## Exploration of a Hot Spring Alkaliphilic Bacterium and its Secretory Protein Pertinent to Self-healing Bio-concrete Material

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Abstract: Hot springs have always drawn attention due to their unique chemical richness and the presence of different microbial communities. The use of hot spring bacteria and its excretory protein in concrete technology is our primary focus. Unavoidable cracks cause a significant reduction in the strength and longevity of concrete. Water and several harmful ions seep through the cracks, initiate corrosion of the reinforcement, and affect the self-life of concrete. Self-repaired concrete will stand for a longer period and thus is gaining interest for constructions purposes. A novel facultative anaerobic and highly alkaliphilic bacterial strain (BKH4; GenBank accession no.: KX622782), which belongs to the family 'Bacillaceae' and homologous (99%) with Lysinibacillus fusiformis was isolated from Bakreshwar hot springs. The isolated coccoid-type Gram-positive bacterium grows well in a defined semi-synthetic medium (pH 12 and 65 °C). This bacterium survives for more than a month inside the concrete environment and shows better efficacy in enhancing compressive strengths (> 50%), ultrasonic pulse velocity (> 25%) and durability of the cementitious mortar when incorporated at a concentration of 10<sup>4</sup> cells per ml of water used. The novel bacterium BKH4 is more effective for the enhancement of the bio-concrete properties. One specific protein was isolated from BKH4 to prepare control and protein-amended cementitious mortar samples, which were subjected to simulate cracks and cured under water for several days. Images and microstructures of the control and protein-incorporated samples established the fact that there was a tiny fingers-like crystalline novel substance developed on the cracked regions. The developed substance was identified as a new phase of silicate compound (Gehlenite) by X-ray and energy dispersive X-ray spectroscopic analyses. The microbial protein enhanced the mechanical strengths and durability of the protein-incorporated samples that were supported by the increments of ultrasonic pulse velocity and compressive strength. The results of sulphate resistance activity, chloride permeability as well as reduction of water permeability and slow water movement (sorptivity test) of the experimental samples confirmed the increased longevity of the protein amended samples. This self-healing phenomenon of the bacterial protein incorporated samples is an added advantage here which is eco-efficient and developed due to the bio-silicification action of the microbial protein incorporated mortar samples. BKH4 bacterium which grows at high alkaline pH (12.0) will add a new dimension to future concrete technology for its usefulness in strength enhancement and durability due to its alkaliphilic and self-healing natures as well as its longer survival ability within a cementitious environment.

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