reading for betaine estimation

No.	Sample ID	Mean O.D. Value	Standard Deviation	Standard Error
1	STD-1(100mg/L)	0.921	0.005508	0.00318
2	STD-2(50mg/L)	0.458	0.012055	0.00696
3	STD-3(25mg/L)	0.234	0.006083	0.003512
4	STD-4(12.5mg/L)	0.101	0.004933	0.002848
5	STD-5(6.25mg/L)	0.056	0.003215	0.001856
6	HYJE003	0.085	0.005568	0.003215
7	HYJE005	0.011	0.003055	0.001764

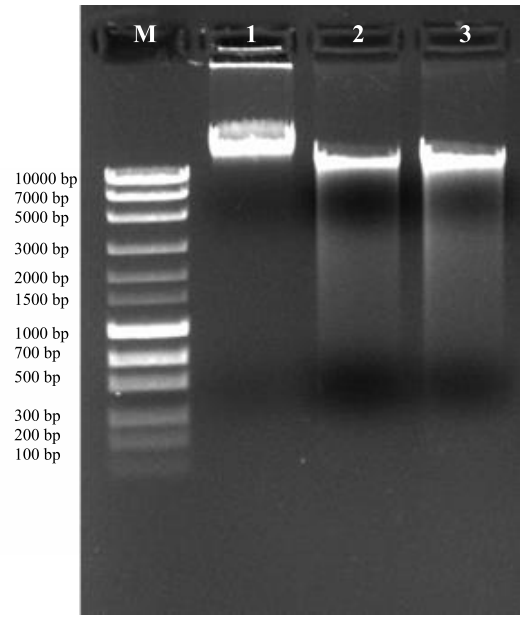

Fig. 6. Extracted DNA quality check on 1.0% agarose gel electrophoresis. M-1Kb DNA ladder, 1- λDNA 200ng, 2- *HYJE003* and 3-*HYJE005*

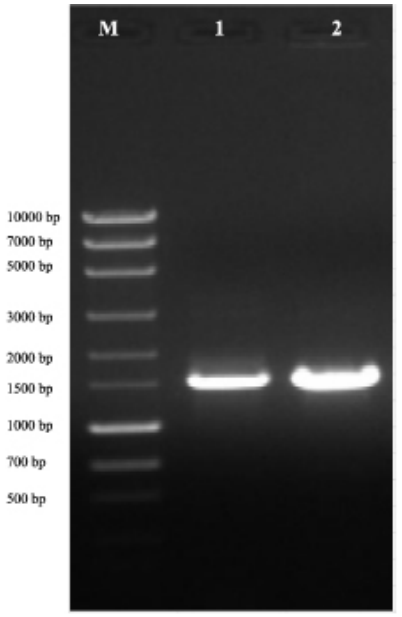

Fig. 7. 16S rRNA PCR gel image of isolated strains showing amplicon size of around 1600 bp (M-1Kb DNA ladder, 1- *HYJE003* and 2-*HYJE005*)

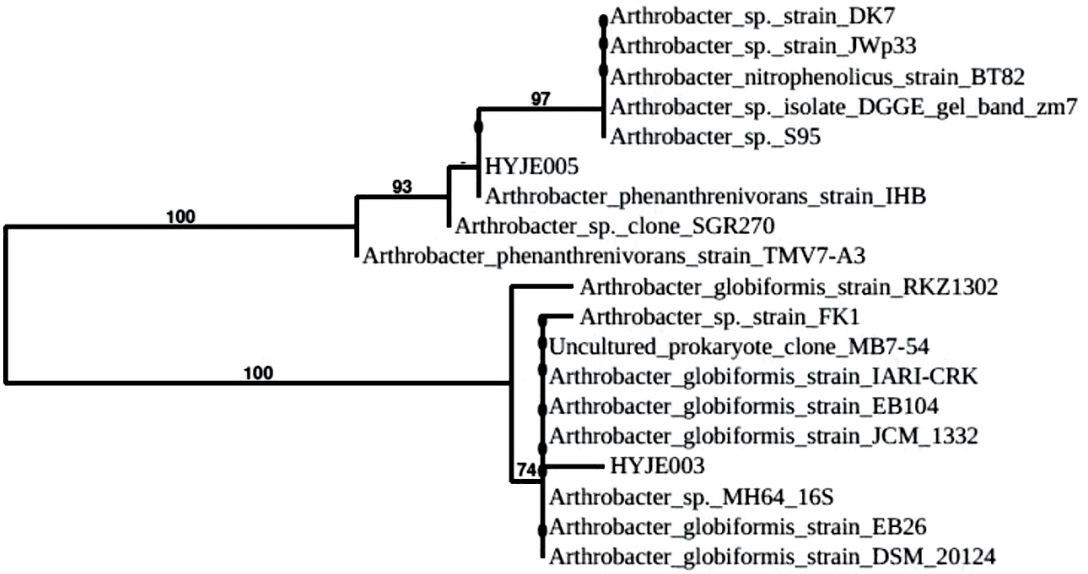

Fig. 8. Phylogenetic tree of *Arthrobacter* sp. *HYJE003* and *HYJE05* based on 16S rRNA sequences. Each branch represents bootstrap values (≥ 50%) for 1000 replications from pairwise neighbor joining

The obtained consensus sequences were analyzed and the 16S rRNA sequence were further used to investigate the identity of the isolates. Based on the concept of sequences similarity[24] and similarity concept of 16S rRNA analysis[25], the isolated strains had 16s rRNA sequence of 1350bp and *HYJE003* shows the 99.85% similarity with the *Arthrobacter globiformis* and the second strain *HYJE005* shows the 99.93% similarity with *Arthrobacter phenanthrenivorans*. The alignment results indicate a significant similarity of the putative strain *HYJE003* DNA sequences

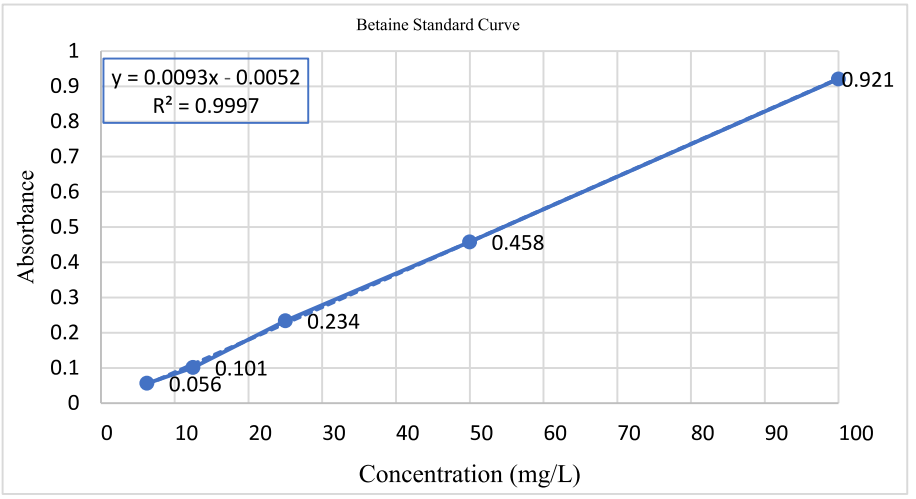

Fig. 9. Betaine estimation through standard curve analysis and concentration of standard were plotted against observance values

(identified in the current study) to the 16S rRNA regions of genius *Arthrobacter globiformis* strains *EB104, EB26, FB24, JCM 1332* and *DCM 20124*[20]. The identified strain *HYJE005* 16S rRNA analysis indicate a significant similarity with *Arthrobacter phenanthrenivorans* strains *IHB, TMV7-A3, SGR270 and S95*[20]. 16S rRNA sequence similarity and phylogenetic tree was constructed with MEGA X using Neighbor joining method, showed isolated strains was closely related to the genus *Arthrobacter*[18] (Fig. 8). The 16S rRNA sequence information was deposited in NCBI GenBank with the consecutive accession numbers of MK968148 and MK968149 for the isolated strain *HYJE003* and *HYJE005* respectively.

Spectrophotometer observance of the betaine reineckate against standard curve relieved 9.8 mg/L and 1.8 mg/L of betaine in the cultures of *HYJE003* and *HYJE005* respectively[4,21] (Table No-4 and Fig. 9). Choline oxidase gene from the *HYJE003* strain was successfully amplified and sequenced. The sequenced choline oxidase gene from the strain *HYJE003* was deposited in NCBI GenBank with the accession numbers of MK988621.

CONCLUSION

In conclusion, the data presented in this study extends our knowledge on the sequential isolation, biochemical and molecular characterization of *Arthrobacter* strains from industrially polluted soil samples and evaluation of their choline oxidase gene potential to convert betaine from choline chloride. Based on conversion result the strain *HYJE003- Arthrobacter globiformis* showed four-fold higher rate of choline to betaine conversion than the strain *HYJE005- Arthrobacter phenanthrenivorans.* Thus, the choline oxidase gene from isolated strain *HYJE003* has shown higher rate of betaine conversion, further research needed for its purified enzyme activity and its commercial feasibility of betaine production.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Camargo, Flavio, Bento, Fatima, C Okeke, Frankenberger, William. Hexavalent Chromium Reduction by an Actinomycete, *Arthrobacter crystallopoietes*. *Biological Trace Element Research*, 2004; **97**: 183-94. https://doi.org/10.1385/BTER:97:2:183
2. Tahri Joutey, Nezha, Sayel, Hanane, Bahafid, Wifak, el ghachtouli, Naima. Mechanisms of Hexavalent Chromium Resistance and Removal by Microorganisms. *Reviews of environmental contamination and toxicology*, 2015; **233**: 45-69. https://doi.org/10.1007/978-3-319-10479-9_2
3. Jones D and Keddie. The Genus Arthrobacter, 2006; pp. 945– 960. The prokaryotes, *Springer*, New York, N.Y, USA. https://doi.org/10.1007/0-387-30743-5_36
4. Ikuta S, Imamura S, Misaki H, Horiuti Y. Purification and characterization of choline oxidase from *Arthrobacter globiformis*. *J. Biochem.*, 1977; **82**(6): 1741-1749. https://doi.org/10.1093/oxfordjournals.jbchem.a131872
5. Teresa Caldas, Nathalie Demont-Caulet, Alexandre Ghazi, Gilbert Richarme. Thermo protection by glycine betaine and choline. *Microbiology*, 1999; **145**: 2543–2548. https://doi.org/10.1099/00221287-145-9-2543
6. Boch, J, Kempf B, Bremer E. Osmoregulation in Bacillus subtilis: Synthesis of the osmoprotectant glycine betaine from exogenously provided choline. *Journal of Bacteriology*, 1997; **176**: 5364–5371. https://doi.org/10.1128/jb.176.17.5364-5371.1994.
7. Sayed M, and Downing J. The effects of water replacement by oral rehydration fluids with or without betaine supplementation on performance, acid base balance, and water retention of heatstressed broiler chickens. *Poult. Sci.*, 2011; **90**: 157–167. https://doi.org/10.3382/ps.2009-00594
8. Honarbakhsh SS, Zaghari M, Shivazad M. Can exogenous betaine be an effective osmolyte in broiler chicks under water salinity stress. *Asian-Australasian Journal of Animal Sci.*, 2007; **20**(11): 1729-1737. https://doi.org/10.5713/ajas.2007.1729
9. Kettunen H, Tiihonen K, Peuranen S, Saarinen MT, Remus JC. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. Comparative Biochemistry and Physiology. *Molecular & Integrative Physiology*, 2000; **130**(4): 759-769. https://doi.org/10.1016/S1095-6433(01)00410-X
10. Murdoch Brenda M., Murdoch Gordon K., Greenwood Sabrina, McKay Stephanie. Nutritional Influence on Epigenetic Marks and Effect on Livestock Production. *Frontiers in Genetics*, 2016; **7**: 182. https://doi.org/10.3389/fgene.2016.00182
11. Joselit Y, Nanobashvili K, Jack-Roberts C. Maternal betaine supplementation affects fetal growth and lipid metabolism of high-fat fed mice in a temporal-specific manner. *Nutr. Diabetes*, 2018; **8**(1): 41. https://doi.org/10.1038/s41387-018-0035-z
12. Luca Rivoira, Sylwia StudziƟska, Malgorzata Szultka, Maria Concetta Bruzzoniti, Bogus³aw Buszewsk. New approaches for extraction and determination of betaine from *Beta vulgaris* samples by hydrophilic interaction liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.*, 2017; **409**: 5133–5141. https://doi.org/10.1007/s00216-017-0461-0
13. Amerah AM. The differences between natural betaine and betaine hydrochloride. *International Poultry Production*, 2013; **22**: 11-13.
14. Govil, Pradip, Govil PK. Distribution and characterization of heavy metals in Jeedimetla industrial area, Hyderabad. *Journal Pollution Research*, 2001; **20**(2): 245-255.
15. Vashist, H, Sharma D and Gupta A. A review on commonly used biochemical test for bacteria. *Innovare Journal of Life Sciences*, 2013; **1**(1): 1-7.
16. Gregersen, T. Rapid method for distinction of gram-negative from gram-positive bacteria. European *J. Appl. Microbiol. Biotechnol*, 1978; **5**: 123. https://doi.org/10.1007/BF00498806
17. Konstantinos Minas, Neil R McEwan, Charles Jamie Newbold, Karen P Scott. Optimization of a high-throughput CTAB-based protocol for the extraction of qPCR-grade DNA from rumen fluid, plant and bacterial pure cultures, *FEMS Microbiology Letters*, 2011; **325**(2): 162–169. https://doi.org/10.1111/j.1574-6968.2011.02424.x
18. Weisburg WG, Barns SM, Pelletier DA and Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, 1991; **173** (2): 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991
19. Davis Gislin , Dorairaj Sudarsanam, Gnanaprakasam Antony Raj, Kathirvelu Baskar. Antibacterial activity of soil bacteria isolated from Kochi, India and their molecular identification. *Journal of Genetic Engineering and Biotechnology*, 2018; **16**(2):287–294. https://doi.org/10.1016/j.jgeb.2018.05.010
20. Vetrovsky T and Baldrian P. The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses. *PLoS ONE*, 2013; **8**(2). https://doi.org/10.1371/journal.pone.0057923
21. Bandelin and pankratz. The Estimation of Betaine and Choline in Mixtures. *J. Am. Pharm. Assoc. Am. Pharm. Assoc.*, 1953; **42**: 442-3. https://doi.org/10.1002/jps.3030420715
22. Chan EC, Steveson L. On the biotin requirement of *Arthrobacter globiformis*. *Canadian Journal of Microbiology*, 1962; **8**: 403-405. https://doi.org/10.1139/m62-053
23. Busse HJ. Review of the taxonomy of the genus *Arthrobacter*. Int. *J. Syst. Evol. Microbiol.*, 2016; **66**(1): 9-37. https://doi.org/10.1099/ijsem.0.000702
24. Bosshard P. Abels PS, Zbinden R, Bottger EC, and Altwegg M. Ribosomal DNA sequencing for identification of aerobic gram-positive rods in the clinical laboratory. *Journal of Clinical Microbiology,* 2003; **41**(9): 4134–4140. https://doi.org/10.1128/JCM.41.9.4134-4140.2003
25. Janda JM and Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, 2007; **5**(9): 2761–2764. https://doi.org/10.1128/JCM.01228-07