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ORIGINAL ARTICLE

Characterization of *Rhodococcus* sp. $A5_{wh}$ isolated from a high altitude Andean lake to unravel the survival strategy under lithium stress

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Abstract Lithium (Li) is widely distributed in nature and has several industrial applications. The largest reserves of Li (over 85%) are in the so-called ''triangle of lithium'' that includes the Salar de Atacama in Chile, Salar de Uyuni in Bolivia and Salar del Hombre Muerto in Argentina. Recently, the use of microorganisms in metal recovery such as copper has increased; however, there is little information about the recovery of lithium. The strain *Rhodococcus* sp. $A5_{wh}$ used in this work was previously isolated from Laguna Azul. The assays revealed that this strain was able to accumulate Li (39.52% of Li/g microbial cells in 180 min) and that it was able to grow in its presence up to 1 M. In order to understand the mechanisms implicated in Li tolerance, a proteomic approach was conducted. Comparative proteomic analyses of strain $A5_{wh}$ exposed and unexposed to Li reveal that 17 spots were differentially expressed. The identification of proteins was performed by MALDI-TOF/MS, and the obtained results showed that proteins involved in stress response, transcription, translations, and metabolism were expressed under Li stress. This knowledge constitutes the first proteomic approach to elucidate the strategy followed by *Rhodococcus* to adapt to Li.

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PALABRAS CLAVE
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Caracterización de las estrategias seguidas por *Rhodococcus* sp. $A5_{wh}$ proveniente de laguna de altura en su adaptación a la presencia de litio

Resumen El litio (Li) es un elemento químico con múltiples aplicaciones industriales. Es considerado uno de los minerales más ampliamente distribuidos en la naturaleza. Sus mayores reservas (más del 85%) se encuentran en el llamado «triángulo de litio»: salar de Atacama, en Chile; salar de Uyuni, en Bolivia, y salar del Hombre Muerto, en Argentina. En los últimos años, el empleo de microorganismos en la recuperación de metales se ha visto incrementado; sin embargo, hay muy poca información sobre la recuperación de Li por esta vía. En este estudio se trabajó con *Rhodococcus* sp. $A5_{wh}$, cepa aislada de Laguna Azul. Los ensayos revelaron que este microorganismo fue capaz de acumular Li (39,52% de Li/g de biomasa en 180 min) y de crecer en presencia de este metaloide hasta una concentración de 1 M. Para comprender los mecanismos implicados en la tolerancia al Li, se llevó a cabo el análisis proteómico comparativo de esta cepa expuesta o no expuesta al Li. Los resultados revelaron 17 *spots* expresados en forma diferencial. La identificación de las proteínas se realizó por MALDI-TOF/MS. Este estudio constituye el primer enfoque proteómico para dilucidar la estrategia seguida por *Rhodococcus* en su adaptación al estrés.

Introduction

Lithium is widely distributed on Earth; seawaters contain a concentration of 0.1–0.2 part per million (ppm), whereas in earth, the concentration has been estimated at 65 ppm. The largest reserves of Li (over 85%) are found in South America specifically Chile, Argentina and Bolivia, which is known as the ''Lithium Triangle'' and includes the Salar de Atacama (Chile), Salar de Uyuni (Bolivia), Salar del Hombre Muerto and Salar del Rincón (Argentina). Argentina is the third world producer of Li, associated with the Li reserves of the Puna salt lakes[22]. Li has many important and interesting uses as heat-resistant ceramics, batteries, pharmaceutical products and it is also required for military, automotive, aircraft, and marine applications. In the last years, the demand for this mineral has increased exponentially[1,34,53,66,72,75]; therefore the recovery is an important subject of study.

The recovery of Li usually involves solar pond evaporation of the brine. Water evaporates and it does not necessarily return as precipitation to the site of origin, because these ponds are in a high desert environment[1,31]. The Puna is a desert, which is high in altitude and with the largest thermal amplitude of the world[3]. The availability of surface water sustains a particular biodiversity and the oldest native people.

An ecologically acceptable and economic alternative is the extraction of metals by means of microorganisms[36], through a process known as ''biomining or bioleaching''[12,13,40,59,60,65,67,74]. Microorganisms are being used for the recovery of metals such as copper[44,51,52,57,61], uranium[32], thorium[77] and gold[28]. However, there is little information on the recovery of Li using microorganisms[72].

Microorganisms have mechanisms of resistance[25,38,42,62] in order to survive in adverse environments, such as metal concentration above acceptable levels. The oldest survival strategy is bacterial biofilm[45,35]. This conformation provides a significant advantage of protection against environmental fluctuations as well as humidity, temperature and pH, concentrating nutrients, among others. Thus, regulation of life depends on the microorganism and the involvement of cellular signal complex networks, in which various proteins that respond to different environmental signs are involved[58]. Moreover, the active efflux of metal from the cytoplasm to the periplasmic space is another mechanism described for metal resistance in gram negative bacteria. It is carried out by ATPases located in the internal membrane of bacteria[62]. In addition, when submitted to stress, cells form chemically reactive molecules containing oxygen such as peroxides, superoxides, hydroxyl radical and singlet oxygen called reactive oxygen species (ROS)[20]. The tricarboxylic acid cycle (TCA) is a metabolic network that acts both as a scavenger and generator of ROS, whose modulation appears to be an important strategy in O_2-dependent organisms to regulate the intracellular levels of ROS. TCA cycle is an important metabolic network used by organisms to survive in an oxidative environment[43].

The high-altitude Andean lakes (HAAL) are unique, not only for their geographical characteristics and wide range of extreme environmental conditions, but also for their abundant biodiversity[3]. The microbial communities that have evolved within these high-altitude aquatic ecosystems tolerate a wide range of chemical and physical stress, such as strong fluctuations in daily temperature, hypersalinity, and variable pH. They have adapted to high levels of UV radiation, a low level of nutrient availability, and high concentrations of heavy metals and metalloids[3,80]. Indigenous extremophiles have apparently developed efficient survival strategies and should be able to produce biomolecules adapted to their unusual living conditions that might represent valuable sources of novel bioproducts (e.g. antioxidants, pigments, extremoenzymes) as it has been shown for other extremophiles[63].

The genus name ''*Rhodococcus*'' is described as aerobic, gram positive, non-motile, mycolate-containing,