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Detection of Calcium carbonate precipitation by soil bacteria and its Infrared spectroscopic analysis

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ABSTRACT: Bacteria are ubiquitous in every habitat on earth, growing in soil and also in deep in the earth's crust. They are able to induce the precipitation of minerals, either by highly controlled phenomenon called microbiologically induced calcite precipitation (MICP). Minerals which are synthesized by biomineralization processes include silica, iron oxides, hydroxyapatite, and calcium carbonate in various polymorph orientations, e.g. calcite, aragonite, and vaterite. Calcium carbonate precipitation, a widespread phenomenon among bacteria, has been investigated due to its wide range of scientific and technological implications. In this study, soil bacteria was isolated, identified for the *in vitro* precipitation of calcium carbonate by optimizing various parameters of the growth and confirmed by Infrared spectroscopic analysis.

Keywords: Biomineralization, calcite, Soil bacteria, Spectroscopic analysis

I. INTRODUCTION

Microorganisms have a geochemical activity which is responsible to a great extent for the deposition of minerals throughout the history of the earth. There are considerable numbers of microorganisms able to induce the extracellular precipitation of a wide range of minerals by "biologically induced biomineralization". Bacteria can change the chemistry of their surrounding environment as a result of bacterial metabolic activity. This, together with the contribution of biological structures such as cells, cell membranes or cell debris and exopolymeric substances- EPS which acts as nuclei for heterogenous crystallization, eventually leads to mineral precipitation [8].

Bacteria are considered as agents that disperse, fractionate or concentrate material via processes such as intracellular deposition, adsorption and fixation at cellular level and precipitation of insoluble extra or intracellular compounds. Bacteria may contribute to mineral precipitation not only actively, but also passively, by serving both the cell, cell structures and the cell debris as a nucleation sites for mineral deposition [5].In this regard, bacteria can induce heterogenous nucleation of minerals by providing not only a surface that lower the energy barriers for mineral precipitation, but also a stereo chemical arrangement of the mineral components. Many studies place special emphasis on the role of microbial extracellular substances (EPS).

Bacterially induced or mediated carbonate mineralization is important in a range of process such as atmospheric CO_2 ,budgeting, carbonate sediment and rock formation, biogeochemical cycling of elements and conservation of ornamental stone. It has been observed that there are number of bacteria capable of producing calcium carbonate, which includes sulphate-reducing bacteria, *Bacillus, Myxococcus, Halobacillus* [10] and *Pseudomonas sp* [16].This is confirmed by numerous laboratory studies, which demonstrate precipitation of different minerals in bacterial cultures [3]. There are numerous study involved in biomineralization which includes standard strains or samples from caves. The aim of this study was to isolate bacteria from soil, identify by performing identification tests, characterization of the isolates and its influence on growth parameters, calcium carbonate precipitation test and its confirmation by spectroscopic analysis.

II. MATERIALS AND METHODS

II. 1. Isolation of Calcium carbonate forming bacteria

Calcium carbonate precipitating bacteria were isolated from from different types of habitats, although they are abundant in soils that are rich in organic material such as cultivated soils collected in a sterile manner and brought to the laboratory [10]. The samples were suspended in a sterile saline solution (0.85% NaCl), serial diluted and plated on to the basal medium. The obtained samples were further processed to identify the

morphology by performing Gram's staining. For fifty two isolates, identification tests were performed such as Catalase and oxidase test and biochemical tests which includes Indole, Methyl red, Voges Proskauer, Simmon's citrate, Triple Sugar Iron , Christensen's Urea Agar , hydrolysis and carbohydrate fermentation tests.

II. 2. Growth Characteristics

Calcium carbonate precipitation depends on various factors such as Cell concentration, pH and temperature on a constant medium that influence on the performance of precipitation. The isolates were maintained in the nutrient medium and the cells were obtained by centrifuging at 8000 rpm and washed twice with 50 mM sodium phosphate buffer.

II. 2.1. Effect of cell concentration

The cell concentration was determined from the standard curve by observing the optical density at an absorbance of 600 nm at regular time interval of 1 hr to 7 hr. Among the isolates, if an OD value of 0.8 to 1.0 was seen, then those isolates were further processed for the calcium carbonate precipitation test [13].

II. 2.2. Effect of pH

The pH of the reactant medium will increase gradually during the process with the increase in the growth. The ability of the isolates to grow at high pH was influenced in enhancing the calcium carbonate precipitation. The active carbonatogenesis induces the growth and shape of the particles that are encasing the calcium carbonate around the bacteria systematically[4].

II.2.3. Effect of Temperature

The growth of microorganisms were usually sensitive to the temperature from 20°C to 30°C. The precipitation test was performed in different temperatures from 15°C to 45°C and studied the optimum temperature that had a positive effect on bacterial precipitation of calcium carbonate, thereby increasing the ability of the strain to form precipitates.

II. 3. Calcium carbonate Precipitation

This test was performed for the isolates of an OD value 0.8. They were inoculated into Triplicate B4 medium(calcium acetate 2.5g, Yeast extract 4g, Glucose 10g, Agar 18g/1000ml of distilled water, pH 7.2) incubated aerobically at various temperatures 15°C, 25°C, 35°C , 45°C for 28 days. After 28 days of incubation, the precipitates was purified by filtration, washed with sterile distilled water and dried at 60°C overnight [6].

II.4. Calcium carbonate Estimation

Precipitated calcium carbonate obtained from the isolates at various intervals of 7,14,21,28 days and measured by EDTA titration method [2]. About 5ml of the culture filtrate was dissolved in 3N hydrochloric acid and 4ml of 5N sodium hydroxide and the final volume was made up to 50ml with distilled water. The final pH should be 12-13. Few drops of hydroxy napthol blue was added as an indicator and the mixture was titrated against 0.05 M EDTA. The end point was an color change from pink to blue was noted and the amount of Calcium carbonate formed was calculated by the volume of EDTA used x 0.005004 x 1000/ml of the sample used.[1].

II.5. FTIR Analysis

The obtained filtrate after dried was analyzed by FTIR (Fourier Transform Infrared Spectroscopy). It was a vibration and rotational state of molecules when subjected to infrared irradiation. This technique was based on the fact that after absorption of IR radiations, the molecule vibrates at many rates of vibrations and forms close packed absorption bands known as I.R absorption spectrum. The I.R spectrum extends over a wide wavelength range. Different bands observed in I.R spectrum corresponds to various functional groups and bonds found in chemical molecules. The I.R spectrum of a molecule considered as its fingerprint and can be used for the identification of the compound.

III. RESULTS

The isolates obtained after performing preliminary and other identification tests were screened and found to be different types of bacteria. Among the obtained isolates, *Bacillus sp* and *Pseudomonas sp* were found to survive in different growth influencing factors such as cell concentration, pH and temperature and precipitate calcium carbonate in an *in vitro* condition. The growth characteristics of *Bacillus sp* and *Pseudomonas sp* were listed in Table I

Table I.
Characteristics of *Bacillus sp*

Characteristics	*Bacillus sp*	*Pseudomonas sp*
Morphology		
Gram staining	Positive	Negative
Shape & arrangement	Rods, chains	Short rods
Capsule	Positive	Negative
Spore	Positive	Negative

Characteristics	*Bacillus sp*	*Pseudomonas sp*
Motility	Positive	Motile
Biochemical tests		
Catalase	Positive	Positive
Oxidase	Negative	Positive
Nitrate	Positive	Positive
Urease	Positive	Positive
Indole	Negative	Negative
Methyl red	Negative	Negative
VP	Positive	Negative
Citrate	Positive	Positive
TSI	K/A, Gas -, H_2S -	K/K, Gas -, H_2S -
Carbohydrate fermentation		
Glucose	Negative	Positive
Fructose	Positive	Negative
Sucrose	Positive	Negative
Lactose	Positive	Negative
Mannitol	Negative	Negative
Hydrolysis		
Starch	Positive	Positive
Casein	Positive	Negative
Gelatin liquefaction	Positive	Negative

These two strains was selected to study on the effect of growth influencing factors such as cell concentrations, pH and temperatures to precipitate calcium carbonate was determined. The growth curve was similar in all the isolates. An OD value of 0.8 at an absorbance of 600nm was obtained in the selected strain and showed no much significant difference between them.(Figure.1). The pH of the medium significantly increased as growth increased and showed similar results in both the strains(Figure.2).The effect of temperature at various levels was determined and showed a significant influence on growth rate at 35°C and 45°C subsequently. Estimation of calcium carbonate was done by EDTA titration method and calcium carbonate precipitated was 22.5% for *Bacillus sp* and 21% for *Pseudomonas sp* respectively(Figure.3).

Figure 1. Effect of Growth

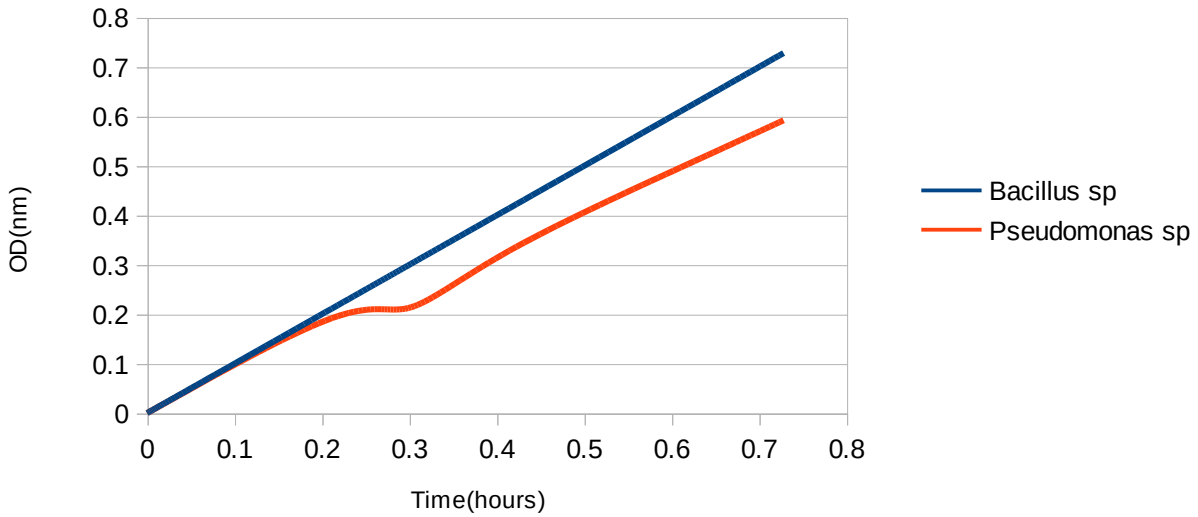


Figure 2. Effect of pH

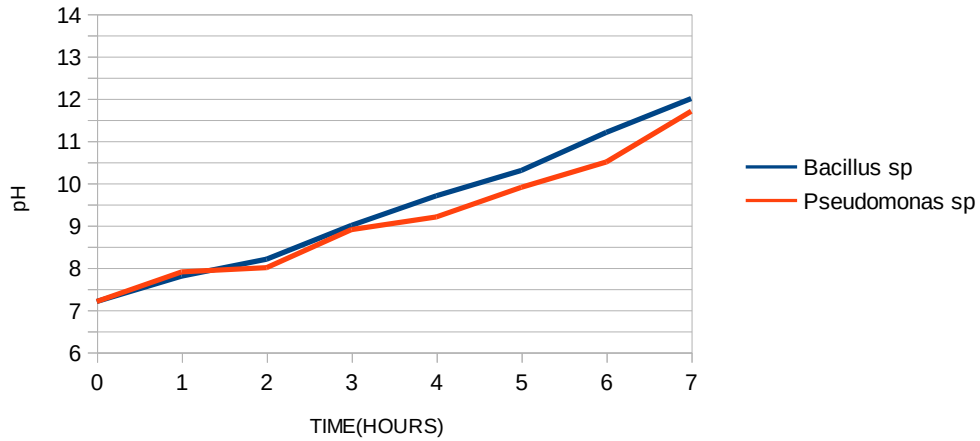
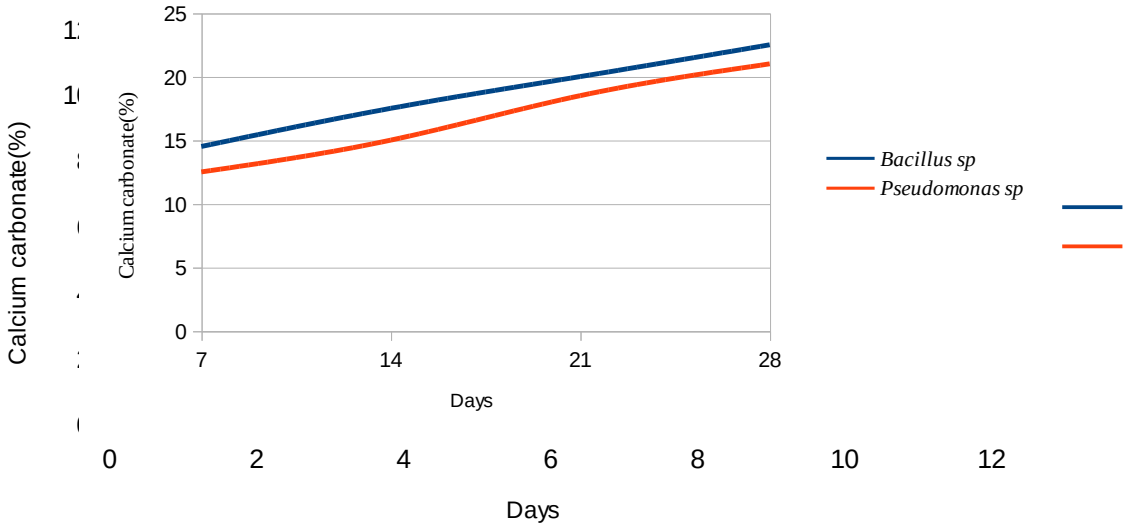


Figure 3.Calcium carbonate precipitation



After 28 days of incubation of calcium carbonate precipitation, FTIR spectroscopy was done to identify the calcium carbonate precipitates as Calcite. The I.R spectrum indicated in-plane bending and out-of-plane bending vibrations of the carbonate group with wave number 712cm^{-1} for *Bacillus sp(Figure 4)* and *Pseudomonas sp* had wave number 713cm^{-1}*(Figure 5)*.It was evident that the obtained calcite crystals when compared with I.R spectra which showed wave number 712 and 725cm^{-1} [7].

Figure 4. FTIR Spectroscopy of *Bacillus sp*

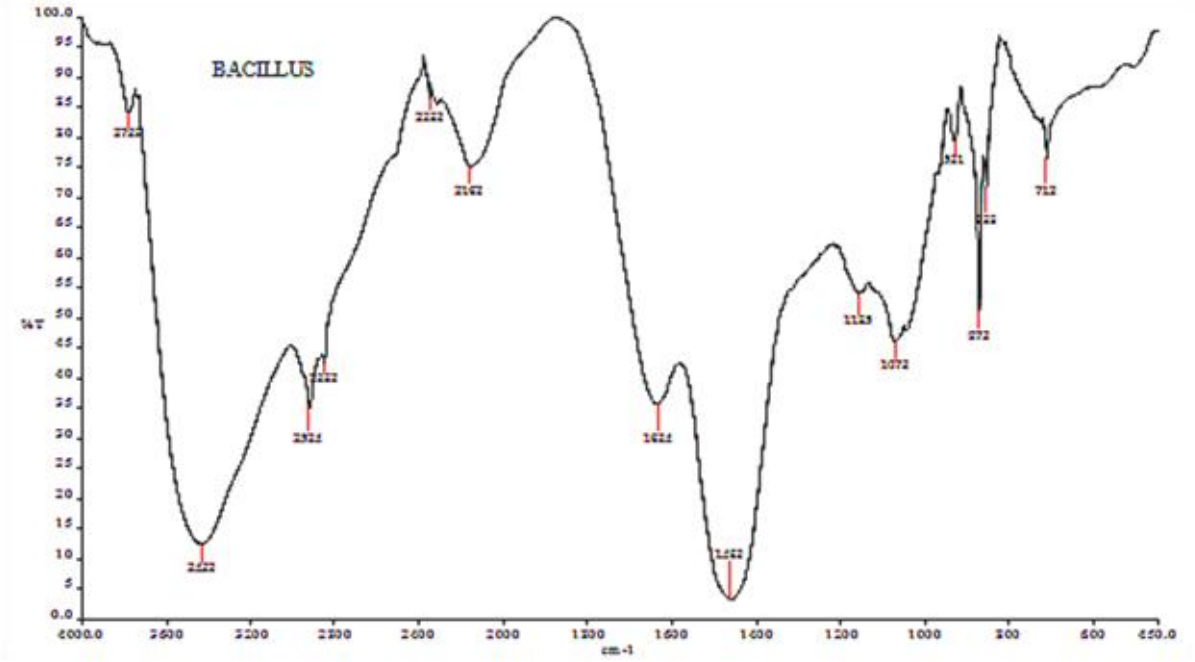
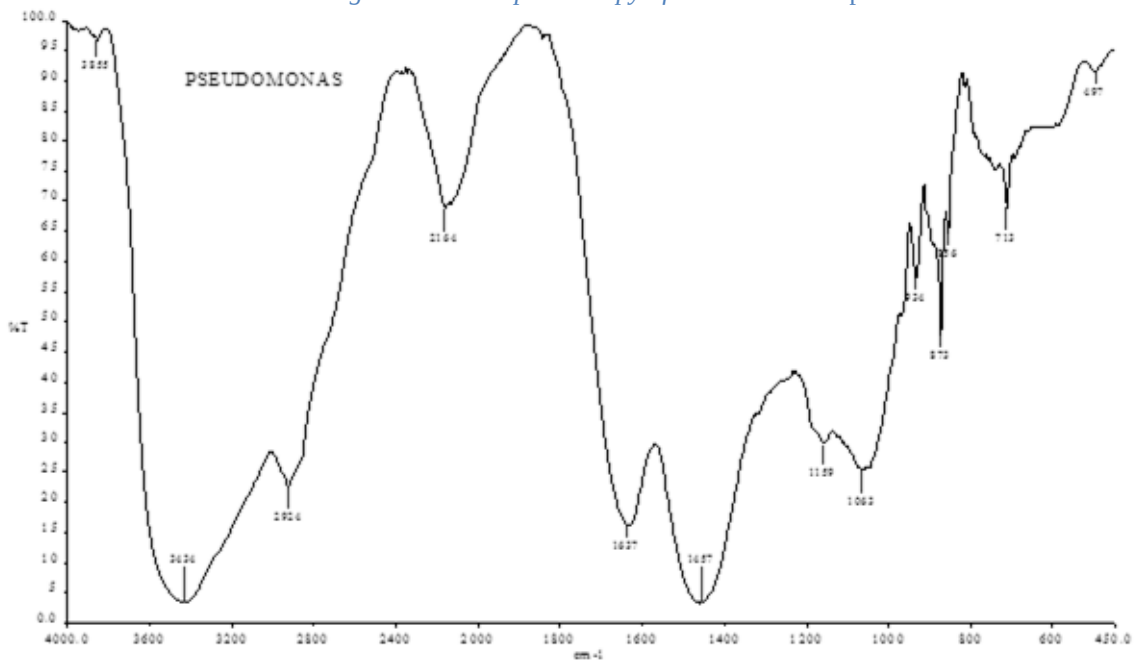


Figure 5. FTIR Spectroscopy of Pseudomonas sp



IV. DISCUSSION

Calcium carbonate biomineralization is a widespread process among organisms from bacteria to Chordata, but it was generally accepted that the mineralization capacities of prokaryotes and eukaryotes are different. Researchers have found that this microbial rate of precipitation was significantly faster than that of chemical precipitation. The chemical $CaCO_3$ precipitation had been controlled by the calcium ions concentration, carbonate concentration, pH and presence of nucleation sites [9]. Humans have the ability to precipitate minerals in the form of bones and teeth continuously. This ability is not only confined to human beings, even bacteria like *Bacillus*, a common soil bacterium can continuously precipitate calcite. This phenomenon is called microbiologically induced calcite

precipitation which comprises of a series of complex biochemical reactions. [15].

Calcium carbonate has been formed in the laboratory in association with different bacterial cultures which includes *Pseudomonas fluorescens* [16]. Microbiologically induced calcite precipitation by the bacterium *Sporosarcina pasteurii (*NCIM 2477)using the industrial effluent of the dairy industry, lactose mother liquor(LML) as growth medium. Calcite precipitation on sand plugging was measured by EDTA titration method and found to be maximum of 28.4% of the total weight of the sand samples plugged by *S. pasteurii* in the growth medium [1]. Growth comparison between *Bacillus subtilis* 168 and its mutant strain FBC5 strains in calcite precipitation was studied and preliminary analysis of EPS extracted from both the biofilms revealed similar FTIR patterns with wave number 1637 and 1544cm $^{-1}$ [12].

The current study demonstrated on isolation of bacteria from soil and its ability to precipitate calcium carbonate against influences of cell concentration, pH and temperature. *Bacillus sp* and *Pseudomonas sp* were isolated and had precipitated calcium carbonate and its efficiency is maximum at pH between 11-12 and temperature of 35°C to 45°C. Estimation of calcium carbonate was done by EDTA titration method and calcium carbonate precipitated was 22.5% for *Bacillus sp* and 21% for *Pseudomonas sp* respectively. The FTIR spectroscopy confirmed the precipitated obtained as calcium carbonate as the vibrations of the carbonate group with wave numbers 712 and 713cm $^{-1}$ for *Bacillus sp* and *Pseudomonas sp* respectively.

V. CONCLUSION

In the last few decades, growing attention has been paid to find out remedies against such degradation and the associated loss of our cultural heritage [11]. Many conservation treatments have been applied to the protection and consolidation of stone before extensive granular disintegration causes loss of surface material and therefore irreversible damage. Biomineralisation process forms precipitation and fills the pores that enhance the mechanical properties in concrete materials such as strength and durability. Calcite-forming bacteria has been reported in various geological environments including limestone caves, and soil. The present study carried out an *in vitro* experiments at an optimal condition emphasized on utilizing *Bacillus sp* and *Pseudomonas sp* in microbial calcium carbonate precipitation has wide applications ranging from bioremediation to conservation of monuments.

REFERENCES

[1] Achal, V., A. Mukherjee, P.C.Basu, M.S.Reddy. Lactose mother liquor as an alternative nutrient source for microbial concrete production by *Sporosarcina pasteurii*. J Ind Microbiol Biotechnol 36 (2009)433-438.

[2] APHA (American Public Health Association) Standard methods for the examination of water and wastewater, 7th edn. American Public Health Association,(1989) Washington, DC.

[3] Beavons.J and N.G. Heatley . J. Gen. Microbiol. 31**(**1962)167-169

[4] Castanier .S, Le Metayer- Levrel G. The role of microbial communities in the degradation and protection of cultural heritage New York, Plenun, (2000)203-218.

[5] Chafetz, H. S., and Buczynski, C. Bacterally induced lithification of microbial mats. Palaios , 7(1992) 277–293

[6] Chiara Barabesi, Alessandro Galizzi, Giorgio Mastromei, Mila Rossi, Elena Tamburini, and Brunella Perito. *Bacillus subtilis* Gene Cluster Involved in Calcium Carbonate Biomineralization. Journal of bacteriology, 189(2006) 228-351.

[7] Gunasekaran, S., Anbalagan, G. and Pandi, S. .Raman and infrared spectra of carbonates of calcite structure. Journal of Raman Spectroscopy, 37(2006) 892-899.

[8] Gonzalez-Munoz, M.T.Omar et al.,Struvite and calcite crystallization induced by cellular membranes of *Myxococcus xanthus* J.Cryst. Growth, 163(1996) 434-439.

[9] Hammes F, Verstraete W. Key roles of pH and calcium metabolism in microbial carbonate precipitation. Rev Environ Sci Biotech 1(2002) 3–7

[10] Jimenez-Lopez .C, F. Jroundi, M. Rodríguez-Gallego, J. M. Arias and M.T. Gonzalez-Munoz. Biomineralization induced by Myxobacteria Communicating Current Research and Educational Topics and Trends in Applied Microbiology(2007) 143-154.

[11] Jroundi F, Bedmar E.J, Rodriguez-Navarro C, Gonzalez-Munoz M.T. Consolidation of ornamental stone by microbial carbanatogenisis, Global Stone Congress. Microb Ecol 60(2010)39-54

[12] Massimiliano Marvasi, Pieter T.Visscher, Brunella Perito, Giorgio Mastromei, Lilliam Casillas-Martinez..Physiological requirements for carbonate precipitation during biofilm development of *Bacillus subtilis* etfA mutant. FEMS Microbiol Ecol 71(2010)341-350

[13] Park, S.J.,Yu-Mi,P.,Chun, W.Y.,Kim, W.J., Ghim, S-Y.Calcite-forming bacteria for compressive strength improvementin mortar.J.Microbiol., Biotechnol. 20(2010) 782-788.

[14] Rodriguez Navarro C, Rodriguez-Gallego M, Ben Chekroun K, Gonzalez-Muñoz MT.Conservation of ornamental stone by *Myxococcus xanthus* induced carbonate biomineralization. Appl Environ Microbiol, 69(2003)2182–2193**.**

[15] Stocks-Fischer S, Galinat JK, Bang S.S Microbiological precipitation of Calcium carbonate. Soil Biol Biochem, 31(1999)1563–1571.

[16] Sushmitha Baskar, R. Baskar ., Mauclaire L., Mackenzie J.A. Microbially induced calcite precipitation in culture experiments: Possible origin for stalactites in Sahastradha caves, Dehradun, India. Current Science, 90(2006) 58-64.