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REVIEW

Microbial Diversity in Phosphate Rock and Phosphogypsum

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Abstract Phosphate rock (PR) and phosphogypsum (PG) contain heavy metals, radionuclides and are exposed to ultraviolet and cycles of desiccation (i.e., Gafsa and Sfax in Tunisia). Nevertheless, an extensive diversity of bacterial species has been identified in such extreme and nutrient-poor environments. This mini-review aims to summarize findings published so far about microbes isolated, enriched from or able to grow on PR and PG. Concerning the ecology of these two complex environments, this survey has underscored the importance of key microbial genera or consortia associated with at least one of these phenotypes: (1) phosphate solubilization, (2) heavy metals tolerance, (3) radiation resistance—positively correlated to desiccation tolerance, (4) sulfur oxidation—associated to polyphosphate stocking and (5) sulfate (SO_4^{2-}) reduction.

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Introduction

The role of microorganisms during phosphogenesis has long been suspected in both modern and ancient phosphorites. Isolation of aerobic, facultative anaerobic and anaerobic microorganisms from phosphorites confirmed the importance of prokaryotic activity in the formation of sedimentary phosphate deposits.

They are capable of mediating metal and mineral bio-precipitation by metabolite production, by changing the physico-chemical micro-environmental conditions around the biomass and also by the indirect release of metal-precipitating substances such as PO_4^{3-}, from organic decomposition or phosphate mineral solubilization [1]; and sulfide (S^{2-}) resulting from sulfate (SO_4^{2-}) reduction performed by Sulfate-Reducing Bacteria (SRB) [2].

They are shown to be involved in all processes related to the transformation of clay minerals such as the formation of clays from metamorphic and sedimentary rocks or from solutions, reversible transitions of different types of clay minerals and their consolidation minerals into sedimentary rocks. The formation of phosphatic mineral deposits remains incompletely understood.

Based on their composition, their origins and their geographical location several varieties of phosphate were described by Cayeux and his successor Vatan [3, 4].

Three and a half thousand million year old phosphate rock (PR) is reported to derive from microbial mats—multi-layered sheets of archaea, bacteria and other microorganisms—and to contain the oldest known fossils of microorganisms. Microbial mats in Modern phosporite

and their fossil are of great interest in the interpretation of early life on Earth [5]. Many authors mentioned the role of fossilized microorganisms in mediating phosphorite formation. Possible apatite bound bacterial microfossils in phosphorites were first described by Cayeux [6], and have since been found in phosphorite out crops of many geological ages. In addition, phosphatic stromatolites provide possible evidence of the microbial mediation of phosphorite mineral. Later, Dahanayake and Krmbein [5] defined these microbial mats as layered communities of bacteria that form cohesive structures.

Dissolved phosphorus (P) can be processed by both macro and microorganisms. Diatomaceous plankton, phytoplankton and zooplankton process and dissolve P in water. The bones and teeth of certain fish absorb P, which are later deposited and buried in the ocean sediment [7].

Phosphate ores are of igneous or sedimentary origin. Concerning the igneous origin which constitutes only 10% of PR are found in Brazil, Canada, South Africa, Finland, Zimbabwe, Uganda, Malawi, Sri Lanka and Russia, especially in the Kola Peninsula. As for the remaining 90% of PR, they are of sedimentary origin and they are located in The United States of America, North Africa, China and The Middle East [8]. The most extensive deposits are the sedimentary PR, which are mined to produce phosphate fertilizer. In PR, around 95% of P is found as calcium phosphate mineral, known as apatite: $Ca_5(PO_4)_3(OH,Cl,F)$ [9].

Phosphate Solubilizing Microorganisms (PSM)

Isolation of Phosphate Solubilizing Microorganisms (PSM)

Phosphate solubilizing capabilities are described in several bacteria belonging to different taxonomic and phylogenetic genera such as *Bacillus*, *Burkholderia*, *Enterobacter*, *Escherichia*, *Paenibacillus*, *Pseudomonas*, *Rahnella* and *Rhizobium* [10]. This solubilization activity is also reported in several *Actinomycetes* namely *Azotobacter chroococcum* [11], *Burkholderia anthina*, *Enterobacter ludwigli*, *Pantoea agglomerans* [12], *Streptomyces cavourensis*, *Streptomyces griseus* and *Streptomyces philanthi* [13]. Other PSM including *Acinetobacter* sp., *Enterobacter* sp., *Klebsiella* sp., *Microbacterium* sp. and *Pseudomonas* sp. were isolated from the rhizoplane of field-grown rice [14]. However, according to many authors, fungi are the most efficient PSM. Mobilization of P from inorganic phosphate materials is generally regarded as one of the most important functions of mycorrhizal fungi [15]. In fact, various forms of inorganic phosphate [16] can be solubilized by microbial species such as *Aspergillus* sp., *Penicillium* sp., *Curvularia* sp. and *Trichoderma* sp. [17].

To bring forth their ability to solubilize phosphate, PSM are routinely screened in the laboratory by a plate essay method using different media such as Pikovskaya (PVK), Sperber or National Botanical Research Institute's Phosphate Growth medium (NBRIP). The morphology and the zone of solubilization of bacteria are different from one medium to another [18].

Nautiyal [19] identified the elements that are essential to the solubilization of phosphate in vitro and their concentration level. Following his investigations, he observed that glucose and tricalcium phosphate ($Ca_3(PO_4)_2$) are essential components of the culture medium, whereas yeast extract and ammonium sulfate ($(NH_4)_2SO_4$) are not. Indeed, he showed that increasing the concentration of yeast extract at a level greater than 0.5 g/l, leads to a decrease in phosphate solubilization, while the dissolution rate increases with increasing glucose concentration in the medium. He also showed that phosphate solubilization is higher when using ammonium sulfate at a concentration of 0.1 g/l. However, an increase in the concentration of magnesium sulfate ($MgSO_4$) from 0.1 to 0.25 g/l increases phosphate solubilization. Following these observations, Nautiyal demonstrated that NBRIP medium—which contains per liter of distilled water: glucose (10 g), $(NH_4)SO_4$ (0.1 g), KCl (0.2 g), $MgSO_4.7H_2O$ (0.25 g) and 5 g of inorganic phosphate—is very effective for studying the solubilization of inorganic phosphate. He showed that the solubilization of phosphate in the NBRIP medium resulted in wider and clearer zones than on other media [19]. The measurement of diameter of the halozone allows evaluating the ability of PSM to solubulize phosphate (Fig. 1)

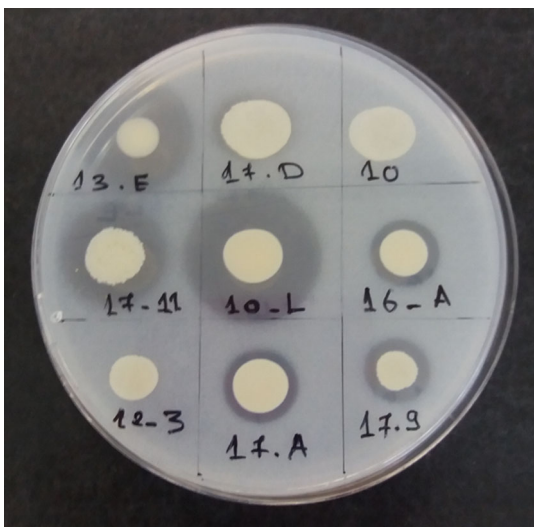

Fig. 1 Plate essay method used for screening of PSM in specific agar medium showing halozon around the bacterial colony

This method can be regarded as generally reliable for isolation and characterization of PSB. In order to confirm the Mineral Phosphate Solubilizing (MPS^+) phenotype, the pure cultures have to be further screened in liquid medium. The phosphate released into culture broth is measured but this estimation has the disadvantage of not taking into account the phosphate used by the cells during growth [20].

The quantitative estimation of phosphate solubilization can be carried out using 10 ml of NBRIP broth medium inoculated with the bacteria at around 10^9 CFU/ml. After incubation, available phosphorus content can be estimated in the culture supernatant using the vanado-molybdate colorimetric method by measuring the absorbance at a wavelength of 420 nm [20].

Mechanisms of Phosphate Solubilization

Inorganic forms of phosphate are transformed into soluble forms, HPO_4^{2-} and $H_2PO_4^-$, by a group of bacteria [21]: *Acetobacter*, acetic acid bacteria, *Klebsiella*, *Serratia*, *Enterobacter intermedium* [22], *Erwinia herbicola*, *Pseudomonas* [20] and *Pantoea* [23]. The mineral phosphate solubilizing (MPS^+) phenotype exhibited by soil bacteria was generally correlated with acidification, chelation, ion exchange reactions and production of a variety of low molecular weight organic acids. Acidification by means of producing organic acids may be the key mechanism attributed to increased phosphate solubilization as revealed by the strong negative correlation observed between the amount of phosphate released and the pH in the culture medium [12]. PSM, particularly those belonging to the genera *Bacillus* sp. and *Pseudomonas* sp. and many others possess the ability to bring insoluble phosphate in soil into soluble forms by secreting organic acids such as citric, gluconic [23–26], lactic [24] malic, succinic [27] and tartaric acids [24] (Table 1). Via their hydroxyl and carboxyl groups, they chelate the cations bound to phosphate and decrease pH in basic soils [26].

In most of the PSB, gluconic acid is one of the prominent organic acids responsible for phosphate solubilization resulting from the direct oxidation of glucose [22, 28, 29]. The enzymes involved in the direct oxidation are oriented in the cytoplasmic membrane. The release of phosphate is accompanied by an acidification of the medium [25, 30].

The production of organic acids is one of the major factors but not the sole factor responsible for bacteria phosphate solubilization [31]. Other studies have shown that another way of solubilization is the release of H^+ to the outer root surface in exchange for cation uptake or with the help of H^+ translocation. ATPase could constitute alternative ways for solubilization of inorganic phosphate. Some bacterial metabolites, different from organic acids, may also contribute to phosphate solubilization through chelation, as well as the production of inorganic acids, such as the sulphidric, nitric and carbonic acids [20], exchange reactions and the formation of polymeric substance [21].

In addition, the role of acid and alkaline phosphatase in phosphate solubilization was also reported [12]. Phosphatase enzymes hydrolyzed organic phosphate to liberate orthophosphate. The released orthophosphate can play a role in the formation of phosphate minerals such as vivianite [$Fe_3(PO_4)_2{\cdot}8H_2O$], strengite ($FePO_4{\cdot}2H_2O$) and variscite ($AlPO_4{\cdot}2H_2O$) [15]. The orthophosphate may arise from organic phosphate degradation, while Fe or Al may arise from microbial solubilization of other minerals [15]. Such formation of phosphate minerals is probably most common in soil [32].

Solubilization Index (SI) is calculated as the ratio of the total diameter (colony and halo zone) to the colony diameter. For instance, bacteria isolated from agriculture soil of Dhanbad area, were tested for their ability to solubilize $Ca_3(PO_4)_2$. Isolates belonging to genus *Bacillus* sp. and *Pseudomonas* sp. showed the maximum phosphate SI of 3.1 and 3.0 in agar plates along with high soluble phosphate production of respectively 373.07 and 368.58 mg l^{-1} in broth culture. Meanwhile, in the culture

Table 1 Organic acids involved in microorganism's phosphate (P) solubilization

Organic acids	Phosphate-solubilizing microorganisms	References
Citric acid	*Pseudomonas* sp.	[24]
Gluconic acid	*Acetobacter* sp.	[24]
	Pseudomonas aeruginosa	[24]
	Pseudomonas cepacia	[25]
	Serratia marcensens	[26]
Gluconic acid, 2-Keto	*Pantoea agglomerans*	[23]
	Rhizobium leguminosarum	[24]
Lactic acid	*Escherichia freundii*	[24]
Malic, succinic acids	*Pseudomonas fluorescens*	[27]
Tartaric acid	*Pseudomonas striata*	[24]

medium a decrease in pH was observed ranging from 3.2 and 6.2 from initial pH of 6.8 to 7.2. This decrease indicated the production of various organic acids by the culture [33]. Clearly, phosphate precipitation depends on many factors such as redox potential, pH value, availability of energy and existence of a suitable nucleation site [1, 34]. Findings of the Phosphate solubulizing activities of many microorganisms were summarized in Table 2.

Phosphate Solubilizing Microorganisms (PSM) in Rock Phosphate (RP)

Apatite ($Ca_5(PO_4)_3(OH,Cl,F)$) is the principal phosphate mineral in most phosphate deposits [35]. Ragot et al. [36] investigated whether microbial community structure in a Natural Alpine Apatite (AP) deposit would differ from the surroundings. They reported that the apatite samples

Table 2 Phosphate solubulizing activities of many microorganisms under in vitro conditions

Species	Source	Culture medium	Organic acid production	Solubilization index (mm)	Incubation time (day)	Final pH	Phosphate sollubilization (μg/ml)	Refernce
Bacteria								
Bacillus sp.	Agriculture soil	Pikovskay's	–	1.7–2.9	7	3.5–4.7	306–368	[33]
Azotobacter chroococcum	Rhizosphere	Pikovskay's	Indole acetic acid	1.3–2.3	–	5.8–5.9	0.94–1.52	[11]
Azotobacter sp.	Agriculture soil	Pikovskay's		1.8	7	5.6–5.7	244–258	[33]
Burkholderia anthina	Green house soil	NBRIP	Gluconic acid, oxalic acid, citric acid	3.5	5	4	More then 600	[12]
Enterobacter ludwigli				3–3.25	4			[12]
Pantoea agglomerans				2.75	3			[12]
Streptomyces cavourensis	Phosphate mines	A synthetic minimum liquid meduim supplemented with 1% of phosphate rock and 40% of root exudates	Indole acetic acid	ND	10	9.14	0.833	[13]
Streptomyces griseus						8.92–9.07	0.377–0.589	[13]
Streptom]yces philanthi						8.86–9.09	0.195	[13]
Acinetobacter sp.	The rhizoplane of field-grown rice	NBRIP	–	6.7	6	4	524	[14]
Enterobacter sp.				3	8	4.5	3	[14]
Klebsiella sp.				4.8	5	4	395	[14]
Microbacterium sp.				2	8	5	97	[14]
Pseudomonas sp.				2.5	8	5.5	132	[14]
Pseudomonas sp.	Agriculture soil	Pikovskay's	–	1.6–3.2	7	3.2–5.4	278–373	[33]
Enterobacter intermedium	Rhizosphere	Broth medium supplemented with glucose, gluconic acid, phospshate rock and Ca CO3	2-Ketogluconic acid	–	2	3	1030	[22]
Mycorrhizal fungi								
Aspergillus sp.	Rhizosphere	Picovskay's	–	–	–	3.5–5.5	0.3–2.66	[17]
Penicillium sp.			–	–	–	3.6–5.2	0.56–1.75	[17]
Curvularia sp.			–	–	–	4.5–5.7	0.41–1.1	[17]
Trichoderma sp.			–	–	–	5–5.4	0.58–1.03	[17]

presented higher water extractable phosphate (up to 3.1 mg/g soil dry weight) and higher concentration of organic acids. In the base of 16S rDNA, gene clone libraries of the AP samples were composed of ten taxonomic groups (*Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Chloroflexi*, *Firmicutes*, *Gammaproteobacteria*, *Planctomycetes*, *Rhizobiales* and *Rhodospirilliales*). The most abundant taxonomic groups belonged to *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Firmicutes* and *Rhizobiales*. *Actinomycetes* are the most filamentous and sporulating bacteria which strongly adhere to the soil particles and establish intimate contacts (endophytic property) with the plants [37]. Sulbarán et al. [30] demonstrated the ability of certain bacterial strains to survive with PR as the sole phosphate source.

Phosphate solubilizing efficiency of fungal strains isolated from tea agroecosystem soil was described and six fungal species were tested: *Aspergillus niger*, *Aspergillus flavus*, *Penicillium funiculosum*, *Trichoderma harzianum*, *Trichoderma citrinoviride* and *Trichoderma asperellum*. The results of $Ca_3(PO_4)_2$ solubilization by the selected isolates showed that *A. niger* and *T. asperellum* were the most efficient strains among the tested fungi, followed by *T. citrinoviride*, though all the fungal strains were found to be good solubilizers [38]. The fungus colonizes the host plant's roots, either intracellularly or extracellularly as in ectomycorrhizal fungi. The phosphate uptake of forest trees is mediated by ectomycorrhizal fungi. Uptake of phosphate from apatite was investigated by Berner et al. [39]. Apatite-amended mesh bags were buried in pairs in the humus layer of Norway forest. After 5 years of apatite exposure, no change was seen in the ectomycorrhizal fungal community structure that colonized these bags. The fungal biomass increased threefold upon apatite amendment. These results suggest that phosphate transfer rates were similar among the different species [39].

Hedh et al. [40] confirmed previous results proving that the major fungal growth in mesh bags was of ectomycorrhizal origin. The effect of apatite amendment on mycelial species composition in a P-poor spruce forest was tested. The most common ectomycorrhizal species found were *Amphinema* sp., *Tomentellopsis submollis*, *Tylospora fibrillosa* and *Xerocomus badius* [40]. Ectomycorrhizal species released elements from apatite and wood ash and accumulated them in the mycelia. Fungal rhizomorphs and mycelia were sampled from sand-filled mesh bags with or without amendment of apatite or wood ash. The mesh bags were buried in forest soil in the field for 13 or 24 months. Identified species were *Paxillus involutus*, *Suillus granulates*, *Thelephora terrestris* and *Tylospora fibillosa*. *P. involutus* was the most common species (31%) and contained the largest amount of calcium (Ca, 2–7 mg/g) [41]. It was mentioned that apatite addition increased the amount of Ca in the rhizomorphs. Ectomycorrhizal fungi can also enhance weathering of certain minerals causing metal release and dissolve a variety of cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb)-bearing minerals.

Phosphate Solubilizing Microorganisms (PSM) in Phosphogypsum (PG)

Different microorganisms were isolated from a PG sample in Tunisia. Bacterial strains were designed BRM15, BRM16, BRM17, BRM18, BRM19 and BRM20 (BRM for BioReMediation). Gram staining of these bacteria reveals that BRM15, BRM19 and BRM20 are Gram positive, while BRM16, BRM17 and BRM18 are Gram negative bacteria [42]. Phosphate solubilization phenotype of bacteria BRM17 and BRM18 was tested in NBRIP medium. It showed the presence of a solubilization halo around bacterial colonies resulting from their ability to solubilize $CaHPO_4$ present in the medium [42].

Heavy Metals Tolerant Microorganisms (HMTM)

Heavy Metals Tolerant Microorganisms (HMTM) in Phosphate (PR)

Strain *Micrococcus* sp. BRM7 was isolated from a phosphate mining region in the South of Tunisia and showed a high tolerance to strontium (Sr). The bacterial isolate *Micrococus* sp. BRM7 showed a high tolerance to Sr [D_{10} (dose for 90% reduction in Colony Forming Units (CFUs)) = 350 mM] with a similar tolerance curve to *Cupriavidus metallidurans* CH34, best known for its high tolerance to a wide range of heavy metals [43].

Heavy metals Tolerant Microorganisms (HMTM) in Phosphogypsum (PG)

Thanks to their ability to remove not only sulfate but also heavy metals from PG [44], several biotechnology procedures based on SRB have been proposed for bioremediation of toxic metals [45]. For example, solubilized sulfate from PG could be reduced to aqueous sulfide by the activity of SRB and the substantial retention of trace elements may be expected by precipitation of insoluble metal sulfides [46, 47]. However, the formation of metal sulfides depends on the concentration of metal-ions, sulfide-ions, pH and redox potential [48, 49].

Several experiments were conducted by Castillo et al. [50] to evaluate the activity and growth of SRB in a metal-rich culture medium (250 mg/l Fe, 75 mg/l Zn and Cu,

10 mg/l Cd) using PG as a bacterial inoculum. During the growth of SRB populations, sulfate was reduced to sulfide and concentrations of metals decreased in the solution. It was reported that the concentrations of Fe (400 mg/l), Cu (80 mg/l) and Zn (150 mg/l) are not toxic for SRB [51, 52]. Bacterial growth and sulfate reduction were possible between 10 and 150 mg/l of initial Zn concentration [53]. Rzeczycka et al. [54] reported that the growth of the SRB community on PG depends not only on Zn concentration (0–80 mg Zn^{2+}/l), but also on the form of zinc.

Radiation Resistant and Desiccation Tolerant Microorganisms (RRDTM)

Radiation Resistant and Desiccation Tolerant Microorganisms (RRDTM) in Phosphate Rock (PR)

RP and PG contain radionuclides such ^{238}U, ^{226}Ra and ^{232}Th, which were the most studied to appreciate radioactivity concentration [55]. PR is characterized by relatively high levels of U and daughter products (e.g., ^{238}U, ^{226}Ra, ^{210}Po and ^{210}Pb); while PG contains high ^{226}Ra, ^{210}Pb and ^{210}Po. SRB isolated from PG waste were particularly effective to Po release expected when sulfide levels did not rise up to 10 μM, in which case Po was precipitated as a metal sulfide [56]. Therefore, the determination of survival rates and D_{10}, F_{10} and H_{10} values—the IR, UV, desiccation dose or period necessary to effect a 90% reduction in CFU, respectively—of prokaryotes, particularly SRB, inhabiting PR is a challenge for future research activities.

Radiation-Resistant and Desiccation-Tolerant Microorganisms (RRDTM) in Phosphogypsum (PG)

Radon-gas-^{222}Rn-producing ^{226}Ra was reported to be the most significant source of PG radioactivity [57]. Alas, ^{222}Rn inflicts considerable damage to internal organs. Accordingly, since 1992, PG overstepping 370 Becquerel (Bq)/kg has been prohibited from all applications. In addition, since 2002, PG was classified as "Technologically Enhanced Naturally Occurring Radioactive Materials (TENORM)" [58]. To the best of our knowledge, there are no RRDTM isolated from PG.

Sulfur-Oxidizing Bacteria (SOB)

The oxidation of sulfur seems to be performed by two groups of microorganisms: the phototrophic and the colorless sulfur bacteria. The difference between these two groups is obviously based on the possession or lack of photosynthetic pigments. The first group is composed of cyanobacteria (known as the blue-green algae) and purple green bacteria which cannot carry out an oxygenic photosynthesis and require anaerobic conditions for growth. The second group is composed of colorless sulfur bacteria which are Gram-negative bacteria including the genus *Thiobacillus* and others. Some are obligate chemolithoautotrophs, some can only oxidize sulfur compounds if they are supplied with an organic carbon source (chemolithoheterotrophs), while others are capable of autotrophic, heterotrophic and mixotrophic growth. It is composed of: (1) the obligate chemolithoautotrophs, the facultative chemolithotrophs, the chemolithoheterotrophs and the denitrifying sulfur bacteria. Colorless sulfur bacteria are found at a wide range of temperature, pH values and degrees of aerobiosis or anaerobiosis, but most of them are dependent on oxygen that's why they often live at the interface between aerobic and anaerobic zones where low concentrations of oxygen (or nitrate) and sulfide can coexist. In these zones, sulfide is usually oxidized by the colorless sulfur bacteria to give sulfate [59].

$$H_2S + 2O_2 \rightarrow 2H^+ + SO_4^{2-}$$

Elemental sulfur, which may be formed as an intermediate, can be reduced to sulfide by sulfur-reducing heterotrophs.

Mechanisms of Sulfur Oxidation and Polyphosphate Stocking

Biochemically, reduced sulfur compounds are converted to sulfite (SO_3^{2-}) and subsequently converted to sulfate (SO_4^{2-}) by the enzyme sulfite oxidase [60]. Some organisms, however, accomplish the same oxidation using a reversal of the APS reductase system used by sulfate-reducing bacteria (see above). In all cases the energy liberated is transferred to the electron transport chain for ATP and NADH production [60]. In addition to aerobic sulfur oxidation, some organisms (e.g., *Thiobacillus denitrificans*) use nitrate (NO_3^-) as a terminal electron acceptor and therefore grow anaerobically.

Parameters causing polyphosphate decomposition and the phosphate release were investigated in *Beggiatoa* strain—a SOB originally described by Sergei Winogradsky, one of the founders of environmental microbiology. The results showed that increasing sulfide concentrations and anoxia resulted in a decomposition of polyphosphate [61]. The latter is a polymer of hundreds of orthophosphate residues linked by phosphor anhydride bonds. In addition to phosphate and energy storage, pH buffering, and metal chelation [62], diverse physiological roles have been attributed to the polyphosphate molecule. It has been recently shown that bacteria, in response to protein-unfolding oxidative stress (i.e., hypochlorous acid (HOCl)),

redirect cellular ATP to polyphosphate resulting in a more than 10,000-foldincrease in stress resistance [63]. The polyphosphate metabolism involved two enzymes encoded by genes with a high degree of sequence conservation [64]. The polyphosphate kinase enzyme catalyzes the reversible conversion of the terminal phosphate of ATP into polyphosphate; whereas, exopolyphosphatase enzyme hydrolyzes polyphosphate liberating inorganic phosphate [62].

Sulfur-Oxidizing Bacteria (SOB) in Phosphate Rock (PR)

The capacity of SOB to solubilize P from PR is drawing the attention of many researchers. In fact, the bacterial sulfur oxidation phenomenon produced high quantity of sulfuric acid which solubilized huge amount of P from PR. In this context, sulfuric acid producing capability of seven *Thiobacillus* strains was used in solubilizing P from PR [65]. For this purpose, an experiment was carried out using PR, *Thiobacillus* strains and elemental sulfur as an energy source. After 40 days of incubation, P solubilization had positive significant correlation with concentration of biologically produced sulfate [65].

In addition to the previously mentioned types of bacteria, sulfur bacteria were found as fossils in recent phosphorite formation and in ancient phosphorite deposits. Possible apatite bound bacterial microfossils have been found in phosphorite of many geological ages [4], showing that microbial activity probably influences the deposition of ancient phosphorites. Indeed, in situ phosphatization has been recently discovered within the vacuolate SOB.

Bailey et al. [66] showed that certain Doushantuo Formation microfossils—one of the oldest beds to contain minutely preserved phosphatic fossils—included structures that can be interpreted as giant vacuolate sulfur bacteria of the genus *Thiomargarita*. Thus, as shown in modern *Thiomargarita*-associated phosphogenic sites, Doushantuo phosphorite precipitation was probably mediated by these bacteria. Because of their large size, sulfur bacteria such as *Beggiatoa* and *Thiomargarita* have a particularly high capacity not only to store sulfur and nitrate, but also to stock P in the form of polyphosphate. Goldhammer et al. [67] reported that the microbial uptake of ^{33}P-labeled phosphate passes rapidly from intracellular polyphosphate into precipitated apatite. *Thiomargarita namibiensis* has been shown to release P from internally stored polyphosphate in pulses creating steep peaks of P in the sediment and thereby inducing the precipitation of phosphate-rich minerals [61]. Phosphate sequestration in apatite occurs under anoxic conditions at a rate of 69–78 nmol cm^{-2} day^{-1} [67].

Sulfate-Reducing Bacteria (SRB)

SRB (about 220 species of 60 genera) are chemolithotrophic bacteria, which use sulfate as terminal electron acceptor during anaerobic respiration. SRB constitute a diverse group of prokaryotes phylogenetically and metabolically versatile and may represent the first respiring microorganisms with subsequent role in the biochemistry of the various environments [45, 68]. SRB have successfully adapted to almost all the ecosystems of the planet and consequently they are widespread in anoxic habitats such as marine sediments, hydrothermal vents and hydrocarbon seeps [44, 45]. SRB can also be found in habitats with extreme pH values such as mining wastewaters, where the pH can be as low as two and in soda lakes, where the pH can be as high as ten [44]. They are also present in aquifers and in engineered systems, such as anaerobic wastewater treatment plants [44, 45].

Because of its versatility and high resistance to heavy metals, this bacterial group has a high potential for biotechnological applications, being used for the treatment of metals and sulfate containing wastes [52, 69, 70] Due to the combined removal of metals and sulfate, as metal sulfides, bioremediation with SRB is considered a promising alternative to the traditional chemical techniques used for the treatment of several types of industrial wastes, namely acid mine drainage [69, 70].

The selection of the carbon source depends to a great extent on the degradability of the organic substrate and on the composition of the bacterial community. Normally, complex organic compounds are not direct substrates for SRB [44, 71]. Thus, SRB are dependent on other microorganisms that degrade these substrates and ferment them to products that can be used as carbon source for SRB [44, 71]. The syntrophic relationships established between various functional groups allowed the degradation of complexes molecules, such as glucose, and the use of the corresponding degradation products by SRB [72]. The coexistence of SRB and fermentative bacteria may be the key factor for the utilization of complex organic substrates for sulfate reduction.

Sulfate-Reducing Bacteria (SRB) in Phosphate Rock (PR)

Although SRB activity in PR is poorly described in the literature, several factors can favor sulfate reduction in phosphorites. In fact, under limiting conditions, PSM—such as *Acinobacteria* and Rhizobiale—secret actively organic acids in order to reduce soil pH and to enhance apatite dissolution. In addition, lactate and acetate were detectable in liquid cultures of several PSM [73]. These

monocarboxylic acids, which are mainly used by SRB as a carbon and energy source, can enhance sulfate reduction activity, particularly in marine sediments under anaerobic conditions. Therefore, in the future, it will be important to structurally and functionally evaluate microbial consortia in PR.

Sulfate-Reducing Bacteria (SRB) in Phosphogypsum (PG)

The chemical composition of PG stockpiles depends on the depth. In fact, surface areas show unsaturated acid and oxidizing condition in opposition to the deeper areas in which saturated, neutral and anaerobic conditions were described [74]. In addition to high concentrations of organic matter in soils and sediments supporting the stack [47], stagnant water near basement could lead to development of anaerobic bacterial communities. Anaerobic conditions in addition to sulfate ions present in PG (about 50%) can favor the activity of SRB. However, SRB activity depends strongly on changes from aerobic to anaerobic conditions throughout the weathering profile of the PG pile. The ability of SRB to proliferate under such extreme conditions is of widespread importance for microbial physiology, bioremediation and industry. Wolicka et al. [75] reported that 66% of PG biotransformation which is introduced into the culture (5 g/l) is obtained by SRB. The reduction of Chemical Oxygen Demand (COD) and SO_4^{2-} was observed in all cultures in which PG was used as the sole electron acceptor and lactate, casein or lactose was the sole carbon source. These cultures use assemblages of anaerobic sulphidogenic microorganisms. In addition to the reduction of COD and SO_4^{2-}, diffractometric studies of the residues confirmed the presence of apatite [76].

Castillo et al. [50] reported that, under anoxic conditions, SRB occur naturally in PG. In a recent study [77], according to 16S rDNA gene clone libraries a community enriched from Portuguese PG was composed by species related to the genera *Desulfotomaculum*, *Desulfosporosinus*, *Sedimentibacter* and *Clostridium*. The SRB Strain E-2^T, for instance, was recovered from marine sediments contaminated by PG wastes near Sfax (Tunisia). The 16S rDNA gene analysis showed that it belonged to the genus *Desulfovibrio*, [78]. Azabou et al. [79] have also isolated an SRB strain from a 6 month enrichment culture in an anaerobic media containing PG as a sulfate source. Phylogenetic analysis of the 16S rDNA gene sequence of the isolate revealed that it was related to members of the genus *Desulfomicrobium* [79].

Thermotogae is a phylum of the domain bacteria which is composed of gram-negative staining, anaerobic, mostly thermophilic and hyperthermophilic bacteria. Recently, some sulfur reducing *Thermotogae* bacteria growing in mesophilic temperatures, named mesotoga, have been identified. *Mesotoga* (strain PhosAc3) has been isolated from an anaerobic reactor fed with cheese whey and PG [80].

From an anaerobic mud of an olive mill wastewater basin contaminated by PG (produced by a Tunisian factory), a new sporulated fermentative bacterium, *Clostridium tunisiense* (strain E1^T), was isolated [81].

The use of PG as sulfate source for the growth and activity of SRB was first reported by Azabou et al. [51]. Subsequently, several authors reported sulfate reduction from PG with pure [79] and mixed cultures [53] by the required addition of different carbon sources, including pure compounds and waste materials [50, 76, 82]. A very recent study [77] showed that PG biotransformation by non-native SRB communities enriched from sludge from wastewater treatment plants was more efficient than the biotransformation achieved with PG naturally occurring SRB. Biotransformation of PG through the activity of SRB can be an effective way to reduce the environmental risks associated to this waste by immobilization of the metals originally present as sulfate into low soluble and thus less mobile metal sulfides.

To conclude, this mini-review is an attempt to survey what is currently known about microbes isolated from or able to grow on two chemically and biologically complex environments, PR and PG. Various microbial phenotypes were discussed in details; and it is suggested that these phenotypes do not correlate obligatory with the environmental conditions of PR and PG. For instance, PR and PG contain simultaneously radionuclides and radiosensitive bacteria. Yet, it is important to remember that many of these microbes recovered from PR and PG may be transient rather than resident on the surfaces. In the future, spectra of temporal and spatial scales and relevant biogeochemical investigations may make it possible to improve our present state of knowledge about PR and PG microbial consortia more promptly than ever. Moreover, previous studies suggest that PR and PG harbour microorganisms that catabolise a wide range of compounds and represent genera of considerable ecological and industrial interest. Analysis of ecologically-related bacteria lodging in these niches to describe their central metabolism and to investigate the occurrence of pertinent classes of enzymes may yield important insights and facilitate the exploitation of these microorganisms in agriculture, bioremediation or biotechnology.

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