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Isolation and Characterisation of Urease Producing Bacteria and their Capacity to Precipitate Calcium Carbonate

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ABSTRACT

The current study was designed to isolate and characterize urease-producing bacteria and to assess their ability to precipitate calcium carbonate. Total eight bacteria were isolated from dung-rich soil samples collected from Dakor, Gujarat. Out of these, two bacterial strains designated as DGDK-3 and DGDK-4 were found to produce a considerable level of urease in the initial screening on the urea agar medium. Based on morphological and physiological tests and more specifically by 16S rRNA gene sequencing analysis, these bacteria were identified as Quasibacillus sp. Strain DGDK-3 and Bacillus sp. Strain DGDK-4. The strains DGDK-3 and DGDK-4 showed 25 IU/ml and 89 IU/ml urease activity, respectively. Also, the efficacy of both strains was tested for calcium carbonate precipitation. Results showed that both the isolates were competent to precipitate a significant level of calcium carbonate. The current work demonstrated that urease-producing bacteria can be utilised in bio-cementationas a crack sealing agent and as a natural stabilizing agent.

Keywords: Urease; Calcite; Quasibacillussp; Dunghills

1. INTRODUCTION

Urease is a nickel-dependent metallo enzyme that catalyzes the hydrolysis of urea to yield ammonia and carbamate. The carbamate is subsequently degraded by spontaneous hydrolysis to produce carbonate and another molecule of ammonia[1]. Functionally, ureases belong to the super family of amidohydrolases and phosphotriesterases. It was the first enzyme that was crystallised in the year 1926 from jack bean[2]. Urease is a cytosolic enzyme found in bacteria, fungi, higher plants, and several invertebrates, but not in animals[2-8]. From a clinical perspective, microbial urease has been extensively studied for its role in virulence factors that cause peptic ulceration, arthritis, urinary stones, and pyelonephritis[1,9].

Despite its negative reputation, microbial urease has extensive applications in clinical diagnosis, biotechnology, construction and agriculture[10-12]. Immobilised urease-based nanoparticles can be used as a biosensor tool for the detection of urea and other target compounds[10]. In fermented alcoholic beverages (red wine, Chinese wine, and white wine) production, microbial urease has been extensively used to remove urea which leads to the production of ethyl carbonate[13-14]. Recently, microbial-induced carbonate precipitation (MICP) using bacterial urease is a promising technique in the field of civil and geotechnical engineering. To reduce the environmental problem, in recent years MICP techniques have been developed as an alternative method for ground improvement[15-16]. Urease-producing bacteria such as *Micrococcus yannanensis*, *Lysinibacillus Sp*[15], *Sporosarcina pasteurii*[17] and *Bacillus megaterium*[18], have been reported as potential players for land improvement and calcium carbonate (calcite) precipitation. The calcite precipitation induced by *Bacillus megaterium BSKAU, Bacillus licheniformis* BSKNAU, *Bacillus flexus* BSKNAU[19], *Bacillus sphaericus* and *Bacillus pasteruii* was reported to be effective in healing the cracks of concrete and increased its compressive strength[20-21].

A large number of bacteria have been reported to produce a significant level of urease[4]. In the majority of studies, the sample used for the bacterial isolation was soil[19,22]. Limited literature was available on the isolation of urease-producing bacteria from dunghills or dung-rich soil, as animal dung is the richest source of substrate urea. Hence, the possibility of urease producers is also extremely high. Therefore, the current study was designed to isolate and identification of urease-producing microaerobic bacteria and to evaluate their ability to precipitate calcium carbonate.

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2. METHODOLOGY

2.1 Sample Collection, Transportation and Bacterial Isolation

Dung-rich soil receives high inputs of urea from urine and dung excreted by livestock. A total of six dung-rich soil samples were obtained from Vada farm Gaushala of Shri Ranchhodraiji Temple, Dakor, Gujarat, India. Samples were collected in sterile sample containers (Tarson, India)by digging at the depth of 15 cm - 30 cm. All collected samples were kept at 4°C and transported to the laboratory.

For the isolation of urease-producing bacteria, 1.0 g of soil sample was diluted in 10 ml of sterile normal saline (0.85%) and put on shaking at 140 rpm for 10 min. The aliquots were taken and serially diluted with normal saline. 100 µl of diluted aliquots were plated on nutrient agar (Himedia, India) medium embedded with 2 per cent sterile (0.22 µm membrane filter - Sartorius AG, Goettingen, Germany) urea (Sigma, USA). Plats were incubated at 30°C for 48 h. Morphologically distinct bacterial colonies were taken and sub-cultured to obtain pure cultures.

2.2 Screening of Urease Producing Bacteria

Bacterial isolates capable to grow on urea supplemented nutrient agar medium were further screened on Urea agar medium (M-PA Agar Base-Himedia, India) supplemented with 2 per cent sterile urea. The urease production was qualitatively monitored through visual examination by changes in the color of the medium from pale yellow to pink-red following incubation[3].

2.3 Species-level Caracterisation of Urease Producing Bacteria

Based on screening results, two bacterial isolates DGDK-3 and DGDK-4 were selected for further characterisation at the species level by 16S rRNA gene sequencing. Genomic DNA was purified using the Bacterial DNA Purification Kit (GeNeiPure™, India). Polymerase Chain Reaction (PCR) was used to amplify the 16S rRNA gene using universal primer set 8F (5'-AGAGTTTGATCCTTGGCTC-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3')[23]. 5 µl 10XPCR buffer, 0.2 mM dNTPs, 1 µM each primer, 2.5 units Taq DNA polymerase, 5 µl DNA templateand sterile MilliQ water to a final volume of 50 µl were used in the reaction mixture. PCR products were purified (GenElute, PCR Clean-Up kit, Sigma) and sequenced at Eurofins Genomics India Pvt. Ltd, India. Initially, 16S rRNA gene sequences were evaluated using the Basic Local Alignment Search Tool (BLAST) nucleotide tool on the NCBI server (http://www.ncbi.nlm.nih.gov/), and the most comparable sequences already in the NCBI database were retrieved. MEGA 5.10 software was used to perform the phylogenetic analysis. The phylogenetic tree was constructed using the neighbour-joining distance method[24].

2.4 Analysis of Urease Activity

Bacterial isolates DGDK-3 and DGDK-4 showing positive results in qualitative screening on the urea agar medium were further selected for the analysis of urease activity. Selected bacterial strains were grown at optimal conditions (pH:7 and temperature: 37 °C) in 200 ml of 2 per cent urea embedded nutrient broth medium with continuous shaking at 140 rpm on the orbital shaker. After 48 h of incubation, 10 ml of culture broth was collected and bacterial cells were separated by centrifugation at 6000 rpm for 10 min. The cell-free broth medium was

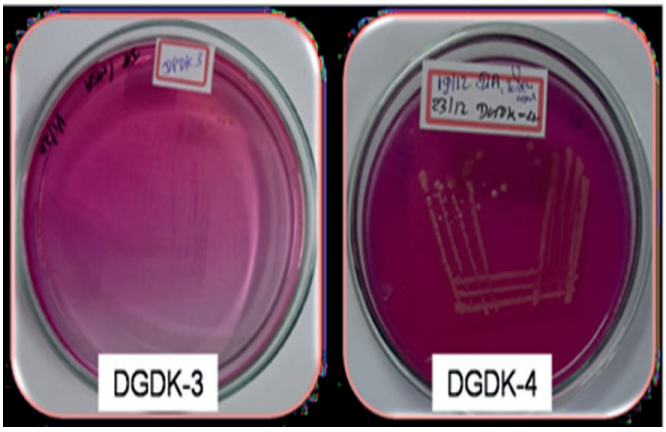
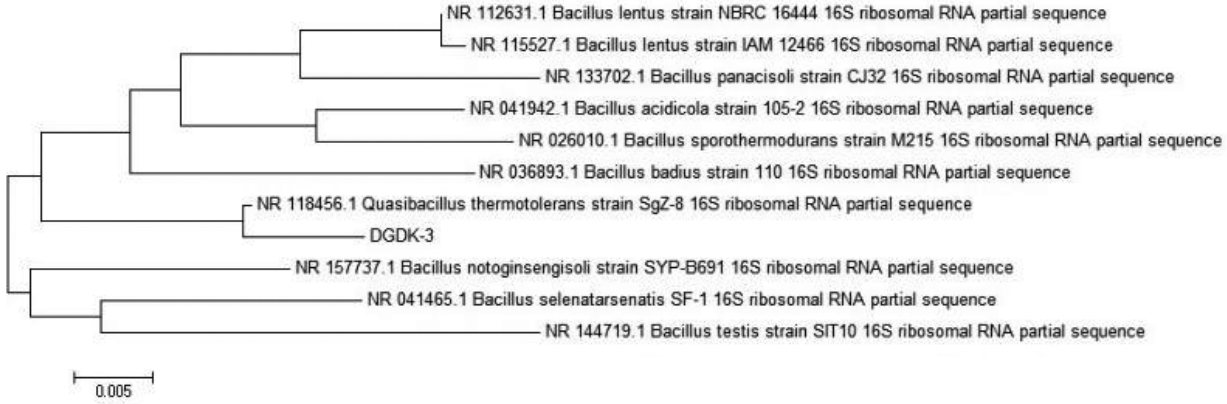


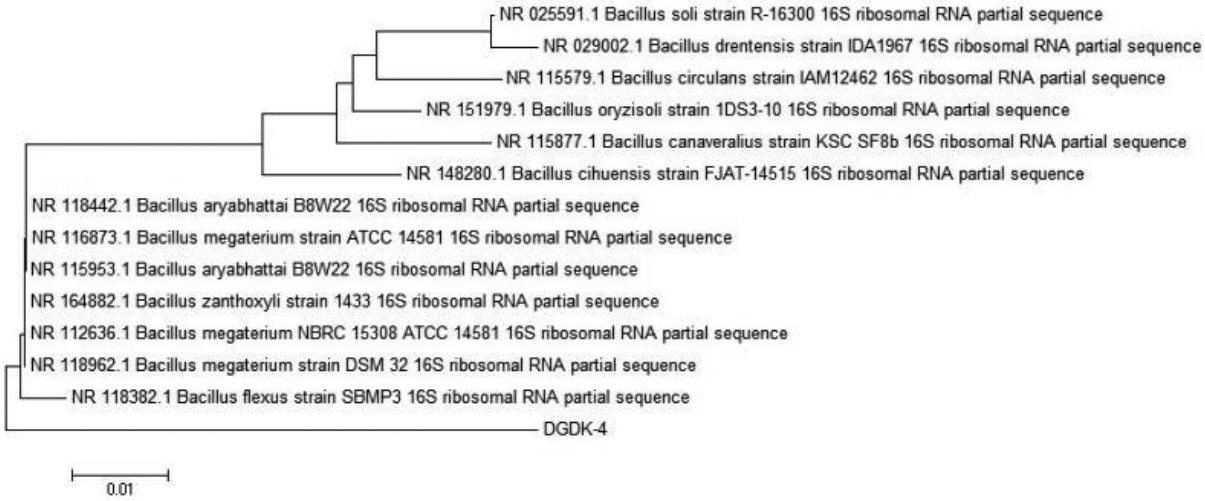
Figure 1. Initial screening of urease producing bacteria on a urea agar plate with phenol red indicator

Table 1. Morphological and physiological characteristics of urease producing bacterial isolates DGDK-3 and DGDK-4

Characteristics	DGDK-3	DGDK-4
Colony on 2% Urea containing nutrient agar	Small, Punctiform, Smooth, Entire, Convex, Moist, Translucent with no pigment	Small, Round, Sooth, Undulate, Convex, Moist, Opaque with no pigment
Gram's reaction	Gram-positive	Gram-positive
Cell shape	Rod	Rod
Motility	-	+
Spore formation	+	+
pH tolerance	5 – 9	4 – 10
Salt tolerance (%)	0 – 6	0 – 8
Temperature tolerance	25 – 60	20 – 40
Catalase	+	+
Oxidase	+	+



(a)



(b)

Figure 2. Phylogram of urease-producing bacteria derived from 16S rRNA gene sequence data: (a) Strain DGDK-3 and (b) Strain DGDK-4 and closely related species.

further assessed for pH using a digital pH meter. The separated bacterial cells were washed 3 times with buffer solution (100 mM Tris/HCl, pH: 8) and finally re-suspended in buffer solution. Re-suspended cells were sonicated and centrifuged at 4°C at 6000 rpm for 10 min. The supernatant was separated. 580 µl of supernatant was mixed with 300 µl of 2 per cent urea solution reagent and incubated at optimal temperature. After 30 min of incubation, the reaction was stopped by adding 500 µl Nessler reagent followed by the addition of 500 µl sterile Milli-Q water[4]. The absorption was measured at 450 nm using a UV/Visible spectrophotometer (Model: 2201, Systronics, India). The absorption of readings was calibrated with buffer (control) by following the same procedure as above. "One unit of urease activity" in terms of urea hydrolysed per minute and expressed as the amount enzyme that increased absorbance by 0.1 units at the above-mentioned wavelength[4].

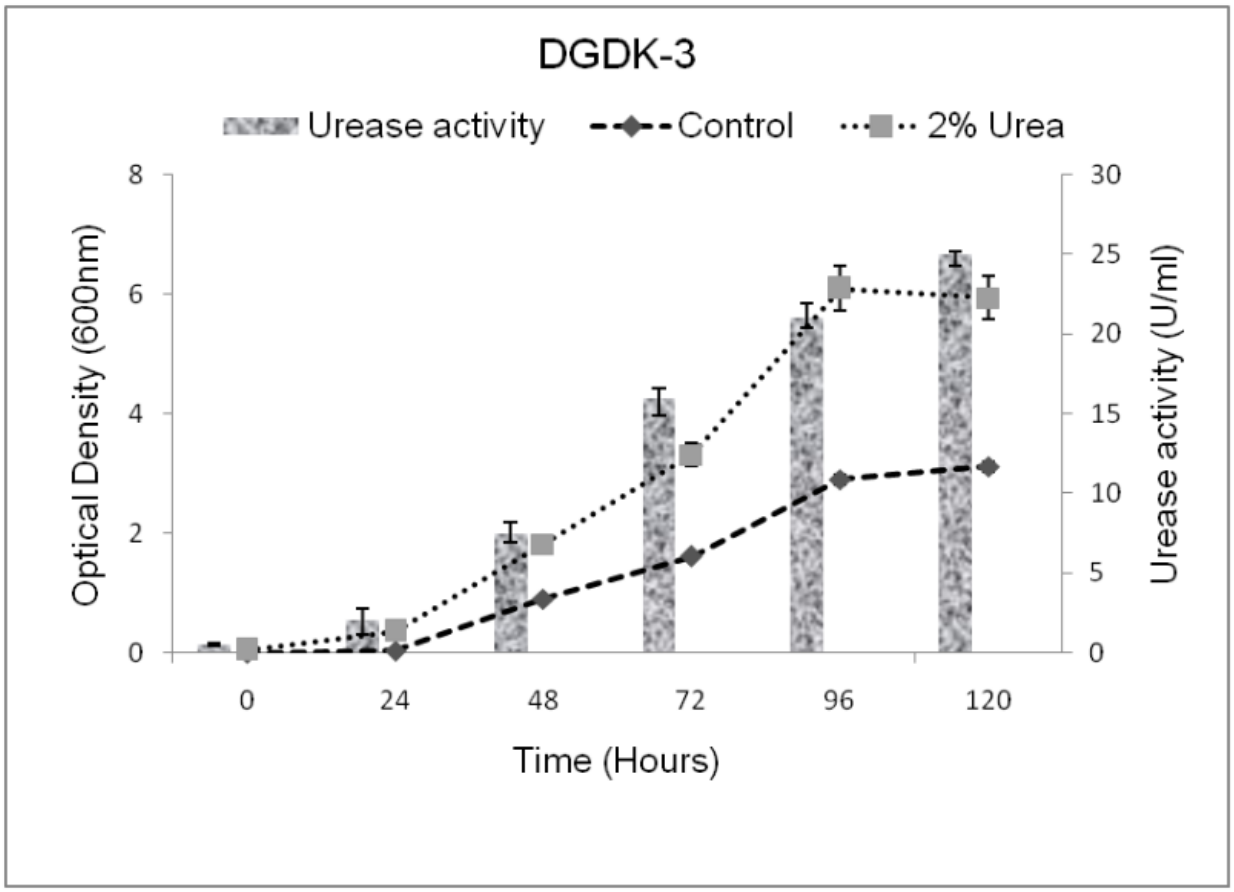
2.5 Calcium Carbonate ($CaCO_3$) Precipitation Test

The selected bacterial strains were examined for their ability to precipitate $CaCO_3$. For the measurement of $CaCO_3$ precipitation, the bacterial strains were grown in 200 ml nutrient broth supplemented with 2 per cent urea and calcium source (2% calcium chloride (Loba Chem, India)) at 37°C. 10 ml broth culture was taken at regular time intervals till the stationary phase of growth reached. The precipitated calcite crystals were separated and washed with distilled water. Calcite precipitates were dried at 60°C in the hot-air oven (Durga Scientific, India) for 48 h, and weighed[3].

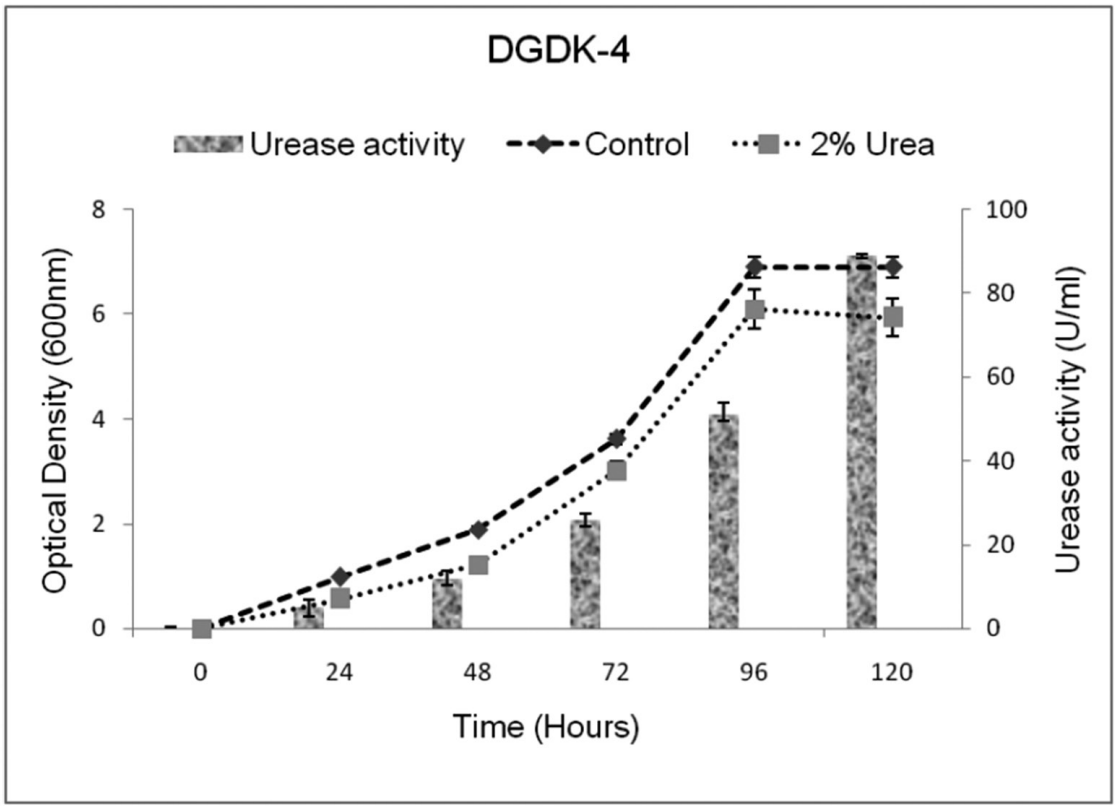
3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Urease Producing Bacterial Isolates

Total eight native bacterial strains were isolated from dung-rich soil samples. Urea agar medium was used for screening urease-producing bacteria. As bacterium starts consuming urea, ammonia is formed which makes the medium alkaline, producing a red-pink colour (Fig. 1) due to the presence of a pH indicator. Out of eight bacterial strains, two were found to produce pink color in initial qualitative screening, which was further screened for characterisation. Morphological characteristics showed



(a)



(b)

Figure 3. Bactetial growth on urea supplemented and urea free control medium plotted against incubationtime, and urease activity (IU/ml).

DGDK-3 and DGDK-4 as gram-positive, rod-shaped bacteria. Morphological and physiological characteristics showed by the isolates are presented in Table 1.

Endospores are unique structural formations produced by certain bacteria which provide them excellent survival capability against adverse hostile and environmental conditions. By forming endospores, bacteria can able to tolerate high physical and chemical stresses induced during concrete mixing[25] and can provide persistent viability for a long period of time (up to 200 years)[19]. When air and water enter through micro-cracks, endospores can germinate into vegetative cells within the concrete. Vegetative cells then start calcite precipitation and restore micro-crakes in concrete[19]. In the present study, both the isolates were capable of forming endospores which may have potential in construction to increase compressive strength.

16S rDNA sequences identified the bacteria as *Quasibacillus Sp.* strain DGDK-3 and *Bacillus Sp.* strain DGDK-4. The genus Bacillus appears to be extensively studied for long-term survival in harsh conditions. Members of this genus are extremely resistant to high temperatures, radiation, chemicals, and desiccation. Moreover, the prevalence of this genus directed us to hypothesize, according to Achal and Pan, 2011[22], that *Bacillus Sp.* play an important role in the natural deposition of carbonate and acquired potentiality in industrial applications[19]. In a study by Jagannathan, *et al.,* 2018[26], 10 per cent of cement was substituted by fly as supplemented by *Bacillus sphaericus.* They observed 10.8 per cent, 29.37 per cent, and 5.1 per cent enhancement incompressive strength, tensile strength, and flexure strength of cement compared to controlled cement without bacterium. In a study, *Bacillus subtilis,* peptone and yeast extract were used as adjuvants in the concrete mixture. Compared to the control, this concrete mixture showed a significant reduction in porosity and permeabilities for water and gases, which further increases the overall strength of concrete[27].

The partial sequence of 16S rRNA genes of *Quasibacillus sp.* Strain DGDK-3 and *Bacillus sp.* strain DGDK-4 were submitted to NCBI Genebank (BankIt) under the accession number MT254409 and MT256087 respectively. The phylogenetic tree derived from 16S rRNA gene sequences of DGDK-3 (1373 base pair) and DGDK-4 (1036 base pair) were aligned closely with the sequences of *Quasibacillus thermotolerans* strain SgZ-8 (Accession number NR118456, sequence similarity 99%) and *Bacillus zanthoxyli* strain 1433 (Accession number NR164882, sequence similarity 95%) (Fig. 2).

3.2 Urease Activity

The comparative growth of isolated bacteria in urea-free media and urea-containing media with urease activities at different time intervals are presented in Fig. 3. Both the isolates were found to produce a significant amount of urease. *Quasibacillus sp.* strain DGDK-3 and *Bacillus sp.* strain DGDK-4 were able to produce 25 IU/ml and 89 IU/ml urease, respectively. Earlier, Khadhim, *et al.,*[28] reported that certain species of *Bacillus* genus were capable to produce 7 IU/ml - 12 IU/ml urease. Certain strains of *Bacillus cereus* were found to produce around 20 IU/ml urease[3]. Urease activity of Bacillus cereus was competent to enhance the compressive strength of cement mortar by promoting biomineralisation[28-29]. *Bacillus haericus* MTCC 5100 showed 32.5 IU/ml[30] and *Bacillus* sp. Strains P9 and P15 showed 6 IU/ml - 42 IU/ml urease activity at a pH range between 4.0-9.0[3]. In the present study, Bacillus sp. strain DGDK-4 was capable of producing large amounts of urease. To the best of our knowledge, the urease activity by *Quasibacillus sp.* has not been previously reported. The present study is the first study that reports urease production by *Quasibacillus sp.*

Both the isolates showed a gradual increase in urease production during the exponential phase, which remains nearly constant during the stationary phase (120-144 h). Another important observation was noticed in the growth of DGDK-3, compared to the control (urea free) medium, DGDK-3 showed optimal growth in urea supplemented medium which suggests that urea favors the growth of this bacterium. A similar observation was reported in a study on Ureaplasmaurealyticum. This bacterium showed remarkable growth on a medium supplemented with an increasing concentration of urea[31]. Most bacteria hydrolyze urea for three purposes (1) to utilise nitrogen, (2) to produce energy, and (3) to increase ambient pH[22]. Hydrolysis of urea by urease or urea amidolyase produces ammonia and carbon dioxide[32]. The release of ammonia increases the pH of the medium which further favors the precipitation of calcium carbonate. The rate of urea hydrolysis is directly proportional to the cell biomass and it is an important factor for the success of MICP[15]

3.3 $CaCO_3$ Precipitation Test

Previous studies showed that it is possible to manage the strength of the treated sand by regulating the number of precipitated minerals[33]. In addition to this, it has been reported that various abiotic (pH, temperature) and biotic (microbes) factors, regulate the amount of calcite precipitation during the MICP process[34]. Hence, it is important to investigate the capability of bacteria to precipitate calcite as it is the main binder material for the MICP process. The amount of calcite precipitation was measured from day one to five of bacterial growth. Both the isolates were capable of producing a significant amount of calcite at the end of 4th day. The calcite precipitation trend for the DGDK-3 and DGDK-4 is shown in Fig. 4. *Bacillus sp.* Strain DGDK-4 produced 1.8 g calcite l^{-1} while *Quasibacillus sp.* strain DGDK-3 produced 0.9 g calcite l^{-1}. At optimum growth conditions, the isolate DGDK-4 has shown doubled capacity to precipitate $CaCO_3$compared to the isolate DGDK-3, signifying the former bacterium a better candidate for sand stabilisation and bio-cementation.

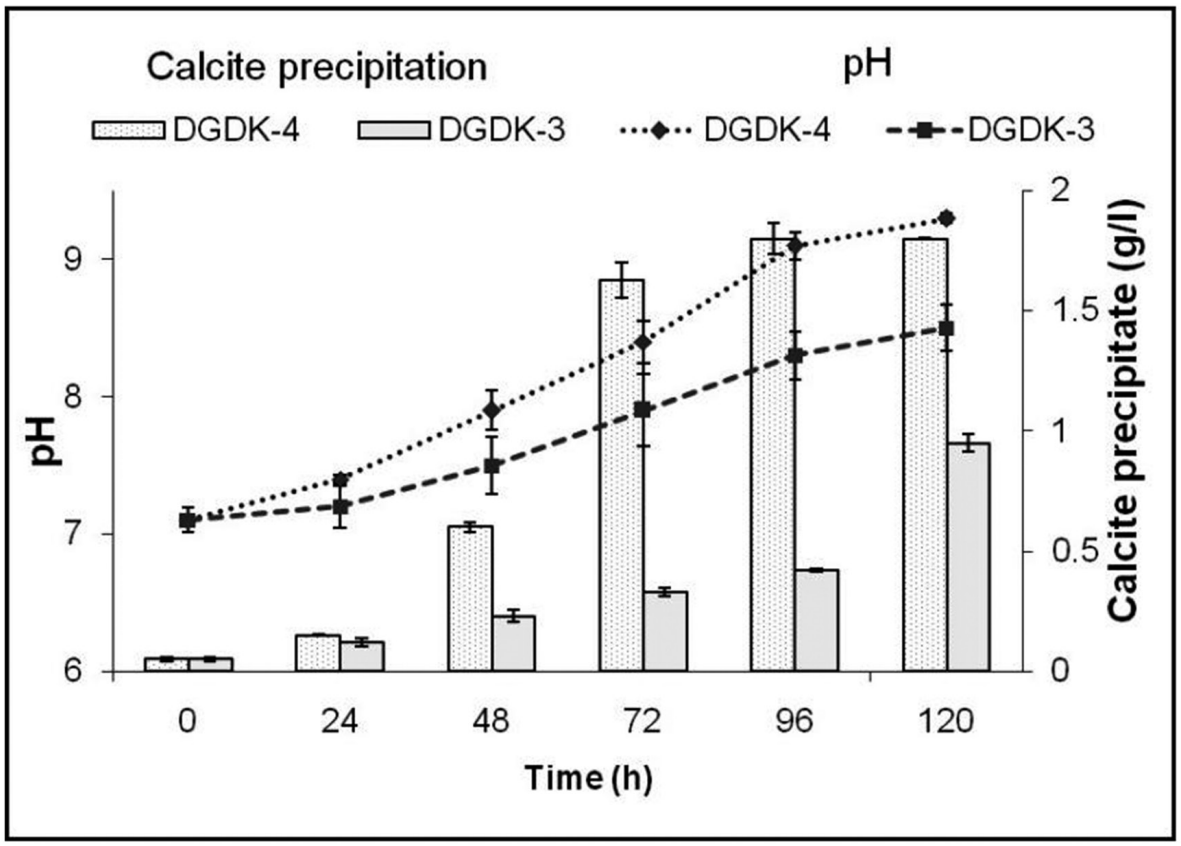


Figure 4. Alteration in pH of the growth medium pH and calcite production by isolates DGDK-3 and DGDK-4 at different time intervals.

4. CONCLUSION

Based on the present work results, it can be concluded that bacterial isolates DGDK-3 and DGDK-4 exhibited high urease activity. 16S rRNA gene sequence analysis identified DGDK-3 and DGDK-4 as species belonging to the genus *Bacillus.* The bacterial isolates also had the ability to produce endospores and also effectively precipitated calcium carbonate. Future recommended studies included urease purification, enzyme activity optimisation and molecular structure examination.

REFERENCES

1. Konieczna, I.; Zarnowiec, P.; Kwinkowski, M.; Kolesinska, B.; Fraczyk, J.; Kaminski, Z. & Kaca, W. Bacterial Urease and its role in long-lasting human diseases. *Curr. Protein Pept. Sci.,* 2012, **13**(8), 789–806. doi:10.2174/138920312804871094.
2. Sirko, A. & Brodzik. R. Plant ureases: Roles and regulation. *Acta Biochim. Pol.*, 2000, **47**(4), 1189-1195.
3. Phang, I.R.K.; Chan, Y.S.; Wong, K.S. & Lau, S.Y. Isolation and characterisation of urease-producing bacteria from tropical peat. *Biocatal. Agric. Biotechnol.,* 2018, **13**, 168-175. doi:10.1016/j.bcab.2017.12.006.
4. Alizadeh, H.; Kandula, D.R.W.; Hampton, J.G.; Stewart, A.; Leung, D.W.M.; Edwards, Y. & Smith, C. Urease producing microorganisms under dairy pasture management in soils across New Zealand. *Geoderma Regional*, 2017, **11**, 78-85. doi: 10.1016/j.geodrs.2017.10.003.
5. Hosseini, M.; Baba, F.; Al-Rubaye, M.T.S.; Fakhari, J. & Al-Musawi, M.H.J. Urease-producing halophilic bacteria isolated from Bahr Al-Milh Salt Lake, Karbala, Iraq. *J. Pure Appl. Microbiol.,* 2017, **11**(2), 711-716. doi: 10.22207/JPAM.11.2.09.
6. Yu, J.J.; Smithson, S.; Thomas, P.W.; Kirkland, T.N. & Cole, G.T. Isolation and characterisation of the urease gene (URE) from the pathogenic fungus Coccidioides immitis. *Gene,* 1997, **198**(1-2), 387-391. doi:10.1016/s0378-1119(97)00342-9.
7. Karplus, P.A.; Pearson, M.A. & Hausinger, R.P. 70 years of crystalline urease: What have we learned? *Acc. Chem. Res.,* 1997, **30**(8), 330-337. doi:10.1021/ar960022j.
8. Patra, A.K. & Aschenbach, J.R. Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: A review. *J. Adv. Res.,* 2018, **13**, 39-50. doi:10.1016/j.jare.2018.02.005.
9. Banerjee, S. & Aggarwal, A. Potential clinical significance of urease enzyme. *European Scientific J.,* 2013, **9**(21), 94-102.
10. Jakhar, S. & Pundir, C.S. Preparation, characterisation, and application of urease nanoparticles for

construction of an improved potentiometric urea biosensor. *Biosens. Bioelectron.,* 2018, **100**, 242-250. doi:10.1016/j.bios.2017.09.005.

11. Dahlen, G.; Hassan, H.; Blomqvist, S. & Carlen, A. Rapid urease test (RUT) for evaluation of urease activity in oral bacteria in vitro and in supragingival dental plaque ex vivo. *BMC Oral Health,* 2018, **18**(89), 1-7. doi:10.1186/s12903-018-0541-3.
12. Qin, Y. & Cabral, J.M.S. Properties and applications of Urease. *Biocatal. Biotransform.,* 2002, **20**(1), 1-14. doi: 10.1080/10242420210154.
13. Kobashi, K.; Takebe, S. & Sakai, T. Removal of urea from alcoholic beverages with an acid urease. *J. Appl. Toxicol.,* 1988, **8**(1), 73-74. doi: 10.1002/jat.2550080112.
14. Ough, C.S. & Trioli, G. Urea removal from wine by an acid urease. *Am. J. enol. Viticult.,* 1988, **39**(4), 303-307.
15. Imran, M.A.; Nakashima, K.; Evelpidou, N. & Kawasaki, S. Factors affecting the urease activity of native ureolytic bacteria isolated from coastal areas. *Geomech. Geoeng.,* 2019, **17**(5), 421-427. doi:10.12989/gae.2019.17.5.421.
16. Danjo, T. & Kawasaki, S. Formation mechanisms of beach rocks in Okinawa and Ishikawa, Japan, with a focus on cement. *Mater. Trans.,* 2014, **55**(3), 493-500. doi:10.2320/matertrans.M-M2013844.
17. Hammad, I.; Talkhan, F. & Zoheir, A. Urease activity and induction of calcium carbonate precipitation by Sporosarcinapasteurii NCIMB 8841. *J. Appl. Sci. Res.,* 2013, **9**(3), 1525-1533.
18. Jiang, N.J.; Yoshioka, H.; Yamamoto, K. & Soga, K. Ureolytic activities of a urease-producing bacterium and purified urease enzyme in the anoxic condition: Implication for subseafloor sand production control by microbially induced carbonate precipitation (MICP). *Ecol. Eng.,* 2016, **90**, 96-104. doi:10.1016/j.ecoleng.2016.01.073.
19. Krishnapriya, S.; Babu, D.L.V. & Arulraj, G.P. *Microbiol. Res.,* 2015, **174**, 48-55. doi:10.1016/j.micres.2015.03.009.
20. Tittelboom, K.V.; Belie, N.D.; Muynck, W.D. & Verstraete, W. Use of bacteria to repair cracks in concrete. *Cem. Concr. Res.*, 2010, **40**(1), 157–166. doi: 10.1016/j.cemconres.2009.08.025.
21. Ramachandran, S.K.; Ramakrishnan, V. & Bang, S.S. Remediation of concrete using micro-organisms. *ACI Mater. J.,* 2001, **98**(1), 3-9.
22. Achal, V. & Pan, X. Characterisation of urease and carbonic anhydrase producing bacteria and their role in calcite precipitation. *Curr. Microbiol.,* 2011, **62**, 894-902. doi:10.1007/s00284-010-9801-4.
23. Khambholja, D.B. & Kalia, K. Seasonal Variation in Arsenic Concentration and its bioremediation potential of marine bacteria isolated from alang-sosiya ship-scrapping yard, Gujarat, India. *Def. Life Sci. J.*, 2016, **1**(1), 78-84. doi: 10.14429/dlsj.1.10088.
24. Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M. & Kumar, S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.,* 2011, **28**(10), 2731–9. doi: 10.1093/molbev/msr121.
25. Sagripanti, J.L & Bonifacino, A. Comparative sporicidal effects of liquid chemical agents. *Appl. Environ. Microbiol.,* 1996, **62**(2), 545-551.
26. Jagannathan, P.; Satya Narayanan, K.S.; Arunachalam, K.D. & Annamalai, S.K. Studies on the mechanical properties of bacterial concrete with two bacterial species. *Mater. Today: Proc.,* 2018, **5**(2), 8875-79. doi:10.1016/j.matpr.2017.12.320.
27. Nguyen, T.H.; Ghorbel, E.; Fares, H. & Cousture, A. Bacterial self-healing of concrete and durability assessment. *Cem. Concr. Compos.*, 2019, **104**, 103340, 1-15. doi:10.1016/j.cemconcomp.2019.103340.
28. Khadhim, H.J.; Ebrahim, S.E. & Ammar, S.H. Isolation and identification of ureolytic bacteria isolated from livestock soil to improve the strength of cement mortar. *Eng. Technol. J.,* 2019, **37** Part C(3), 319-326. doi: 10.30684/etj.37.3C.3.
29. Maheswaran, S.; Dasuru, S.S.; Murthy, C.; Rama, A.; Bhuvaneshwari, B.; Kumar, V.R. & Sandhya, S. Strength improvement studies using new type wild strain Bacillus cereus on cement mortar. *Curr. Sci.,* 2014, **106**(1), 50-57.
30. Singh, A.K.; Singh, M. & Verma, N. Extraction, purification, kinetic characterisation and immobilisation of urease from Bacillus sphaericus MTCC 5100. *Biocat. Agri. Biotechnol.,* 2017, **12**, 341-347. doi:10.1016/j.bcab.2017.10.020.
31. Kenny, G.E. & Frank, A.D. Effect of Urea Concentration on Growth of Ureaplasmaurealyticum(T-Strain Mycoplasma). *J. Bacteriol.,* 1977, **132**(1), 144-150.
32. Strope, P.K.; Nickerson, K.W.; Harris, S.D. & Moriyama, E.N. Molecular evolution of urea amidolyase and urea carboxylase in fungi. *BMC Evol. Biol.,* 2011, **11**(80), 1-15. doi:10.1186/1471-2148-11-80.
33. Putra, H.; Yasuhara, H.; Kinoshita, N. & Hirata, A. Application of magnesium to improve the uniform distribution of precipitated minerals in 1-m column specimens. *Geomech. Geoeng.,* 2017, **12**, 803-813. doi: 10.12989/gae.2017.12.5.803.
34. Keykha, H.A.; Asadi, A. & Zareian, M. Environmental factors affecting the compressive strength of microbiologically induced calcite precipitation treated soil. *Geomicrobiol. J.,* 2017, **34**(10), 889-894. doi: 10.1080/01490451.2017.1291772.

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