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Recovering of Cracks in Concrete to Enhance Its Strength by *Bacillus licheniformis* - A Biomineralization Study

Oshin Victor[1], [*]Freeda Christy C[2], Kavitha S[3] and Padmanabhan D[4]

[1 &2] Former PG Scholar, Department of Civil Engineering
[2]Associate Professor, Department of Civil Engineering
[3]Assistant Professor, Department of Biotechnology
[4]Former Research Scholar, Department of Biotechnology
Oshinvictor12@gmail.com, freeda@karunya.edu, kavibiotec@karunya.edu,
pathumicro@gmail.com
Karunya Institute of Technology and Sciences, Coimbatore 641 114, Tamil Nadu, India

Abstract

Concrete is a strong and durable material composed of cement, aggregate and water which is commonly used in construction. Concrete performs better under compression, but as the concrete is stressed internally, cracks are formed. The water and other salts penetrate through the cracks in concrete which may induce corrosion and reduce the durability of concrete. In such cases, the cracks and splits formed can be filled within itself with some bacteria by biomineralization process in order to enhance the properties of concrete. In our study, we examined the characteristics of few microbes that are extracted by mineral precipitation process especially to fill the cracks in the concrete. In the present investigation, gram positive spore shaping microorganism, such as Bacillus licheniformis, is used in concrete. It was found that the modulus of rupture of concrete were enhanced by more than 50%. Therefore, this ecofriendly bioconcrete technology can be very well used to protect concrete structures. The results obtained from the above experiment are discussed in this research article

Key words: Self-recovering, *Bacillus licheniformis* , bioconcrete, MICCP

1. INTRODUCTION

Concrete is a material which is predominant building material in construction industries. When the concrete is stressed internally because of load, it initiates multiple cracks in the concrete structures which will lead to different problems. Various reasons are found in the literature for internal cracking of concrete structures. Internal stresses in concrete may be due to shrinkage, solidification of defrost responses and mechanical compressive or ductile responses, Gandhimathi etal [1]. The corrosive chemicals such as water and chloride ions may enter directly through these cracked concrete structures and in due course of time this will result in the severe deterioration of steel bars corrosion in the reinforced concrete structures. Over the past few decades, bioconcrete technology is being developed worldwide to self-repair the concrete cracks. Therefore, research work is needed to study the suitable microorganism in order to precipitate sufficient calcium carbonate (calcite) from the concrete to fill the cracks found in concrete by suitable microbial mechanisms in the presence of moisture. This method of using microbes in bacterial concrete in order to fill the micro cracks formed by microbial induced calcium carbonate precipitation (MICCP) is known as bio mineralization. The concrete having spore forming bacteria which precipitate calcite continuously is said to be bacterial concrete Richard A. Raiders etal [2, 3]. Microbial calcite precipitation occurs mainly due to ureolytic activity and bio-mineralization of bacteria, [4]. Significant research on precipitation of carbonate by ureolytic bacteria [5]. Urease positive bacteria has been found to influence the precipitation of calcium carbonate by the production of the enzyme urease. This urease enzyme can be used to hydrolyse urea to carbon-di-oxide and ammonia, which may enhance the carbonate absorption in concrete by maintaining suitable pH [6]. Previous

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techniques were based on microbial mineral precipitation led to the use of micro-organism to cure the cracks in concrete. Different microorganisms were used to increase the compressive strength of cement mortar and for the remediation of cracks in concrete. Jasira Bashi et al [7] experimented on bio-concrete specimens with different bacteria and observed that the strength gained as a result of sprouting of filler material inside the cement - sand matrix pores. The compressive strength of bio concrete was increased upto 30% *using B. pasteurii* and 52% by using *B. Sphaericus*. Mayur Shantilal Vekariya et al [8] identified the bacteria *Bacillus* as the self-healing agent along-with synthetic polymers together with epoxy treatment. Microbial concrete technology is reported as better than the other conventional technologies because of its eco- friendly nature, self-recovering behaviour and durability in construction field. Seshagiri Rao et al [9] used *Bacillus* bacteria as self-healing agent in concrete structures as it lowered the water absorption, sorptivity and porosity values. Chintalpudi and Rama Mohan et al [10] observed that with mixing of alkaliphilic aerobic microorganism *Bacillus subtilis JC3* in cement mortar samples along-with water resulted in the enhancement of compressive strength of cement mortar via. 25% in 28 days. Ruben Boelens et al [11] used alkali-resistant bacteria in the concrete to self-heal the concrete and it took 40 days. Henk Jonkers and Schlangen, [12] investigated the potential of different species to precipitate calcite that produce endospores, which healed the cracks in concrete extensively. De Belie and De Muynck [13] produced a bioceramic material from mixing of bacteria in a silica gel. This was used to bridge the cracks found in the concrete. Based on the continuous research work carried out around the globe, various modifications have been made from time to time to overcome the problems associated with the bioconcrete technology. Several researchers investigated that the bio-mineralization in cement concrete can result in self -healing phenomena. In the present study, the potential bacterium was identified as *Bacillus licheniformis* as self- recovering bacteria in concrete. The results of using these bacteria in concrete structures are discussed in the forthcoming sections.

2. BACTERIAL STUDY

The availability of bacteria from the source was identified by gram staining test and the peptidoglycan layer which preserves the shape and rigidity of the bacterial study was carried out. Bacterial culture of *Bacillus sp.* was cultivated by preparing the nutrient broth with the ingredient of 1 gm/l of peptone with 1gm/l of NaCl along with yeast extract 0.6 gm/l which acts as nitrogen source for bacterial growth. 200 ml of distilled water is added and adjusted to a neutral pH of 6.8 at 25^{O}C. The nutrient broth was first autoclaved at 121°C for 15 minutes and the bacterial samples were inoculated into it and incubated in a shaker at 37°C for 24 hours. The samples were also tested for urease activity, their morphology and gram reaction. The bacterial isolates were characterized by conventional physiological and biochemical characterization tests. With the identified bacteria *Bascilius licheniformis,* the microbial induced calcium carbonate precipitate was studied to recover the concrete cracks formed. The enhancement of mechanical properties of the bio-concrete were studied with the bacteria *Bascilius licheniformis* with calcium lactate of 0%, 5% and 10% in the concrete.

3. RESULTS AND DISCUSSION

The microbial induced calcium precipitation (MICCP) by urealytic bacteria was carried out to study the calcium carbonate precipitation to recover the cracks in concrete. Also in-vitro test for calcium carbonate precipitation in cement mortar was studied. Then the bio –concrete was studied for the improvement of the mechanical properties of concrete. The bacteria was isolated, cultivated and its survival in alkaline medium was made by urease test method as concrete is in alkaline medium with pH value 13. Urease test was carried out by preparing urea broth by adding 20gms/l of urea with 5gms/l of NaCl, 2 gms/l of mono Potassium phosphate, 1gm/l of peptone and Dextrose, 0.012 gm/l of Phenol red and 150 gms/l of agar. A loop full of *Bacillus licheniformis* was inoculated into the urea broth and incubated at 37°C for 24-48 hrs. Obtained the broths from the incubator and observed the colour change as indicated in Fig 1.

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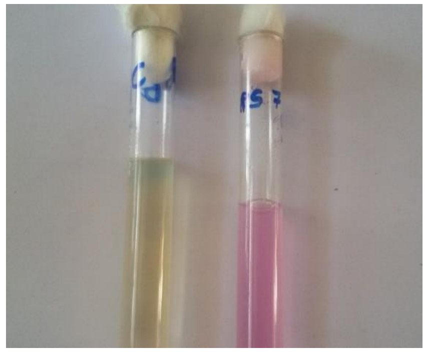

Fig. 1 Colour changes to Pink

The colour changes from light orange to pink, because of the change in the alkaline medium (ie) pH value, as ammonia changes the medium basic and the ammonia formation by urealytic activity was indicated in the reaction (1, 2). This indicates that the bacteria, *Bacillus licheniformis* can be survived in the alkaline environment as the concrete is alkaline.

$$H_2N\,CONH_2 + H_2O \xrightarrow{Urease} NH_3 + H_2NCOOH \quad \text{-----} (1)$$
Urea + water ammonia + Carbamic acid

$$H_2NCOOH + H_2O \longrightarrow NH_3 + H_2CO_3 \quad \text{-------} (2)$$
Carbamic acid + water ammonia +Carbonic acid

The presence of bacteria influences the precipitation of calcium carbonate by the production of a urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of the pH and carbonate concentration in the bacterial environment [14]. The hydrolysis of urea involves microbial process and a wide variety of microorganisms produce the urease enzyme which makes it ideally suited for biotechnological applications. Precipitation of calcium carbonate crystals occurs by heterogeneous nucleation on the bacterial cell wall. The suitability of bacteria in concrete and its cell wall was identified by Gram staining method. This test classify the microorganisms into Gram Positive bacteria with thick walled peptidoglycan and Gram Negative bacteria with thin walled peptidoglycan. Bacterial smear was structured on a glass slide. Smear was flooded with crystal violet (CV) for 60 sec. It was then washed lightly in water and the excess crystal violet was eliminated. Later it was flooded with Gram's iodine for 60 sec. Smear was then decolorized with ethanol for 10 sec and washed with water. Counterstaining with safranin for 60 sec and washed with water to dispose of the immoderate stain. Safranin stains them red. The interaction of bacterial enzymes changes the colour with differential substrates inside the medium. Stain dark purple retain the primary dye called Crystal Violet in the cell wall. Finally samples were visualized underneath the microscope at different magnification and observed for the Gram response and morphology of the bacterial cells had been indicated in Fig. 2.

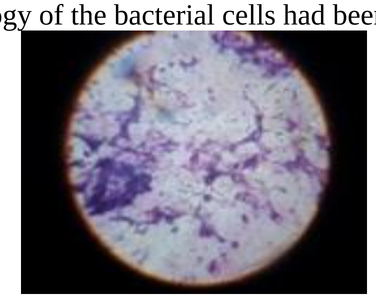

Fig. 2 Crystal Violet in the cell wall indicates Gram-Staining Positive

The crystal violet and iodine form an insoluble complex that is bound to the thick layer of peptidoglycan of Gram positive bacteria that appears blue or dark purple in colour. This thick wall gets dehydrated as it undergoes decolourization and the cell pores are closed as it shrinks which prevents the stain to move out

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from the cell. As the bacteria Bacillus lichineformis showed Gram positive staining indicated the thick walled peptidoglycan which preserves the cell walls of the bacteria and its shape in the alkaline concrete.
Then the study was carried out for bio-mineralisation process which influences the $CaCO_3$ crystal formation and the ureolytic activity are greatly affected with calcium concentrations, pH value and dissolved inorganic carbon concentrations. The bio minerals are formed by the chemical process with the influence of urease [15]. These parameters impact the carbonate particles focus and constant calcium carbonate development [16]. Microbes fill in as nucleation destinations in the biomineralization processes, for precipitation of calcium carbonate with the microorganisms. These parameters incredibly influence either the ureolytic movement or $CaCO_3$ crystal formation. For the bacterial growth, the hydrogen ion concentration of an organism's environment plays the major role. It limits the fusing of bacterial enzymes which is responsible for synthesizing the new protoplasm. Since concrete is in highly alkaline condition, the bacteria identified should survive in the highly alkaline condition. So the identified bacteria, *Bacillus licheniformis* were grown in the nutrient agar of different pH range of 7 to 9 prepared in a conical flask and sterilized in an autoclave at 121°C for 15 minutes. Then, it was poured in the nutrient medium into 4 petri plates each with different pH as shown in Fig 3.

(a) pH – 7 **(b) pH – 7.5** **(c) pH – 8** **(d) pH – 9**

Fig. 3 (a) – (d) : *Bacillus licheniformis* survival at various pH

In the stir of cementing, the *Bacillus licheniformis* suspension was streaked into each petri plates and incubated at 37°C for 24hrs. From Fig.3, the development of microscopic organisms at different pH was observed. *Bacillus licheniformis* survived in the high alkaline condition. Hence, it may be very well utilized in the concrete to plug the cracks.
In order to recover the cracks in the concrete, the bacteria present in the concrete should be able to convert the soluble organic nutrients into insoluble inorganic calcite crystals which seals the cracks [9]. Calcium carbonate precipitation test were carried out by preparing controlled B4 medium for mineral precipitation with 60 gms/l of calcium acetate, 12 gms/l of yeast extract and 12 gms/l of glucose. Later the bacterial sample were grown aerobically in 50 ml of modified B4 medium with bacterial inoculums. All bacterial stains were incubated at 30^{O}C up to 3 weeks. The precipitated carbonates were collected on Whatttman No.1 filter paper by filtration, washed with sterile distilled water and air dried at 37^{O}C. The crystals precipitated were collected and the amount of carbonate crystals precipitated by different isolates were estimated. Microstructure analysis of the precipitated carbonate crystals was carried out by SEM as shown in Fig 4.

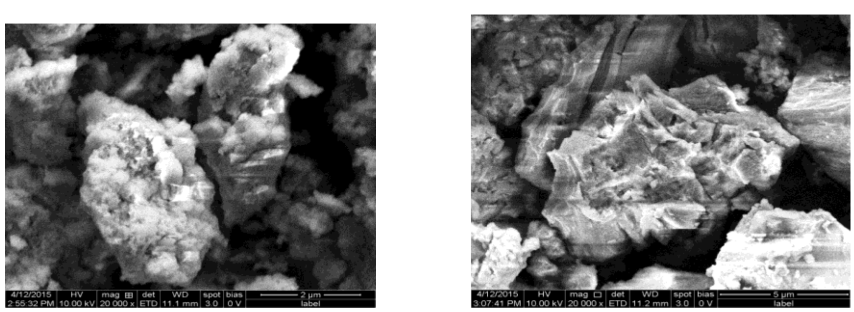

Fig. 4 SEM micrographs of different morpohologies of calcite crystal precipitated

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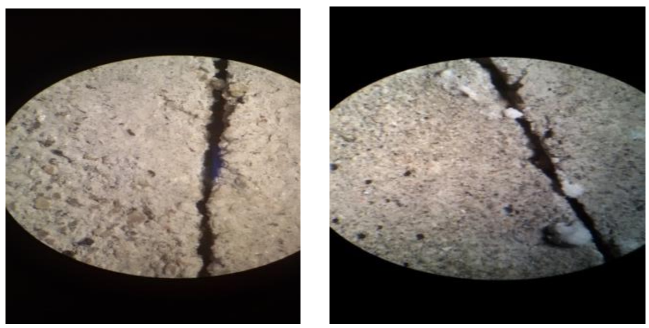
From Fig. 4, the SEM analysis with *Bacillus licheniformis* confirmed the presence of distinct calcite crystal precipitation in the bacterial samples which improves the bonding property in concrete. The presence of crystalline calcite associated with bacteria indicated that bacteria serve as nucleation sites during mineralization process [17]. The calcium carbonate precipitation of bacteria in the bio concrete was studied in cement mortar by In-vitro method. Cement mortar samples were prepared with aggregate to cement ratio as 2:1 with 0.5 water to cement ratio. These concrete mixture is prepared in a petri plate and kept for drying. It is taken as control specimen. Concrete mixtures with different bacterial concentrations were then prepared in different petri plates with bacterial culture added to the water content. Cracks were created manually on the semi set cement mortar. Then the cement mortar was dried at room temperature and observed the crack healing capacity under microscope after incubating the cement mortar for 7 days. The microbial induced calcite carbonate precipitation was formed as shown in Fig. 5.



(a) **(b)**

Fig. 5 (a) Crack in cement mortar
(b)$CaCO_3$ precipitation in cement mortar

Bacteria on fresh crack surfaces become activated due to water ingression, start to multiply and precipitate minerals such as calcite ($CaCO_3$) which eventually sealed the crack. With the identified bacteria, the bio-concrete was prepared with the M20 mix. Two sets of samples were prepared as one set of concrete with bacteria as bioconcrete, the test mix and the other set of concrete without bacteria as the control mix. The identified bacteria, Bacillus licheniformis along with the calcium-based nutrient known as calcium lactate in 5%, 10%, 15% as nutrient were added to the ingredients of the concrete at the mixing stage of the fresh concrete as shown in Figure 6, 7 and 8. The bacteria that was mixed to the concrete involves two mechanisms, bacteria acts as a catalyst and calcium lactate i.e. the mineral precursor converts it to calcium carbonate minerals as indicated in reaction (3).

$$2Ca(CHO_2)_2 + 2O_2 + \text{Bacteria} \xrightarrow[\text{oxygen}]{} 2\,CaCO_3 + 2CO_2 + 2H_2O \qquad \text{---------------} (3)$$

Calcium Lactate + Bacteria →Calcium Carbonate + carbondioxide + water

Fig. 6 Bacteria *Bacillus licheniformis* with Calcium Lactate

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Fig. 7 Bacteria added to the mix

Concrete, an alkaline material with pH as high as 13. This alkalinity imparts concrete the protection against corrosion. Since *Bacillus licheniformis* survived in high alkaline environment, study was carried out on bioconcrete and with the addition of the bacteria *Bacillus licheniformis* along with calcium lactate as nutrients by 5%, 10% and 15% weight of cement at the stage of preparation of the concrete by mixing the spore suspension in concrete mixing water for microbial activities to form inorganic solids as indicated in Fig 8 (a). As the crack appears in the concrete, water enters through the crack it activates the dormant (inactive) bacteria *Bacillus licheniformis* by the process of metabolically mediated calcium carbonate precipitation and it recovers the concrete cracks. Calcium hydroxide being soluble in nature dissolves in excess water and comes out from cracks as leaching, indicated in Fig 8 (b). The cracks were plugged because by Bacillus *licheniformis* in concrete and it was healed as indicated in Fig. 8 (c). A biological approach of self-recovery in concrete is one such crack remediation methods in which bacteria *Bacillus licheniformis* induce bio-minerals during their growth and metabolism and fills the cracks and pores in the concrete.

(a)

(b)

(c)

(a) Crack in the concrete
(b) Calcium carbonate formation
(c) Cracks filled in by *Bacillus licheniformis* precipitation
Fig. 8 Recovery of crack in concrete

Initially the crack width was measured using micrometer and the crack width was observed as 0.3 mm as shown in Fig. 9

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Fig. 9 Crack width measuring using micrometer

In self-recovering concrete, active metabolic conversion of calcium nutrients takes place with *Bacillus licheniformis* in the presence of moisture content and the crack width was reduced from 0.3mm to 0.05mm. The cracks were plugged in the concrete to about 83%. This 'self- recovering' property brings about water tightness and thus restricts the infiltration of destructive substances into concrete structures and improves the concrete durability. Then the flexural strength of the concrete was studied as it plays a major role in the structural design of pavement concrete. The flexural strength is referred as the modulus of rupture of the concrete. Modulus of rupture were performed on 100 x 100 x 500mm prisms as per ASTM C 78 [18] as shown in Fig.10

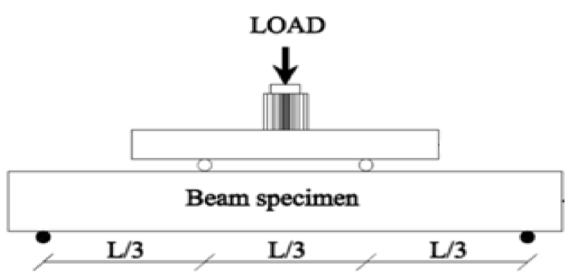

Fig. 10 Flexural testing

Concrete specimens for beams were cast with M_{20} grade concrete with w/c ratio of 0.50. The bacteria *Bacillus licheniformis*, with calcium lactate were added as 5%, 10% and 15% as nutrient in the M_{20} concrete mix. The modulus of rupture of concrete were determined by third-point loading in the flexural testing method and the results were indicated in Table 1.

Table 1: Modulus of rupture of concrete, f_b (N/mm^2)

	Control mix	Control mix with calcium lactate			Bio concrete with *Bacillus licheniformis* and calcium lactate		
	0%	5%	10%	15%	5%	10%	15%
Sample 1	3.52	3.62	3.82	3.92	5.00	5.68	5.68
Sample 2	3.57	3.72	4.02	4.02	5.39	6.47	6.57
Sample 3	3.62	3.72	3.92	3.82	5.24	6.57	6.47

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From Table 1, as the internal cracks occurred, the bacteria became activated and precipitate calcium carbonate and plugs the cracks which enhanced the flexural strength of the bioconcrete. The Modulus of Rupture of the concrete and the bioconcrete with the bacteria '*Bacillus licheniformis'* were determined and indicated in Fig. 11.

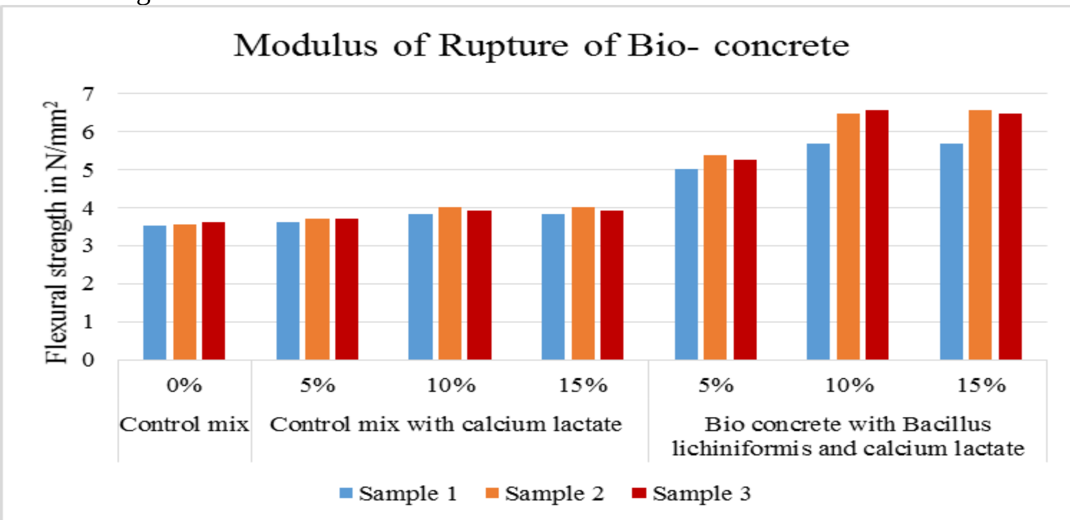

Fig. 11 Modulus of Rupture of Bio concrete

It was observed from the Fig.11 that the cracks were plugged through bio-mineralization and the flexural strength of the concrete was increased twice with the addition of 10% and 15% of *Bacillus licheniformis* in the concrete. The *B. licheniformis* increased the flexural strength of about 48% in this study whereas in the study conducted by Anandasu et al [19] the tensile strength of bio concrete using B. subtilis increased 38.17% and B. sphaericus increased 31.14%. Stronger physical bond between the rough-textured aggregate and the cement paste is responsible for the increased tensile strength [20]. The ecofriendly nature of bio concrete may be used in construction industries.

4 CONCLUSIONS

In this study, based on the phenotypic and biochemical characterization, the bacteria identified was *Bacillus licheniformis* isolated from estuary sediments as self- recovering agent.

- *Bacillus licheniformis* grew well in minimal salt medium without any additional carbon source which proved to be safe and cost effective.
- *Bacillus licheniformis* had influenced the precipitation of calcium carbonate by the creation of urease enzyme as well as the crystal image which improved the bonding property.
- *Bacillus licheniformis*, self - recovered the concrete by improving the hydrated structure of cement concrete.
- The crack formed in the concrete was filled with the calcium carbonate precipitate and the pore size was plugged upto 83%.
- The addition of 10% and 15% *Bacillus licheniformis* in concrete enhanced the modulus of rupture of concrete by 48% and 52% respectively. The ecofriendly nature of bio concrete may be used in construction industries.

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Data Availability Statement:

The Phylogenetic affiliation of each 16SrRNA sequence was carried out and the pylogenetic tree was constructed. The potential bacterium was identified as *Bacillus licheniformis* under accession number KJ933861 deposited in GenBank. The weblink for NCBI submission of PS-6 [URL:https://www.ncbi.nlm.nih.gov/nuccore/KJ933861].

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