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The environmental bacterium Pseudomonas resinovorans MOB-449 (MOB-449) was initially isolated by our group in order to analyze its use in Mn bioremediation processes. To this end, the MOB-449 strain was characterized according to its Mn(II) oxidation and biofilm formation capacities. These studies showed that MOB-449 is capable of oxidizing the metal only when it grows under static conditions and, unlike other Mn-oxidizing bacteria (MOB), it shows higher oxidation efficiency at 18°C than at 30°C (the optimal growth temperature). The focus of this work was to investigate the Mn(II) oxidation mechanism, especially at low temperatures. First of all, MOB-449 biofilm growth and development were analyzed in Lept medium, in the presence or absence of Mn(II). In both cases, a positive effect of the metal was detected at 18°C. This effect was accompanied by Manganese Oxide formation suggesting that the bacterium could obtain energy to grow through this process. Further, in vitro Mn(II) oxidase activity assays were performed with total protein extracts obtained at 18°C and different concentrations of uncoupling agent 2,4 Dinitrophenol (DNF). Results showed that the higher the concentration of DNF, the greater the Mn(II) oxidase activity, suggesting that Mn(II) oxidation could be a process that uses MOB-449 to obtain energy. In this context, and based on previous reports associating cytochromes with Mn(II) oxidation in other MOB, in silico searches of cytochrome terminal oxidase complexes present in MOB-449 sequenced genome were performed. MOB-449 genome denoted the presence of four cytochrome c terminal oxidases: the cbb3-1 oxidase (Cbb3-1), the cbb3-2 oxidase (Cbb3-2), the aa3 oxidase (Aa3), and the alternative-aa3 oxidase (Aa3). Specific oligonucleotides were designed to evaluate the expression levels of these four terminal oxidases by RT-qPCR. The results showed that genes analyzed that encode subunits of the four cytochrome c oxidases showed increased expression at 18 °C in the presence of Mn(II). Subsequently, to found if specific cytochromes may oxidize Mn(II) delivering the electron to the cytochrome c terminal oxidase, the presence of cytochromes in total biofilm protein extracts of MOB-449 grown at 18 °C was determined. To this end, extracts obtained in the presence and absence of Mn(II) were separated by polyacrylamide gels electrophoresis and cytochromes were evidenced via their intrinsic peroxidase activity by using 3,3',5,5'-Tetramethylbenzidine (TMBZ) and H₂O₂. In this way, a band of greater intensity was detected in the extracts with Mn(II) and the proteins present in this band will be identified by Mass Spectrometry. To conclude, the results obtained propose the Mn(II) oxidation as a form of chemolithotrophic metabolism of MOB-449 which could be vital for cellular functions at 18°C. However, future studies will be necessary to be able to elucidate with greater certainty the mechanism involved.

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DECIPHERING THE LIGHT SIGNAL TRANSDUCTION MECHANISM IN Staphylococcus

aureus

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Staphylococcus aureus, Pseudomonas aeruginosa, and Acinetobacter baumannii have been recognized by the WHO and the CDC as critical human pathogens. These microorganisms belong to the ESKAPE group, so named since they are capable of "escaping" antibiotic treatments. The infections caused by these pathogens result in a dramatic increase in the costs of medical care. Previous results from our laboratory have shown that these microorganisms can sense and respond to light. In S. aureus, light has been shown to modulate important pathogenicity determinants such as alpha toxin-dependent hemolysis, as well as virulence in an epithelial infection model, which could have implications in human infections. Light also regulates persistence, metabolism, and the ability to kill competitors such as C. albicans, in this microorganism. To our knowledge, the ability of S. aureus to sense and respond to light constitutes a newly described physiological trait. These pathogens could sense light to synchronize their behavior with the circadian rhythm of their hosts, likely as a strategy to optimize infection development. Identification of the photoreceptors involved in light sensing in S. aureus would provide important insights into the light signal transduction cascade. Despite no traditional photoreceptors were found encoded in its genome, we identified the presence of three putative proteins containing GAF domains. GAF domains have been shown to be part of phytochromes and cyanobacteriochromes along with other domains such as PHY and PAS. While in two of them the GAF domain encompasses the full-length protein sequence, suggesting a new photoreceptor architecture, the last one harbors a GAF N-terminal domain associated with a C-terminal histidine kinase. The genomic environment of each putative photoreceptor was determined, and genes such as LuxR, involved in a quorum-sensing regulation; and DegU, identified as a response regulator of bacterial motility, virulence and biofilm formation, were found in their close proximity. Recent results from our group show that motility in S. aureus is not only modulated by blue light, but also by red and green lights. This is compatible with multiple GAF photoreceptors as they exist in two thermally stable states interconvertible by light, absorbing in different regions of the spectrum. Moreover, the presence of three cysteine residues were observed, residue shown to be essential for binding of the bilin chromophore. In this sense, it is interesting to note that S. aureus produces Staphylobilin as a product of heme metabolism. In addition, we amplified the DNA fragments encoding these putative photoreceptors from S. aureus USA300 strain, and subcloned them into the expression vector pET-TEV, to corroborate that they are active photoreceptors upon light absorption. Finally, proteomic results are discussed which suggest new pathways modulated by light in S. aureus such as cell wall synthesis and recycling.