DISCUSSION
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Muscovite is a K-bearing mineral the belongs to the mica group of silicate minerals. Muscovite is commonly found in metamorphic rocks (Schist’s and gneisses) sedimentary rocks, and igneous rocks (such as granite). During natural weathering the void spaces inside muscovite lattice, changes in inter layer spacing and drying of lattice occurs due to the presence of CaCO₃ and release K⁺ from muscovite. This creates an alkaline environment. Further, by nature Igneous rocks which contains Na⁺ or K⁺ content are alkaline in nature.

Potassium is the third major essential nutrient for plant growth. So Potassium is added to soil in the form of Potassic fertilizers. India ranks fourth after USA, China, and Brazil as for as the total consumption of K- fertilizers in the world is concerned (FAI.2007). The muscovite contains a significant amount (8-12% K₂O) of Potassium (Nishanth and Biswas, 2008). Further, the sub surface environment mimics ‘concrete’ environments in terms of non availability of O₂, nutrients etc. With all these in mind, this study has focussed on simple methods of isolating potential microbes from the muscovite ore, and subsequently tested their potential in mobilization of K/P from the mineral and their bio mineralization ability.

The microbial studies pertaining to deep surface often contain unique assets, and they are ideal for several biotechnological and environmental applications.

Further the microbial studies pertaining to Muscovite ore are very scarce. The choice of indigenous microbes for this investigation is essentially due to possibility of their better acclimatization to the bio beneficiation environments.

In the present study isolation and preliminary characterization of indigenous alkaliphiles to muscovite mine coupled with a molecular approach in which case PCR amplification of 16S rDNA sequence based bacterial identification was attempted. Further their chemical signatures were also analysed.

Alkaliphilic prokaryotes, in their rich phylogenetic diversity and metabolic varsality are central participants in useful bioprocessing settings (Sorokin et al., 2008; Sarethy et al., 2011). A range of pH values are used by different investigation to define extreme alkaliphiles, alkaliphiles and alkaline tolerant bacteria. Extremely
alkaliphilic bacteria are generally defined as those that grow at an external pH ≥10 (the more extreme strains growing at pH ≥12), moderate alkaliphiles as those that can grow in the pH 9-10 range, the alkaline tolerant bacteria as those that can survive and grow sub optimally at~ pH 9 (Krulwich and Ita). It has always been a very interesting and challenging area to explore microbes that resides in extreme environment. Alkaliphiles were isolated using culture dependent analysis. Microbial communities in subsurface muscovite mine have escaped our attention so far. These mines harbour unique extreme environments for microorganisms, both natural and anthropogenic, including extreme temperature pressure, low oxygen concentration toxic heavy metals oligotrophic conditions, low water availability and pH. The physical and chemical characterization of the sample also revealed its nature.

Selective isolation of alkaliphiles were done using PPYG medium with a pH of 10.5 and above (Gee et al., 1980; Horikoshi, 1999). Which in the present study has been maintained using sterile sodium carbonate. It has been reported that the pH of the medium can be increased using higher concentration of sodium carbonate (Grant and Horikoshi, 1989; Kobayashi and Horikoshi, 1980). Hence the sodium salts are favoured as the compounds for pH change for growth of alkaliphiles (Horikoshi 1991).

The bacteria primary belonging to the phylum Firmicutes. This phylum consists of gram positive cell wall structure. Scientist once classified the Firmicutes to include all gram positive bacteria, but have recently defined them to be of a core group of related forms called the low G+C group. They have round cells or rod like forms. Many Firmicutes produce endospores, which are resistant to dessication and can survive extreme environments. They are found in various environments. Surveys of gene sequence diversity from environment DNA have indicated that fewer than 1% of bacterial species are cultivable at present (Giovannoni and Stingl 2005). So, traditional laboratory based studies of pure culture isolates have been blind to over 99% of bacterial diversity as happy in our study. A likely explanation is that the overly plates/medium we used did not support the growth of these microorganisms and inability to culture the vaste majority of microorganisms under laboratory conditions (Gans et al., 2005). Representatives of phylum Firmicutes were more. A possible explanation is that some representatives of this phylum such as bacteria of the genus Bacillus use as survival strategy in a rapid growth when there are nutrients in abundant quantities, which is known as strategy (Alas R. M. Bants, R. (1999)
“Microbial evolution and biodiversity the origins of life fourth book news Inc. Portland”). These strategists are not good competitors, having rapid multiplication as their only adaptation advantage in relation to competitors. They prevail only if there is a great quantity of available nutrients in low-competition areas. Consequently, there are huge variations in their populations. Strategists are usually found in unstable environments suffering transitions.

Exploring bacterial diversity is basically done by amplifying rRNA genes, from DNA samples isolated from different habitat. 16S rRNA genes are among the evolutionary most conserved macromolecules. The sequences are then compared to each other and to the 16S rRNA sequences from known species if no close match to an existing 16S rRNA gene sequence is found, then the test sequences thought to represent a new bacterium and is listed in Genbank as “uncultured bacterium”. Even in well studied, discrete places like the human mouth, new groups of uncultured bacteria continue to be discovered all the time. A newly identified organism has to be isolated and cultured in the lab to be described further. The accumulating database of 16S rRNA sequences, which now includes over GQ375226.1, HM006903.1, HQ831404, HM071942.1, JN 644502.1, AB 5253891 and HQ231210.1 (16S rRNA) provides an extra incentive to focus on microbial diversity of extreme microbial flora whose unidentified sequence similarities can be matched with these reported sequences to find the close and divergent relatives of microbial ecosystem. Due to this vast availability of data and reliability of technique it is one of the most used methods of species identification for large bacterial populations. That is why we have chosen the 16S rRNA gene sequencing for identification of all bacterial isolates.

In our study 16S rRNA based identification revealed that our isolates are SVUNM4, SVUNM8, SVUNM9, SVUNM11, SVUNM13, SVUNM14 and SVUNM15. The genus *Bacillus subtilis* is composed of rod shaped, endospore forming bacteria that are members of the phylum ‘Firmicutes’. Owing largely to the fact that they are common inhabitants of soil and aquatic sediment, species within the genus are widespread in nature and are found in virtually every environment. Our study reinforces the observation based on the morphological, physiological; biochemical or other properties (Alejandro *et al.*, 2009; Roberts *et al.*, 1994, 1996; Chun and Bae, 2000; Palmisano *et al.*, 2001; Ruiz-Garcia *et al.*, 2005a,b; Gatson *et al.*, 2006).
The genus *B. mylotrophics* is one of the earliest methylotrophic organisms with Gram–positive bacterium. Later it was renamed as “*Bacterium methylicum*”. The aerobic non spore forming, facultative methylotroph. These methylotrophic strains of this genus displays a strong resistance to high methanol concentration (Munuswamy *et al*., 2010). This bacterium was also best studied biocontrol, plant–growth promoting and Bioremediation activities. The genus *Peanibacillus* was proposed by reclassification of 11 *Bacillus* species by Ash *et al*., (1993). Since the description of the genus, continuous reclassification of some other *Bacillus* species and description of new species have increased considerably the number of species belonging to the genus *Peanibacillus* (Heyndrickx *et al*., 1996a, Shida *et al*., 1997b). The genus comprises at least 60 species with validly published names, including the recently described species, *Paenibacillus xylanilyticus* (Rivas *et al*., 2005a) *Paenibacillus phyllospharae* (Rivas *et al*., 2005b) *Paenibacillus hodagayensis* (Takeda *et al*., 2005) *Paenibacillus barcinonensis* (Sanchez *et al*., 2005). In this study we report on the taxonomic characterisation of a *Paenibacillus* like bacterial strain from alkaline muscovite mica from India.

The genus *Stenotrophomonas* was first described by Palleroni and Bradbury (1993) and, at present, the genus comprises the five species *Stenotrophomonas acidaminiphila* (Assih *et al*., 2002). *S. Africana* (Drancourt *et al*., 1997), *S. maltophilia* (Hugh, 1981; Palleroni and Bradbury 1993), *S. nitritireducens* (Finkmann *et al*., 2000) and *S. rhizophila* (Wolf *et al*. 2002). *Stenotrophomonas maltophilia*, formerly known as *Pseudomonas* or *Xanthomonas maltophilia*, is an aerobic, glucose non fermentative, Gram-negative *Bacillus* (Denton and Kerr, 1998; Muder *et al*., 1987; Palleroni *et al*., 1993) that is frequently isolated from water, soil, animals, plant materials and hospital equipment (Borner *et al*., (2003); Gandu *et al*., (1996) ). *Stenotrophomonas koreensis* isolated from compost (Yang *et al*., 2006), *Stenotrophomonas daejeonensis* (Lee *et al*. 2011) *Stenotrophomonas pavanii* (Ramos *et al*. 2011), and *Stenotrophomonas terrae* and *Stenotrophomonas humi*, nitrate- reducing species were isolated from soil samples (Heylen *et al*., 2007). *Stenotrophomonas chelatiphaga*, isolate sewage sludge, was validly published (Kaparullina *et al*., 2009, 2010). This bacterium is generally considered to be an opportunistic pathogen, (Denton and Kerr, 1998; Muder *et al*., 1987; Khardori *et al*., 1990; Elting and Bidey, 1990; Marshall *et al*., 1989; Zuravleff and Yu, 1982) causing various infections, including bacteraemia, urinary tract infections,
respiratory tract infections, skin and soft tissue infections, endocarditis meningitis and ocular infections (Murder et al., 1987; Khadori et al., 1990; Zuravleff and Yu, 1982; Holmes et al., 1979; Morrrison et al., 1986). Although S. maltophilia causes mainly nosocomial infections, (Denton et al., 1998; Calza et al., 2003) community-acquired infections may also occur (Heath et al., 1995). It was also commonly isolated from patients with cystic fibrosis (Marchac et al., 2004; San Gabriel et al., 2004). Standard microbiology reference data currently indicate that S. maltophilia is an oxidase-negative bacterium. Although Stenotrophomonas is ubiquitous, the type species Stenotrophomonas maltophilia was preferentially recovered from the rhizosphere of plants like wheat, oat, cucumber, maize and cabbage (Berg et al., 1996). Due to the beneficial interactions with plants which promote plant growth, the gram-negative bacterium has become important for biotechnological applications in agriculture. For instance, S. maltophilia is applied for biological control of fungal plant diseases (Berg et al., 1996; Dunne et al., 2000) and supports plant development on marginal Soil (Taghavi et al., 2009). Furthermore, the property of metabolizing a broad range of organic compounds in conjunction with a high metal tolerance makes it attractive for bioremediation purposes (Alonso and Martinez, 2000). In the last years, S. maltophilia has also emerged as a human pathogen in immunosuppressed patients (Looney et al., 2009). Although not highly virulent, the bacterium can cause various bacteraemic infection diseases or pneumonia and has been isolated from cystic fibrosis patients (Gross et al., 2004). S. maltophilia produces numerous hydrolytic enzymes like chitinases, glucanases, lipases, laccases and proteases (Ryan et al., 2009). The latter are known to contribute to the biocontrol activity. For instance, an extracellular protease from strain G-2 was shown to be an important factor in virulence against a plant-parasitic nematode (Huang et al., 2009). Overproduction of extracellular proteolytic activity by mutagenesis of strain W81 resulted in significantly enhanced suppression of the phytopathogenic fungi Phytium ultimum (Dunne et al., 1997). Besides, that clinical sources extracellular serine proteases were isolated and characterized from clinical source also (Windhorst et al., 2002; Nicoletti et al., 2010).

The genus of Bacillus licheniformis is a Gram-positive, spore-forming bacterium widely distributed as a saprophytic organism in the environment. This species is a close relative of Bacillus subtilis, an organism that is second only to
Escherichia coli in the level of detail at which it has been studied. Unlike most other bacilli, which are predominantly aerobic, B. licheniformis is a facultative anaerobe, which may allow it to grow in additional ecological niches. Certain B. licheniformis isolates are capable of de-nitrification; the relevance of this characteristic to environmental de-nitrification may be small, however, as the species generally persists in soil as endospores (Alexander M et al., 1977). B. licheniformis can be differentiated from other bacilli on the basis of metabolic and physiological tests (Logan and Berkeley, 1981; O'Donnell et al., 1980) however, biochemical and phenotypic characteristics may be ambiguous among closely related species. Recent taxonomic studies indicate that B. licheniformis is closely related to B. subtilis and Bacillus amyloliquefaciens on the basis of comparisons of 16S rDNA and 16S-23S internal transcribed spacer (ITS) nucleotide sequences (Xu and Cote, 2003). Lapidus et al., (2002) recently constructed a physical map of the B. licheniformis chromosome using a PCR approach, and established a number of regions of colinearity where gene content and organization were conserved with the B. subtilis genome. B. licheniformis is an industrial organism used for the manufacture of enzymes, antibiotics, and chemicals and also plays an important role in nutrient cycling. The organism was never reported to be pathogenic for either animals or plants and is used extensively for large-scale industrial production of exoenzymes as it can secrete large quantities of proteins of up to 20–25 g/l (Schallmey et al., 2004). The alkaline serine proteases (subtilisins) that are manufactured with B. licheniformis and also with Bacillus pumilus and Bacillus subtilis have a primary application as additives to household detergents. Their annual output has been estimated to about 500 metric tonnes of pure enzyme protein (Schallmey et al., 2004). Other products that can be produced by fermentation of B. licheniformis strains are amylases (Declerck et al., 2000; Yuuki et al., 1985) and the topical antibiotic bacitracin (Froyshov and Laland, 1974). The hosts B. licheniformis and Bacillus clausii are also extremely important for commercial processes for heterologous exoenzymes as they frequently exhibit higher enzyme yields than B. subtilis (Schallmey et al., 2004). B. licheniformis belongs to the B. subtilis group (group II) of the genus Bacillus together with other well-known species whose complete genome sequence has been determined. These are Bacillus anthracis (Read et al., 2003), Bacillus cereus (Ivanova et al., 2003; Rasko et al., 2004), Bacillus thuringiensis, the alkaliphilic species Bacillus halodurans (Takami and Horikoshi, 2000; Takami et al., 2000) and B. subtilis (Kunst et al., 1997). In the
context of an extensive comparative genomics of this group of organisms and because of the biotechnological importance of the organism, we sequenced the genome of *B. licheniformis* and present a first analysis of data derived from the annotated sequence.

The genus *Brevibacillus* was established in the year 1996, derived by a genetic reclassification of strains previously allotted to the *Bacillus brevis* group. *B. brevis* was first described in and reclassified as a species belonging to the novel genus, *Brevibacillus*, along with nine other species (Migula, 1900, Shida *et al.*, 1996). Results of a gene sequence study by Shida *et al.*, (1996) demonstrated that the *B. brevis* cluster includes ten species i.e., *B. brevis*, *B. agri*, *B. centrosorus*, *B. choshinensis*, *B. parabrevis*, *B. reuszeri*, *B. formosus*, *B. borstelensis*, *B. laterosporus* and *B. thermorumber*. Currently the genus *Brevibacillus* includes 20 species with published names ([http://www.bacteriocict-fr/b_brevibacillus.html](http://www.bacteriocict-fr/b_brevibacillus.html)). *Brevibacillus* has several biotechnological applications. *B. choshinensis* is a Gram – positive bacterium which has exceptional capacity for heterologous protein expression. It is an excellent host for intracellular protein production, frequently producing intracellular protein in soluble form in the cytoplasm without forming inclusion bodies .The *Brevibacillus* expression system enables highly efficient production of target protein in the secreted form. Pramila *et al.*, 2012 reported *B. parabrevis* that producer hydrophobic LDPE Films –Ye *et al.*, (2013) investigated TPT (Triphenyltin) biosorption by *B. brevis*. Yang and Lee (2007) isolated pene phenol degrading *Brevibacillus* species *Brevibacillus* species are potential biocontrol agents. Baoyu *et al.*, (2007) used *Brevibacillus* species for nematode control. Hassi *et al.* (2012) reported antimycobacterial activity. Sunita *et al.*, 2010 reported *Brevibacillus* species that can produce a wide variety of metabolites with antifungal activities, which can control plant diseases.

Yasar Yildiz *et al.*, (2013) reported to the exopolysaccharides production by *B. thermorubes* Usha kiran *et al.*, (2012) reported that *B. brevis* extends the shelf life of curd and was able to set curd within 5 hours of incubation at a temperature of 42°C. There are also reports on aldehyde oxidase production from *Brevibacillus* specie Mey -43: (Yoshifermi *et al.*, 2008). Sridevi and Prabhune (2009) isolated *Brevibacillus* species from a hot water spring near Konkan, Maharusra, India and standardized the fermentation conditions for the intracellular production of bile salt hydrolase. Thus *Brevibacillus* can be exploited as an excellent tool for structural and functional Biology.
Bacteria can potentially promote solubilization of K during plant growth that is in agreement with the findings of Ullman et al. (1996), Welch et al. (1999), Hutchens et al. (2003), He and Sheng (2006), and Song et al. (2007). The decomposing ability of the bacteria could be attributed to the production of protons, organic acids, siderophores, exopolysaccharides, and organic acids (Groudev 1987; Grayston et al. 1997; Welch et al. 1999; Liermann et al. 2000; Rogers and Bennett 2004).

This suggests that organic acids and siderophores could play a crucial role in the solubilization of elements such as K, Si, and Fe from the liquid medium containing acid-leached soil, muscovite, and biotite. Similarly in our study ‘K’ is released from ‘K’. Generally an inverse relationship was observed between pH value of culture medium and concentration of produced by strains JK2 to JK7. Similar, inverse relationship between pH and soluble ‘K’ content was reported by earlier by Sheng et al., (2008). In contrast, in our study of the pH of the liquid medium containing muscovite and biotite did not change during the incubation (unpublished data) that is probably due to CaCO3 and MgCO3 impurities present in these minerals which would be released into the medium following weathering of muscovite and biotite fact that calcium and magnesium act as buffer, the culture are more alkaline, thereby prevent the culture from being acidified by production of organic acids. Many microorganisms in soil are able to solubilize unavailable forms of K in silicate minerals, such as micas and orthoclases, by excreting organic acids, which either directly dissolve rock K or chelate silicon ions to bring K into solution (Groudev 1987; Friedrich et al. 1991; Bennett et al. 1998). It was postulated that causes responsible for microbially K solubilization may involve a combination of complexation reactions between organic acids and siderophores. Both the mineralogy and chemical composition of different K sources seem to affect growth as well as metabolism of strains consequently induced significant increases in K solubilization. Chemical composition of silicate minerals may determine their susceptibility to microbial K mobilization biotite is the most susceptible medium in weathering or decomposing procedure. Biotite contains higher amounts of Fe, Mg, and Ca than muscovite do, and ions of these metals can be chelated by organic acids that produced by bacterial strains. Therefore, the higher Fe, Mg, and Ca contents in biotite may permit a faster rate of K solubilization by bacteria.
Mortar and concrete forms major components in the construction industry as it is cheap, easily available and convenient to cost. But drawback of these materials is that it cracks under sustained loading and due to agree environmental agents which ultimately reduce the life of structure which are built using this materials (Chahal et al., 2012; Arunachalam 2010). This process of damage occurs in the early life of the building structures and also during its life time. Synthetic materials like epoxies are used for remediation. But they are not compatible costly, reduce aesthetic appearances and need constant maintenance. In the biosphere, bacteria can function has geochemical agent promoting the dispersion fractionation and concentration of materials. Microbial mineral precipitation is resulted from metabolic activities of micro organisms. Based on this biominerology concept, an attempt has been made to develop bio concrete/biomotar materials incorporating on enrichment culture of alkaliphic bacteria with in cement sand motar/concrete. The result showed a significant increase in compressive strength. The astonishing advantage of this bio concrete is its self healing capacity.

The process of microbial mineral plugging in porous media was induced by Stocks-Fischer et al. (1999) by using an alkaliphilic soil microorganism, *B. pasteurii*. It was found that urease activity is high in alkaline pH, where calcite precipitation is favourable. They suggest that microbial calcite precipitation process can be used in remediation of surface and subsurface of porous media. Ramakrishna et al. (1999) studied the role of *B. pasteurii* in crack-filling by using artificially cracked cement mortar beams. Microscopic observations confirmed the microbial calcite precipitation in cement. In another study, Ramachandran et al. (2001) used *B. pasteurii*, for remediating cracks and fissures in concrete utilizing microbiologically induced calcite (CaCO$_3$) precipitation. It was reported that the effect of bio-deposition improves the durability of cement mortar/concrete specimens. It was also observed that deposition of CaCO$_3$ crystals decreased the water absorption of the sample depending on the inherent porosity of the specimen leading to a decrease in the carbonation rate by about 25–30%. It is essential to improve the strength and durability of concrete where similar biomimetic techniques have been used worldwide. However, they did not find any improvement in strength in the cement mortar using *Escherichia coli*. Van Tittelboom et al. (2010) studied biological repair technique using ureolytic bacteria such as *Bacillus sphaericus*, which were able to
precipitate CaCO\textsubscript{3} by conversion of urea into ammonium and carbonate. Park \textit{et al.} (2010) studied the microbiological CaCO\textsubscript{3} precipitation ability to improve the compressive strength of concrete. He isolated four calcite-forming bacteria (CFB) located from seven environmental concrete structures. They observed that the bacterial species belonging to \textit{Arthrobacter crystallopoietes} improved the compressive strength of concrete cubes. Another strain (\textit{Bacillus subtilis}) was used by Afifudin \textit{et al.}, (2011) in the formation of calcium silicate hydrated gel by means of adsorbing silicate using chemically modified \textit{B. subtilis} (CMBS). They reported 28\% improvement in the compressive strength of CMBS incorporated concrete compared to control concrete with optimum concentration of 10\textsuperscript{6} cells/ml. Similarly, in our study all the seven isolates showed overwhelming compressive strength increase over control without bacteria and control without calcium lactate.

In addition to their multitude applications of alkaliphiles, in our study we also found added innovative applications of our isolates which needs indepth study.