Exiguobacterium aquaticum CS6 a potent calcite solubilizer isolated calcite isolated from marble slurry

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Abstract **- Marble industries are growing in pace with the increasing demand of marble stones. During the mining process the slurry is discharged as a waste either to a vacant land or to a surface water source in the nearby areas. The current investigation was aimed to detect calcite solubilizing bacteria from marble slurry collected from dumping yard in Sukher, Udaipur, India. Isolation was performed on nutrient agar medium using pour plate method. Calcite solubilizing efficiency (CSE) and index (CSAI) of the isolate was tested on 0.1% calcite agar medium and incubation was done at 37°C for 10 days. The isolate was identified on the basis of morphological, biochemical and molecular methods. Isolate CS6 was appeared on nutrient agar medium after an incubation of 24 hours at 37°C. The colony appeared as orange, pinpointed and with convex elevation. It showed clear zone of 32 mm diameter after 10 days of incubation indicating calcite solubilizing activity. The maximum CSE and CSAI observed were 166% and 260 respectively. Isolate CS6 was identified as Exiguobacterium aquaticum CS6 (accession no. MK353511). The isolate showed remarkable calcite solubilizing activity and can be further analyzed for its possible use in restoration of marble slurry contaminated soil.**

Index Terms **- *Exiguobacterium aquaticum* CS6, Calcite Solubilizing bacteria, Marble slurry, Nutrient agar.**

INTRODUCTION

Rajasthan is known as mineral majestic state as more than 50 types of minerals are produced. The state is endowed with vast deposits of natural rocks known as "stones" in local parlance and a few important ones amongst them are granite, marble, sandstone, limestone, slate and quartzite. The galaxy of the stone found in Rajasthan and its vide applicability has inspired many at home and outside. Marble – generally a white based elegant looking stone, geologically a thermally metamorphosed rocks belonging mainly to Precambrian rock formations of Rajasthan, spread over in 16 belts in 15 districts of the state is much sought after stone. The ever-increasing popularity of the marbles of Rajasthan and growing demand led to the increase in the number of marble quarries which results in generation of huge marble waste.

Mine waste deposits have been turned as a global problem (Hudson-Edwards *et al*., 2011; Bini, 2012) Besides environmental threats, mine wastes are considered hazardous substances, which can induce a risk to human health (Ghorbel *et al.,* 2010; Zhao *et al*., 2012; Wahsha *et al*., 2014). This mining waste may be utilized for product formation (Shah *et al*., 2015) or strategies for restoration can be developed (Bini *et al.,* 2017).

Marble slurry wastes predominantly, consist of around 99% calcium carbonate. Because of insoluble nature it adversely affects soil fertility (Mahdi *et al.,* 2011) and human health (Rana *et al.,* 2015). Udaipur Marble Processors Association has identified various dumping sites at Sukher and Chitrakoot Nagar near to Khelgaon. Marble slurry generated during cutting, processing and polishing is dumped into this site regularly which occupies huge landmass as waste of land. Considering these facts an attempt has been made to isolate efficient calcite solubilizing bacterial strain.

MATERIALS AND METHODS

Collection of samples

Marble slurry sample was collected from dumping yard (DYI) (24° 39' 39.88'' N, 73° 43' 24.53'' E) located in marble industrial area, Sukher, Udaipur (India). Isolation was done on nutrient agar.

Determination of calcite solubilizing activity:

Calcite solubilisation was determined on calcite agar medium supplemented with 0.1% $CaCO_3$ using pour

plate method (Rana *et al.,* 2015). The plates were incubated at 37° C for 10 days. Bacterial isolate producing clear-halos around the colony was considered to have calcite-solubilizing activity.

The calcite solubilizing efficiency (CSE) (Nguyen *et al.,* 1992) and calcite solubilizing activity index (CSAI) (Rana *et al.,* 2015) of calcite solubilizing bacteria was calculated using following formula:

$$\text{Calcite solubilizing efficiency (CSE)} = \frac{\text{Halo zone diameter}}{\text{Colony diameter}} \times 100$$

$$\text{Calcite solubilizing activity index (CSAI)} = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

Characterization of calcite solubilizing bacteria

The molecular characterization of calcite solubilizing bacteria was done by 16S rRNA gene sequencing. The genomic DNA of the isolates CS6 was isolated by Pospeich and Neikmann's method (1995). The 16S rRNA sequence of DNA was amplified using universal primers, 27F (5'-AGAGTTTGATC CTGGCTCAG-3') and 1492R (5'CGGTTAC CTTGTTACGACTT-3') (Weisberg *et al.,* 1991)

using PCR. The amplified products were submitted to Bangalore GeneiPvt. Ltd., Bangalore (India) for sequencing. The partially sequenced genomes of the isolates were compared with available standard sequences of bacterial lineages in the NCBI Genbank using nBLAST program.

RESULTS AND DISCUSSION

Isolate CS6 was appeared on nutrient agar medium after an incubation of 24 hours at 37°C. The colony appeared as pin-pointed, orange, circular, smooth with convex elevation. The isolate CS6 was appeared as Gram positive small rods. The diameter of clear zone for calcite solubilisation, CSE and CSAI was gradually increased with an increase of incubation period from first day to 10th day. The data for the same has been presented in Table 1.

The maximum diameter of halo zone measured was 32 mm including colony diameter (12 mm) at 37°C after an incubation of 10 days. The maximum CSE and CSAI observed were 166% and 260 respectively. On the basis of partial 16S rRNA sequencing isolate CS6 was identified as *Exiguobacterium aquaticum* CS6 (accession no. MK353511)

In the present study CSAI observed for *Exiguobacterium aquaticum* CS6 was 260. Similar findings were reported by Rana et al. 2015. They determined calcite solubilisation activity of bacteria isolated from cow dung. For *Pseudomonas* species CSAI was observed in the range of 111 to 266.7. They explained that calcite solubilisation was due to the secretion of citric acid, oxalic acid and sanazine pigment. This may be the probable reason for calcite solubilisation observed in the present study.

Table 1: Calcite solubilisation activity of *Exiguobacterium aquaticum* CS6

Incubation Period (Days)	Day 1	Day 3	Day 5	Day 7	Day 10
Colony Diameter (mm)	5	8	10	12	12
Calcite solubilizaion Diameter of clear zone including colony diameter (mm)	8	17	23	30	32
Calcite solubilizing efficiency (CSE) %	60	112	130	150	166
Calcite solubilizing activity index (CSAI)	160	212	230	250	260

CONCLUSION

Exiguobacterium aquaticum CS6 is a potent calcite solubilising strain. It showed fairly high CSE and CSAI. It can be further analysed to study the molecular mechanism of calcite solubilisation. Studies in pot experiments can be carried out to explore its possible use in restoration of marble slurry contaminated land.

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