Antonie van Leeuwenhoek (2018) 111:1479–1491
https://doi.org/10.1007/s10482-018-1031-y

CrossMark

REVIEW

Metabolic modelling and flux analysis of microorganisms from the Atacama Desert used in biotechnological processes

Valeria Razmilic · Jean Franco Castro · Francisca Marchant · Juan A. Asenjo · Barbara Andrews

Received: 3 January 2018 / Accepted: 25 January 2018 / Published online: 2 February 2018
© Springer International Publishing AG, part of Springer Nature 2018

Abstract Metabolic modelling is a useful tool that enables the rational design of metabolic engineering experiments and the study of the unique capabilities of biotechnologically important microorganisms. The extreme abiotic conditions of the Atacama Desert have selected microbial diversity with exceptional characteristics that can be applied in the mining industry for bioleaching processes and for production of specialised metabolites with antimicrobial, antifungal, antiviral, antitumoral, among other activities. In this review we summarise the scientific data available of the use of metabolic modelling and flux analysis to improve the performance of Atacama Desert microorganisms in biotechnological applications.

Keywords Actinobacteria · Atacama Desert · Bioleaching microorganisms · Flux balance analysis · Metabolic flux analysis · Metabolic modelling · Specialised metabolites

Valeria Razmilic, Jean Franco Castro, and Francisca Marchant are Joint first authors.

V. Razmilic · J. F. Castro · F. Marchant ·
J. A. Asenjo · B. Andrews (✉)
Department of Chemical Engineering and Biotechnology, Centre for Biotechnology and Bioengineering (CeBiB), Universidad de Chile, Santiago, Chile
e-mail: bandrews@ing.uchile.cl

Introduction

Microorganisms tolerant to extreme conditions display unique characteristics useful for biotechnological applications, such as bioleaching bacteria adapted to extreme acidic conditions and specialised metabolite producing actinobacteria adapted to thrive with scarce organic matter availability among other extreme abiotic factors. The Atacama Desert of northern Chile is known for being the driest and oldest on Earth (Hartley et al. 2005), the extreme conditions of temperature, pH, pressure, salinity, heavy metal toxicity, and radiation levels present in this biome, impose a selection pressure over the microorganisms that coevolved with it (Azua-Bustos and González-Silva 2014). Examples of microorganisms with unique traits isolated from the Atacama Desert are copper solubilising bacteria and archaea (Latorre et al. 2016) and specialised metabolite producer streptomycetes (Rateb et al. 2011a; Schulz et al. 2011).

The study of the metabolism of microorganisms used in biotechnological processes can be addressed by metabolic modelling through flux analysis for exploiting their biotechnological potential. A metabolic model capable of simulating the internal metabolic fluxes and predicting key microbial growth parameters can be constructed using information drawn from genome annotation (Bobadilla Fazzini et al. 2013; Hold et al. 2009). The usage of these models is of great interest for investigating metabolic networks such as the contextualization of

experimental data (Castro et al. 2013), guidance for rational design of metabolic engineering experiments (Kim et al. 2014), directing hypothesis-driven discovery (Campodonico et al. 2016), study of multi-species relationships (Merino et al. 2015, 2016), and network property discovery (Ates et al. 2011; Feist and Palsson 2008).

This review will be focused on mathematical models of metabolic networks developed for microbial strains of the Atacama Desert with applications in bioleaching and specialised metabolite production. We will summarise the scientific data available on the use of metabolic modelling and flux analysis in biotechnological applications.

An overview to mathematical modelling for studying the metabolism of microorganisms

Mathematical modelling of metabolic networks aids the study of the metabolic behaviour of a microorganism of biotechnological importance, providing an analytical platform for the contextualization of experimental data and prediction of cellular processes. Metabolic models are based on genome annotation and experimental data and they can range from the central metabolism of the cell up to genome scale representations (Kim et al. 2008). Modelling approaches are used to quantitatively describe intracellular metabolic fluxes at pseudo-steady state that are difficult to determine experimentally.

Metabolic flux analysis (MFA) of metabolic networks

MFA gives an insight into the metabolism of a microorganism by calculating the internal metabolic fluxes of a given metabolic network under the assumption of pseudo-steady state. Empirically, the pseudo-steady state is achieved during the exponential growth phase of a microorganism where there is no accumulation or depletion of intracellular metabolites. MFA provides a framework for analysing physiological characteristics of the cell such as, identification of rigid and flexible nodes in the metabolic network, identification of alternative pathways, calculation of non-measured extracellular fluxes, and calculation of maximum theoretical yields (Stephanopoulos et al. 1998).

The intracellular metabolic fluxes are estimated based on a mathematical model that is constructed using the stoichiometric coefficients of each metabolic reaction considered in the network of the microorganism under study. The stoichiometric coefficients are arranged in a stoichiometric matrix S, where each column m corresponds to a metabolic reaction and each row n corresponds to the stoichiometric coefficient of a metabolite considered in the reaction: negative values indicate consumption of a substrate, positive values indicate generation of a product, and zeros are placed for those metabolites that are not participating in the metabolic reaction. The unknown intracellular fluxes are arranged in a vector of intracellular metabolic fluxes $v(x)$ that are constrained by the stoichiometric matrix S (Antoniewicz 2015). During the pseudo-steady state there is no accumulation of intracellular metabolites, thus the mass balance is given by the Eq. 1:

$$S \cdot v(x) = 0 \tag{1}$$

The system is complemented with a set of extracellularly measured rates q, which correspond to consumption of substrates, generation of products and/or the specific growth rate. These rates are used as input for the calculation of the metabolic fluxes $v(x)$, represented by the Eq. 2:

$$S \cdot v(x) = \begin{bmatrix} q \\ 0 \end{bmatrix} \tag{2}$$

The result of MFA is a net flux distribution map of the network, including estimated rates $v(x)$ for each biochemical reaction under a pseudo-steady state assumption (Stephanopoulos et al. 1998). As the number of reactions n is always greater than the number of pathway metabolites m there is a certain degree of freedom f in the set of algebraic equations, represented by Eq. 3:

$$f = n - m \tag{3}$$

According to the number of measured rates, the system can be determined, overdetermined or underdetermined and thus different approaches are used to resolve it (Stephanopoulos et al. 1998). The system becomes determined and the solution is unique if f is exactly the number of measured fluxes q, in turn the

system becomes overdetermined if *f* is higher than the number of measured fluxes *q*, so the system has extra equations that can be used for testing the consistency of the overall metabolic flux distribution in the network, the accuracy of the flux measurements, the validity of the pseudo-steady state assumption and the calculation of more accurate values for the unknown intracellular fluxes. Finally, the system is underdetermined if *f* is lower than the number of measured fluxes *q*, therefore there is not a unique solution for the system, in consequence the unknown fluxes can be determined only if additional constraints are introduced or an overall optimization criterion is imposed in the metabolic balances (Stephanopoulos et al. 1998).

Stoichiometric models that consider only the main metabolic pathways of a microorganism usually can be solved by simple linear algebra, since the system is either determined or overdetermined. To obtain an observable system some assumptions are made to decrease *f* and avoid linear-dependency. For example, sequential reactions are lumped into a single reaction step by eliminating intermediate metabolites that do not participate in other reactions of the network, without affecting the flux results obtained. To avoid linear dependences in the stoichiometric matrix, only one cofactor is included in the metabolic model.

In the case of stoichiometric models at the genome-scale, there are more unknown variables than equations and the system is always underdetermined and can be solved by flux balance analysis (FBA). The solution for the system is found through optimization of an objective function using linear programming when a solution space is defined by constraints such as the mass balance at pseudo-steady state and the definition of lower and upper bound of each reaction (Orth et al. 2010) (Fig. 1).

Use of flux analysis to study microorganisms used in biotechnological processes

To date there are a few examples of the use of MFA and FBA applied to microorganisms isolated from the Atacama Desert to study their metabolic capabilities and behaviour under different experimental conditions. Some examples will be described in the following sections.

Metabolic modelling of strains used in the bioleaching processes

Bioleaching is the use of a consortium of microorganisms to facilitate the extraction and recovery of metals from primary ores and concentrates. Microorganisms used in bioleaching processes are generally autotrophic and capable of obtaining their energy by oxidising reduced forms of Fe^{+2} ion or sulphur compounds (or both) to provide Fe^{+3} and protons, in a low pH environment. The production of protons keeps the pH low and thus, the Fe ions in solution. Pyrite (FeS_2) is only attacked by Fe^{+3} ions and therefore only dissolved by Fe^{+2} oxidizing bacteria or archaea (Schippers et al. 2014). During the FeS_2 bioleaching process, the production of extracellular polymeric substance (EPS) is crucial (Gehrke et al. 1998), as it aids bacterial adhesion to the mineral surface allowing oxidative attack on the sulphur (Sand and Gehrke 2006).

Bioleaching consortia occur naturally in mining environments that select for microorganisms with these specialised characteristics (Latorre et al. 2016; Rawlings and Johnson 2007). Characterisation of the 16S rRNA gene regions of culturable bacteria and archaea found in Chilean mines revealed low species richness, where the predominant bacterial genera are *Acidithiobacillus* and *Leptospirillum*, while *Ferroplasma* is the prevailing genus of archaea (Latorre et al. 2016). These bacterial strains, such as *Acidithiobacillus thiooxidans* strain Licanantay (Ohata et al. 2013), *Acidiphilium multivorum* strain Yenapatur, *Leptospirillum ferriphilum* strain Pañiwe, *Acidithiobacillus ferrooxidans* strain Wenelen (Sugio et al. 2009) and *Sulfobacillus thermosulfidooxidans* strain Cutipay (Travisany et al. 2012), have enhanced capacity to extract and solubilise copper, compared to their publicly available counterparts (Latorre et al. 2016).

Below, we summarise the metabolic models developed for bioleaching bacteria and their uses.

Metabolic model for the S^0 oxidizing bacterium, A. thiooxidans *strain Licanantay*

A. thiooxidans is a mesophilic chemolithoautotrophic proteobacterium that grows at an optimum pH 2.0–3.0, also it can obtain its energy from the oxidation of reduced inorganic sulphur compounds (RISC) and

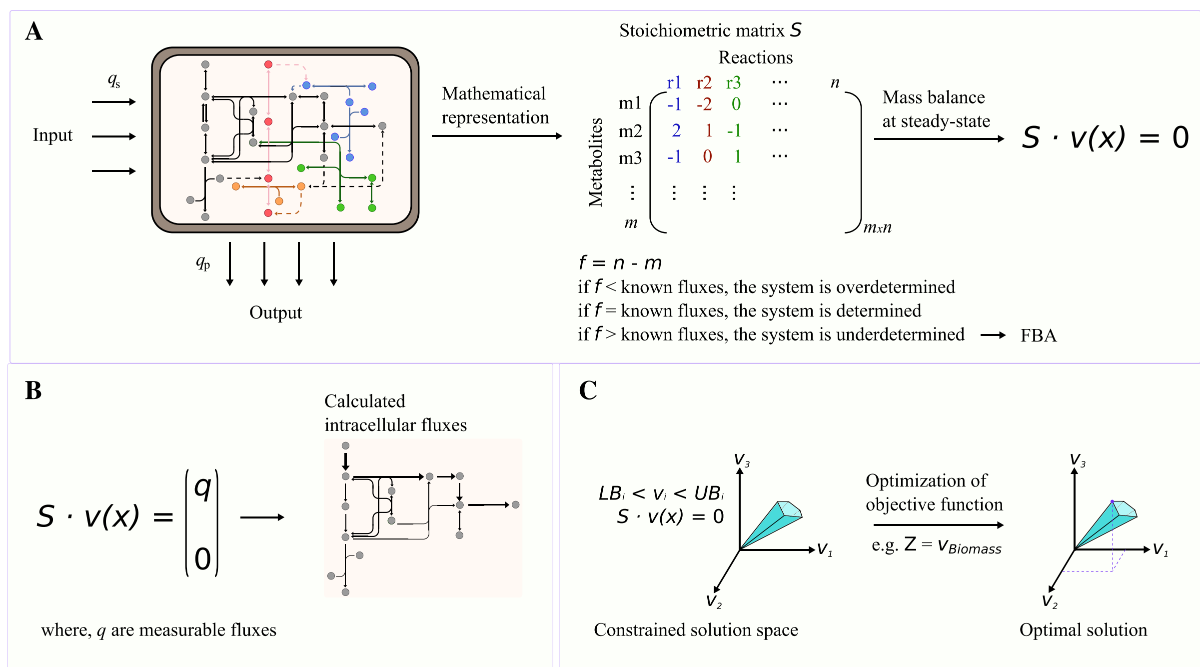

Fig. 1 Schematic representation of metabolic flux analysis (MFA) and flux balance analysis (FBA). **a** The metabolic pathways of microorganisms are represented mathematically in a stoichiometric matrix *S*. The system can be solved under the assumption of pseudo-steady state by two approximations depending on the degrees of freedom *f* of the system: **b** calculation of the intracellular fluxes for an overdetermined or determined system is performed using known measurable fluxes and **c** for an underdetermined system the optimal solution is obtained through optimization of an objective function *Z* within a constrained solution space given by the lower bound (*LB*) and upper bound (*UB*) of each reaction in steady-state through FBA

uses CO_2 as a carbon source. The genome of *A. thiooxidans* strain Licanantay presents additional elements compared to *A. thiooxidans*, not associated to bioleaching, that can be associated with adaptation to its environment. Also, Travisany et al. (2014) identified a core of 139 putative bioleaching genes involved in mechanisms of RISC oxidation, heavy metal resistance, biosynthesis of Fe–S clusters, heme, glutathione, cysteine, and NAD, and energy related processes represented by NADH dehydrogenase and ATP synthetase.

The first experimentally validated metabolic model for *A. thiooxidans* strain Licanantay was developed based on its genome sequence and the available literature (Bobadilla Fazzini et al. 2013). The metabolic model comprises 190 metabolites and 181 reactions including central metabolism, synthesis of amino acids, nucleotides, biosynthesis of biomass and included enzymatic and chemical reaction able to reproduce oxidation of RISC, such as the sulphur oxygenase reductase (SOR) and the sulphur-oxidizing (Sox) systems, and the electron transfer arising from RISC oxidation.

In the case of simulating the oxidation of S^0 via simultaneous SOR and sulphur dioxygenase (SDO) activities, the model predicted a biomass yield of 0.012 gDW $mmol^{-1}$ of S^0, very close to the experimental value (0.013 gDW $mmol^{-1}$ of S^0) (Konishi et al. 1994). The model predicted that 99% of the flux was through the SDO system rather than the SOR, since this system is considered the only source of sulfhydryl (HS^-) in the model, which is a precursor for cysteine formation, an important building block for biomass formation. This prediction seems biologically plausible since the substrate is readily available for SDO that is reported to be a periplasmic enzyme whereas SOR is a cytoplasmic enzyme (Rohwerder and Sand 2003). Using the same substrate, the model could not predict intracellular sulphur storage, which has been experimentally observed, probably because this phenomenon takes place in non-exponential growth phases as observed for *A. ferrooxidans* ATCC 23,270 (Beard et al. 2011) and therefore, is not

captured by the model. Additionally, no flux through the tetrathionate hydrolase (TTH) was detected indicating that the tetrathionate ($S_4O_6^{-2}$) formed is rapidly reduced to thiosulfate by a chemical reaction.

When simulating the oxidation of $S_4O_6^{-2}$, using the experimental maximum specific substrate consumption rate, the model predicted that 49% of the sulphur uptake goes to sulphur storage in the cellular compartment. The simulations predicted a biomass yield (0.052 gDW $mmol^{-1}$ $S_4O_6^{-2}$) close to the experimentally observed value (0.041) with no sulphur storage when the stoichiometry of the reaction catalysed by TTH considered no production of S^0, as observed for a $S_4O_6^{-2}$ decomposing enzyme from *A. thiooxidans* ON107 (Tano et al. 1996). Thus, the model developed for *A. thiooxidans* could be a useful tool for the optimization of biomass production under different metabolic conditions.

Metabolic model for the Fe^{+2}, S^0, and RISC oxidizing bacterium, Acidithiobacillus ferrooxidans

A. thiooxidans ATCC 23270 is a chemolithoautotrophic bacterium, isolated from an acidic black coal mine effluent from Pennsylvania, USA. It can obtain energy from the oxidation of Fe^{+2}, RISC, hydrogen (Drobner et al. 1990), and formate (Pronk et al. 1991), and it can fix CO_2 from the environment. Representatives of this species and closely related microorganisms, have been isolated from the Atacama Desert (Drees et al. 2006; Korehi et al. 2013; Sugio et al. 2008).

Two stoichiometric models have been developed using the genome of *A. ferrooxidans* strain ATCC 23270. The first model included the central metabolic pathways and the lithotrophic catabolism (oxidation of Fe^{+2}, S^0 and RISC), comprising 62 reactions and 78 metabolites. The model was built using a criterion that at least 50% of the genes encoding for the enzymes of a pathway had to be identified in the genome of *A. ferrooxidans* strain ATCC 23270 to accept that pathway in the model (Hold et al. 2009). On the other hand, a genome-scale model for this strain, named *i*MC507, was built to study its metabolism and its bioleaching properties. The model included 587 reactions, 573 metabolites and 507 genes, which were distributed over 42 subsystems and three different cellular compartments: extracellular, periplasm and cytoplasm (Campodonico et al. 2016).

The stoichiometric model developed by Hold et al. (2009), was used to simulate the bacterium behaviour during the oxidation of Fe^{+2} and was able to reproduce verified experimental data, for instance the metabolic model predicted 96.2% of the incoming electrons being transferred to the electron downhill pathway compared to 95% estimated in the literature (Levicán et al. 2002). Similarly, the experimentally observed value of maximum oxygen uptake rate was 0.093 mol O_2 gDW^{-1} (Boon 1996), which compares very well with that of 0.096 mol O_2 gDW^{-1} calculated by the model. With regards to catabolism, the model covers the consumption of 75% of the energy in pumping the electrons out of the cell in the uphill pathway being the dominating energy consumer (61% of total). This clearly shows the growth limiting influence of available energy in *A. ferrooxidans*. The maintenance reaction accounted for a consumption of 25.1 ATP mmol gDW^{-1} h^{-1}, a value in the same order of magnitude than that for *E. coli* (18.9 ATP mmol gDW^{-1} h^{-1}) (Hempfling and Mainzer 1975). As a result of the simulations, the TCA cycle functioned at low levels in the backwards direction, compared with heterotrophic organisms. Thus, the model is able to simulate the main aspects of the metabolism and allows further investigation and potential improvement of bioleaching processes.

The genome-scale model, *i*MC507, constructed by Campodonico et al. (2016) was used to study the gene essentiality, the proton translocation metabolism, the electron transfer metabolism using either Fe^{+2}, $S_4O_6^{-2}$ or $S_2O_3^{-2}$ in aerobic growth, the carbon metabolism using Fe^{+2} as electron donor, and the extracellular polymeric substance (EPS) production coupled to growth.

Gene essentiality under aerobic conditions considering each of the different electron donors was evaluated by FBA. In all cases, approximately 68% of all possible single gene knock-outs were lethal to the organism. The number of essential reactions of oxidative phosphorylation and sulphur metabolism when evaluating $S_4O_6^{-2}$ and $S_2O_3^{-2}$ was higher than for Fe^{+2}. The majority of the reactions of the sulphur metabolism were non-essential during growth with all electron donors tested. These reactions were mainly associated to RISC metabolism where several metabolic routes can be used to oxidise sulphur compounds to provide electron motive force.

Simulation of aerobic growth using H_2CO_3 as a carbon source allowed the study of the electron transfer metabolism using either Fe^{+2}, $S_4O_6^{-2}$ or $S_2O_3^{-2}$. FBA for studying the central carbon metabolism considered Fe^{+2} as electron donor since Fe^{+2}, $S_4O_6^{-2}$ and $S_2O_3^{-2}$ share similar pathways. A high flux through the Calvin cycle, which incorporates CO_2 through the ribulose-bisphosphate carboxylase (RUBISCO) reaction was predicted, which is in agreement with previously reported expression analysis (Esparza et al. 2010). The flow of electrons split at the rusticyanin branch point and most of them were transferred to oxygen via cytochrome oxidase that creates a proton motive force, and 3% of them were transferred via cytochrome oxidase bc1 and NADH ubiquinone reductase to the formation of NADH. NADH is further utilized for carbon fixation (Campodonico et al. 2016). This result was consistent with previously reported information that less than 5% of electrons go to NADH ubiquinone reductase (Ferguson and Ingledew 2008).

The model predicted a different flux distribution for $S_4O_6^{-2}$ and $S_2O_3^{-2}$ metabolisms. The proton transport was predicted to be 40% lower in the $S_2O_3^{-2}$ than in the $S_4O_6^{-2}$ metabolism. In turn, the SO_4^{-} transport was 13% lower in the $S_4O_6^{-2}$ metabolism.

The production of EPS coupled to growth pointed out that reactions fumarate hydratase (associated to *AFE_2673*) and malate dehydrogenase (associated to *AFE_3000*) were candidates for genetic knock-out that would allow production of EPS coupled to growth, independently of the presence of a CO_2 external transporter.

Thus, the model *i*MC507 allowed the understanding and the identification of metabolic engineering targets of *A. ferrooxidans* ATCC 23270 from a comprehensive model-driven perspective.

Metabolic model for *Leptospirillum ferrooxidans*

L. ferrooxidans is a chemolithoautotrophic bacterium, capable of oxidising Fe^{+2} and FeS_2, as energy sources (Rawlings et al. 1999). Also, it is capable of assimilating nitrogen from different sources, it can either reduce atmospheric N_2 to NH_4 using the nitrogenase enzyme complex, or assimilate NH_4 from the culture media, which is taken into the cell by ammonia permeases (Norris et al. 1995; Parro and Moreno-Paz 2004; Parro et al. 2007; Tyson et al. 2004).

A stoichiometric model for *L. ferrooxidans* was developed based on the genome sequence of *A. ferrooxidans* strain ATCC 23270, comprising 86 reactions describing the main catabolic and anabolic metabolic pathways (Merino et al. 2010). The results of the simulations revealed that the TCA cycle is running in a reductive manner, since it would be inefficient for *L. ferrooxidans* to use the oxidative TCA cycle releasing the CO_2 needed for growth. Simulations of growth on Fe^{+2} predicted a biomass yield of 0.007 mol-C mol^{-1} Fe^{+2}, which is similar to the experimentally determined value (Breed et al. 1999; Mignone and Donati 2004; van Scherpenzeel et al. 1998). The model could be used to create new strategies to enrich the bioleaching process.

Metabolic model for a mixed culture of Leptospirillum ferriphilum *and* Ferroplasma acidiphilum

L. ferrooxidans is an acidic chemolithoautotrophic bacterium, which can obtain energy and grow from Fe^{+2} oxidation, and is able to fix CO_2 from the atmosphere (Coram and Rawlings 2002). On the other hand, *F. acidiphilum* is a mesophilic, acidophilic archaeon, which can utilise Fe^{+2} and FeS_2 as energy sources and can also fix CO_2 (Pivovarova et al. 2002). Members of the genus *Ferroplasma* are capable of growing chemoorganotrophically on yeast extract and chemomixotrophically on yeast extract and Fe^{+2} (Dopson et al. 2005).

Species of the genera *Leptospirilum* and *Ferroplasma* are dominant in microbial communities of bioleaching environments, however there is uncertainty about how these organisms interact with each other, hence the importance of developing a metabolic model to gain insights into their interactions and their behaviour in microbial communities (Merino et al. 2015).

A mathematical model for a mixed culture of *L. ferriphilum* and *F. acidiphilum* was constructed by combining experimental information available, including that from the draft version of the genome sequence of *Ferroplasma acidarmanus* Fer1 and an existing stoichiometric model for a similar species of *L. ferriphilum*, *L. ferrooxidans* (Merino et al. 2010).

Growth of *F. acidiphilum* was modelled by enabling the use of Fe^{+2} as energy source and EPS secreted by *L. ferriphilum* as carbon source (Merino et al. 2015).

The metabolic pathways taken into account for *L. ferriphilum* included the assimilation of CO_2 and ammonia fixation through the TCA cycle in a reductive manner and the glutamate dehydrogenase pathway, respectively. The synthesis of EPS by the Leloir pathway that was identified in *A. ferrooxidans* (Barreto et al. 2005) was incorporated into the metabolic model, and it was assumed that the formation flux of EPS by *L. ferriphilum* was equal to its degradation flux by *F. acidiphilum*. Other pathways such as fuelling reactions, synthesis of precursor metabolites, biosynthesis of building blocks, and macromolecule biosynthesis were considered to be analogous to those included in the metabolic model of *L. ferrooxidans* (Merino et al. 2010). For *F. acidiphilum* the model considered experimental information that glucose is converted to pyruvate via the Embden-Meyerhof-Parnas (EMP) pathway since the enzymes fructose 1,6-bisphosphatase and phosphoglycerate mutase were upregulated under chemomixotrophic conditions in *F. acidiphilum* Y^T (Dopson et al. 2005). The stoichiometric model also included anaplerotic reactions to generate oxaloacetate, both the oxidative and the non-oxidative branches of the pentose phosphate pathway and the TCA cycle. Iron oxidation was approximated to the one of *F. acidarmanus* Fer1 for chemomixotrophic growth (Dopson et al. 2005). The model considered protein DNA, RNA, lipid and carbohydrate biosynthesis and biomass formation. EPS was expressed as glucose-6-phosphate in both models. The underdetermined system of stoichiometric equations was solved using linear programming through FBA.

Simulations of a mixed culture on ferrous iron resulted in a predicted yield of 0.0016 mol-C mol^{-1} Fe^{+2} for *L. ferriphilum* and 0.037 mol-C mol^{-1} Fe^{+2} for *F. acidiphilum*, which are values very close to those obtained experimentally for *L. ferriphilum* (Gahan et al. 2010) and for *F. acidiphilum* (Golyshina et al. 2000). Another conclusion of this simulation was that the oxidative branch of the pentose phosphate pathway in *F. acidiphilum* was inactive, which led to the hypothesis that the non-oxidative branch for pentose formation is fed from fructose 6-phosphate of the EMP pathway by the transketolase activity.

Simulation of a pure culture of *F. acidiphilum*, considering CO_2 assimilation through the reductive acetyl-CoA pathway, Fe^{+2} oxidation as energy source and suppressing the EPS consumption flux, showed that growth was not possible and an input of an organic compound such as glucose was required, reaffirming a chemomixotrophic growth as described by Dopson et al. (2005). In turn, simulation of growth of *F. acidiphilum* on different substrates allowed the identification of serine as an important precursor that increases its specific growth rate.

Knock-out simulations were carried out to check the essential enzymes needed to support growth, using as input data the consumption rates of CO_2 and Fe^{+2} for *L. ferriphilum* and consumption rates of EPS and Fe^{+2} for *F. acidiphilum*. Under these conditions, all the enzymes of the reductive TCA cycle were essential to support growth because *L. ferriphilum* fixes CO_2 in this way. The knock-out of the enzyme ribose-5-phosphate isomerase was also found to be lethal for cell growth. Respiratory enzymes and the ATP synthetase are indispensable for growth. *F. acidiphilum* required the enzymes of glycolysis for growth, because this pathway is its only way to obtain energy from organic carbon sources. The knock-out of enzymes involved in Fe^{+2} oxidation resulted in a slight decrease in cell growth, due to the chemo-organotrophic capacities of this microorganism. TCA cycle enzymes are essential except succinyl-CoA synthetase, because the model considered an alternative reaction to generate succinate from lysine synthesis. The predictions made by the model were consistent with literature information, indicating a good performance of both metabolic models.

The metabolic model described above was refined with experimental data of specific growth and Fe^{+2} consumption rates of pure cultures of *F. acidiphilum* strain BRL-115 and of a mixed culture of *F. acidiphilum* with *L. ferriphilum* strain BRL-111 (Merino et al. 2016), strains that were isolated from Chilean mine sites (Merino, MP, personal communication). The experimental Fe^{+2} consumption rate for the mixed culture and a calculated EPS specific production rate for *L. ferriphilum* were used as input for the simulations. The maximization of the growth of both microorganisms was used as objective function within the metabolic model developed by Merino et al. (2015). As a result, a reasonable behaviour of the metabolic fluxes and a good correlation of the

predicted rates with the experimental data were obtained. For example, the specific consumption rate of Fe^{+2} for *L. ferriphilum* was much higher than that obtained for *F. acidiphilum*, since *L. ferriphilum* requires Fe^{+2} for growth, unlike *F. acidiphilum*. These authors propose that the model of a mixed culture of two bioleaching strains can be used to plan strategies to inactivate dispensable routes, to increase the activity of key metabolic pathways and to define an optimal composition of the culture medium.

Actinobacteria from the Atacama Desert, producers of specialised metabolites

Specialised metabolites refer to the bioactive compounds produced by microorganisms that play an important role in the development of medicines, cosmetics and other products. Several actinobacteria have been isolated from the Atacama Desert, surprisingly they produced new specialised metabolites with diverse bioactivities that included antibacterial, antifungal, antiviral, and antitumoral compounds. For example, *Lentzea* sp. strain H45, formally classified as the type strain of *Lentzea chajnantorensis* (Idris et al. 2017), is the producer of lentziosides (Wichner et al. 2016), *Streptomyces leeuwenhoekii* C34^T, C38, and C58, and *Streptomyces* sp. DB64, are producers of chaxamycins and chaxalactins (Rateb et al. 2011a, b), atacamycins (Nachtigall et al. 2011), chaxapeptin (Elsayed et al. 2015), and abenquines (Schulz et al. 2011), respectively.

Genome-scale model of S. leeuwenhoekii *strain C34^T*

The high-quality genome sequence of this bacterium showed a great potential for the production of several specialised metabolites, including polyketides, non-ribosomal peptides, and ribosomal peptides (Gomez-Escribano et al. 2015), and allowed the identification of the biosynthesis gene cluster of chaxamycins (Castro et al. 2015) and chaxalactins (Castro 2015). The inherent ability of *S. leeuwenhoekii* C34^T to produce novel specialised metabolites motivated the elaboration of a genome-scale model (Razmilic 2017).

The genome-scale model of *S. leeuwenhoekii* C34^T was built de novo using the high-quality genome sequence (Gomez-Escribano et al. 2015). The model, named *i*VR1007, consisted of 1722 reactions, 1463 metabolites and 1007 genes which included the carbohydrate, amino acid, nucleotide, fatty acid, and energy metabolisms. Also, specialised metabolite biosynthetic pathways produced by *S. leeuwenhoekii* C34^T were incorporated, such as desferrioxamine, ectoine, chaxamycins, and chaxalactins. The model was validated with experimental information of 89, 54 and 23, different carbon, nitrogen and phosphorus sources, respectively, achieving 83.7% accuracy.

Metabolic engineering targets that would enhance the production of specialised metabolites were predicted using FBA and setting biomass production as the objective function within *i*VR1007. Hence, gene knock-outs and gene overexpressions that predicted an improvement in the production of chaxamycins, chaxalactins and other specialised metabolites were identified (Razmilic 2017). Some of the identified knock-out targets were the genes *sle03600* (homoserine *O*-acetyltransferase) associated to the cysteine and methionine metabolism, and *sle39090* (trehalose-phosphate synthase). The theoretical deletion of the gene *sle03600* produced more availability of acetyl-CoA that could be converted to malonyl-CoA and used as precursor/extender units for specialised metabolite biosynthesis. The deletion of *sle39090* resulted in more UDP-glucose availability for the biosynthesis of 3-amino-5-hydroxybenzoic acid (AHBA) the precursor molecule of chaxamycins.

The overexpression of the gene encoding for UTP-glucose-1-phosphate uridylyltransferase (*sle41020*), the genes encoding for methylmalonyl-CoA mutase (*sle28060*, *sle28760*, *sle22410*, or *sle22940*), or the acetyl-carboxylase complex (*sle47660* and either *sle27560*, *sle44630*, *sle39830*, or *sle59710*), resulted in higher fluxes to AHBA, *S*-methylmalonyl-CoA and malonyl-CoA, respectively.

Thus, metabolic engineering targets for improving specialised metabolites biosynthesis using *i*VR1007 were identified, generating in silico over-producer strains.

Prospects for mathematical modelling using bacteria isolated from the Atacama Desert for biotechnological applications

The extreme characteristics of the Atacama Desert, such as pH, redox potential, salinity, UV-radiation, low organic matter content, low water availability, and the presence of a wide spectrum of toxic compounds,

Table 1 Reports on microorganisms from the Atacama Desert with known or potential biotechnological applications

Application	Organism	Isolation site	Activity	References
Biofuel production	*Dunaliella salina* SA32007	Salar de Atacama	Triglycerides-enriched lipids production	(Arias-Forero et al. 2013)
Bioremediation	*Nitrincola* sp. strain A-D6	Salar de Ascotán	Reduce and extrude arsenic	(Valdés et al. 2014)
	Pseudomonas arsenicoxydans CCUG 58201^T	Valle Camarones	Oxidation of As^{+3} to As^{+5}	(Campos et al. 2010)
Bioleaching	*Acidithiobacillus ferrooxidans* strain Wenelen; *Acidithiobacillus thiooxidans* strain Licanantay	Salar de Atacama	Enhanced Cu extraction from chalcopyrite	(Ohata et al. 2013; Sugio et al. 2009)
	Strains from the *Acidithiobacillus*, *Alicyclobacillus* and *Sulfobacillus* genera	Bahía Chañaral	Bioleaching with seawater salt concentrations	(Korehi et al. 2013)
Cosmetics, nutritional supplements, anti-biofoulings, enzyme production	*Dunaliella salina* strain Conc-007	Salar de Atacama	Production of β-carotene	(Gómez and González 2004)
	Bacillus spp., *Halobacillus* spp., *Thalassobacillus* sp., *Salinicoccus* sp., *Pseudomonas* sp., and *Halomonas* sp.	Salar de Atacama, Valle de La Muerte	Producers of amylase, protease, lipase, DNase, xylanase, and pullulanase enzymes	(Moreno et al. 2012)
	Vibrio spp. strain C9 and C33, and *Pseudomonas* sp. strain C30.	Bahía San Jorge	Probiotic to promote the growth of *Argopecten purpuratus* larvae	(Riquelme et al. 1997)
	Bacillus pumilus	Bahía San Jorge	Production of anti-biofoulings that inhibit the adhesion of microalgal mix	(Leyton et al. 2017)
	Chlorophyta sorokiniana strain CH03	Laguna de Chaxa	Production of nutrients and bioactive compounds	(Hayashida et al. 2017)
Biomedicine	*Streptomyces leeuwenhoekii* strain C34^T	Laguna de Chaxa	Production of chaxalactins A-C and chaxamycins A-D with antibiotic and antitumoral activities	(Rateb et al. 2011a, b)
	Streptomyces leeuwenhoekii strain C38	Laguna de Chaxa	Production of atacamycins A–C with antitumoral and phosphodiesterase (PDE-4B2) inhibitory activities	(Nachtigall et al. 2011)
	Streptomyces sp. strain C1	Atacama Desert soil	Production of antibiotics and therapeutic compounds	(Leirós et al. 2014)
	Streptomyces sp. strain DB634	Salar de Tara	Production of abenquines A–D with antibacterial antifungal and phosphodiesterase (PDE4b) inhibitory activities	(Schulz et al. 2011)
	Streptomyces leeuwenhoekii strain C58	Laguna de Chaxa	Production of chaxapeptin, an antiproliferative compound	(Elsayed et al. 2015)
	Fusibacter ascotence ATCC BAA-2418	Salar de Ascotán	Arsenic sulphide nanoparticles production for selective effects on cancer cell lines	(Demergasso et al. 2017)
	Coculture of *Aspergillus fumigatus* and *Streptomyces bullii*	Atacama Desert soil	Production of antitumoral, antihistaminic and inhibitor of chitin synthase compounds	(Rateb et al. 2013)
	Lentzea sp strain H45	Chajnantor plateau	Production of lentzeosides A–F, inhibitors of HIV integrase	(Wichner et al. 2016)

has allowed the selection of poly-extremophile microorganisms with potential biotechnological applications (Bull et al. 2016). The various biotechnological applications of these microorganisms include production of specialised metabolites and the use of whole cells in either pure culture or consortia in the bioenergy, bioleaching, medicine and/or bioremediation fields (Table 1).

The use of metabolic models helps our understanding of the metabolism of these microorganisms to exploit their biotechnological potential, enabling a rational design of metabolic engineering experiments, definition of optimal culture medium, and evaluation of the effect of genetic or environmental perturbations on the metabolism of the cell (Kim et al. 2008). We believe that the great extension and different poly-extreme habitats of the Atacama Desert harbour microbial diversity with biotechnological potential waiting to be discovered. Therefore, future bio-prospection of this extreme environment could enable the discovery of new biomolecules for the development of biotechnological products as well as efficient microorganisms with application in the mining industry. Metabolic modelling has proven to be a useful tool to study and design strategies aimed at improving the productivity of microorganisms involved in bioleaching processes and production of specialised metabolites.

Acknowledgements The authors want to thank the Basal Programme of the National Commission for Scientific and Technological Research (CONICYT, Chile) for funding the Centre for Biotechnology and Bioengineering (CeBiB, Project FB0001) and from the RCUK-CONICYT International Research Project Bioprospecting the Atacama Desert: the discovery and enhancement of novel therapeutic drugs from actinomycetes (Newton Fund JIC CA586). FM would like to thank CONICYT for National Doctoral Scholarship No. 21171090.

Conflict of interest The authors declare that they have no conflict of interest.

References

Antoniewicz MR (2015) Methods and advances in metabolic flux analysis: a mini-review. J Ind Microbiol Biotechnol 42:317–325. https://doi.org/10.1007/s10295-015-1585-x

Arias-Forero D, Hayashida G, Aranda M, Araya S, Portilla T, García A, Díaz-Palma P (2013) Protocol for maximizing the triglycerides-enriched lipids production from *Dunaliella salina* SA32007 biomass, isolated from the Salar de Atacama (Northern Chile). Adv Biosci Biotechnol 4:830–839. https://doi.org/10.4236/abb.2013.48110

Ates Ö, Oner ET, Arga KY (2011) Genome-scale reconstruction of metabolic network for a halophilic extremophile, *Chromohalobacter salexigens* DSM 3043. BMC Syst Biol 5:12. https://doi.org/10.1186/1752-0509-5-12

Azua-Bustos A, González-Silva C (2014) Biotechnological applications derived from microorganisms of the Atacama Desert. Biomed Res Int 2014:7. https://doi.org/10.1155/2014/909312

Barreto M, Jedlicki E, Holmes DS (2005) Identification of a gene cluster for the formation of extracellular polysaccharide precursors in the chemolithoautotroph *Acidithiobacillus ferrooxidans*. Appl Environ Microbiol 71:2902–2909. https://doi.org/10.1128/aem.71.6.2902-2909.2005

Beard S, Paradela A, Albar JP, Jerez CA (2011) Growth of *Acidithiobacillus Ferrooxidans* ATCC 23270 in thiosulfate under oxygen-limiting conditions generates extracellular sulfur globules by means of a secreted tetrathionate hydrolase. Front Microbiol 2:79. https://doi.org/10.3389/fmicb.2011.00079

Bobadilla Fazzini RA, Cortes MP, Padilla L, Maturana D, Budinich M, Maass A, Parada P (2013) Stoichiometric modeling of oxidation of reduced inorganic sulfur compounds (RISCs) in *Acidithiobacillus thiooxidans*. Biotechnol Bioeng 110:2242–2251. https://doi.org/10.1002/bit.24875

Boon M (1996) Theoretical and experimental methods in the modelling of bio-oxidation kinetics of sulphide minerals. Doctoral thesis, Technical University of Delft

Breed AW, Dempers CJN, Searby GE, Gardner MN, Rawlings DE, Hansford GS (1999) The effect of temperature on the continuous ferrous-iron oxidation kinetics of a predominantly *Leptospirillum ferrooxidans* culture. Biotechnol Bioeng 65:44–53

Bull AT, Asenjo JA, Goodfellow M, Gómez-Silva B (2016) The Atacama desert: technical resources and the growing importance of novel microbial diversity. Annu Rev Microbiol 70:215–234. https://doi.org/10.1146/annurev-micro-102215-095236

Campodonico MA, Vaisman D, Castro JF, Razmilic V, Mercado F, Andrews BA, Feist AM, Asenjo JA (2016) *Acidithiobacillus ferrooxidans*'s comprehensive model driven analysis of the electron transfer metabolism and synthetic strain design for biomining applications. Metab Eng Commun 3:84–96. https://doi.org/10.1016/j.meteno.2016.03.003

Campos VL, Valenzuela C, Yarza P, Kämpfer P, Vidal R, Zaror C, Mondaca M-A, Lopez-Lopez A, Rosselló-Móra R (2010) *Pseudomonas arsenicoxydans* sp nov., an arsenite-oxidizing strain isolated from the Atacama desert. Syst Appl Microbiol 33:193–197. https://doi.org/10.1016/j.syapm.2010.02.007

Castro JF (2015) Identification of the chaxamycin and chaxalactin biosynthesis genes through genome mining of *Streptomyces leeuwenhoekii* C34 and heterologous production of chaxamycins in *Streptomyces coelicolor* M1152. Doctoral thesis, University of Chile

Castro JF, Razmilic V, Gerdtzen ZP (2013) Genome based metabolic flux analysis of *Ethanoligenens harbinense* for

enhanced hydrogen production. Int J Hydrog Energy 38:1297–1306. https://doi.org/10.1016/j.ijhydene.2012.11.007

Castro JF, Razmilic V, Gomez-Escribano JP, Andrews B, Asenjo JA, Bibb MJ (2015) Identification and heterologous expression of the chaxamycin biosynthesis gene cluster from *Streptomyces leeuwenhoekii*. Appl Environ Microbiol 81:5820–5831. https://doi.org/10.1128/AEM.01039-15

Coram NJ, Rawlings DE (2002) Molecular relationship between two groups of the genus *Leptospirillum* and the finding that *Leptospirillum ferriphilum* sp. nov. dominates South African commercial biooxidation tanks that operate at 40 °C. Appl Environ Microbiol 68:838–845. https://doi.org/10.1128/aem.68.2.838-845.2002

Demergasso C, Escudero L, Serrano AE (2017) Method for producing arsenic sulphide nanostructures from a microorganism and produced nanostructures. WO Patent No. 2017100959

Dopson M, Baker-Austin C, Bond PL (2005) Analysis of differential protein expression during growth states of Ferroplasma strains and insights into electron transport for iron oxidation. Microbiology 151:4127–4137. https://doi.org/10.1099/mic.0.28362-0

Drees KP, Neilson JW, Betancourt JL, Quade J, Henderson DA, Pryor BM, Maier RM (2006) Bacterial community structure in the hyperarid core of the Atacama Desert, Chile. Appl Environ Microbiol 72:7902–7908. https://doi.org/10.1128/aem.01305-06

Drobner E, Huber H, Stetter KO (1990) *Thiobacillus ferrooxidans*, a facultative hydrogen oxidizer. Appl Environ Microbiol 56:2922–2923

Elsayed SS, Trusch F, Deng H, Raab A, Prokes I, Busarakam K, Asenjo JA, Andrews BA, van West P, Bull AT, Goodfellow M, Yi Y, Ebel R, Jaspars M, Rateb ME (2015) Chaxapeptin, a lasso peptide from extremotolerant *Streptomyces leeuwenhoekii* strain C58 from the hyperarid Atacama Desert. J Org Chem 80:10252–10260. https://doi.org/10.1021/acs.joc.5b01878

Esparza M, Cardenas JP, Bowien B, Jedlicki E, Holmes DS (2010) Genes and pathways for CO_2 fixation in the obligate, chemolithoautotrophic acidophile, *Acidithiobacillus ferrooxidans*, carbon fixation in *A. ferrooxidans*. BMC Microbiol 10:229. https://doi.org/10.1186/1471-2180-10-229

Feist AM, Palsson BØ (2008) The growing scope of applications of genome-scale metabolic reconstructions using *Escherichia coli*. Nat Biotechnol 26:659. https://doi.org/10.1038/nbt1401

Ferguson SJ, Ingledew WJ (2008) Energetic problems faced by micro-organisms growing or surviving on parsimonious energy sources and at acidic pH: I. *Acidithiobacillus ferrooxidans* as a paradigm. Biochim Biophys Acta 1777:1471–1479. https://doi.org/10.1016/j.bbabio.2008.08.012

Gahan CS, Sundkvist J-E, Dopson M, Sandström Å (2010) Effect of chloride on ferrous iron oxidation by a *Leptospirillum ferriphilum*-dominated chemostat culture. Biotechnol Bioeng 106:422–431. https://doi.org/10.1002/bit.22709

Gehrke T, Telegdi J, Thierry D, Sand W (1998) Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching. Appl Environ Microbiol 64:2743–2747

Golyshina OV, Pivovarova TA, Karavaiko GI, Kondrateva TF, Moore ER, Abraham WR, Lunsdorf H, Timmis KN, Yakimov MM, Golyshin PN (2000) *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the Archaea. Int J Syst Evol Microbiol 50:997–1006. https://doi.org/10.1099/00207713-50-3-997

Gómez PI, González MA (2004) Genetic variation among seven strains of *Dunaliella salina* (Chlorophyta) with industrial potential, based on RAPD banding patterns and on nuclear ITS rDNA sequences. Aquaculture. https://doi.org/10.1016/j.aquaculture.2003.11.005

Gomez-Escribano JP, Castro JF, Razmilic V, Chandra G, Andrews B, Asenjo JA, Bibb MJ (2015) The *Streptomyces leeuwenhoekii* genome: *de novo* sequencing and assembly in single contigs of the chromosome, circular plasmid pSLE1 and linear plasmid pSLE2. BMC Genomics 16:485. https://doi.org/10.1186/s12864-015-1652-8

Hartley AJ, Chong G, Houston J, Mather AE (2005) 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile. J Geol Soc 162:421–424. https://doi.org/10.1144/0016-764904-071

Hayashida G, Schneider C, Espíndola L, Arias D, Riquelme C, Wulff-Zottele C, Díaz-Palma P, Rivas M (2017) Characterization of a Chlorophyta microalga isolated from a microbial mat in Salar de Atacama (northern Chile) as a potential source of compounds for biotechnological applications. Phycol Res 65:202–211. https://doi.org/10.1111/pre.12176

Hempfling WP, Mainzer SE (1975) Effects of varying the carbon source limiting growth on yield and maintenance characteristics of *Escherichia coli* in continuous culture. J Bacteriol 123:1076–1087

Hold C, Andrews BA, Asenjo JA (2009) A stoichiometric model of *Acidithiobacillus ferrooxidans* ATCC 23270 for metabolic flux analysis. Biotechnol Bioeng 102:1448–1459. https://doi.org/10.1002/bit.22183

Idris H, Nouioui I, Asenjo JA, Bull AT, Goodfellow M (2017) *Lentzea chajnantorensis* sp. nov., an actinobacterium from a very high altitude Cerro Chajnantor gravel soil in northern Chile. Antonie Van Leeuwenhoek 110:795–802. https://doi.org/10.1007/s10482-017-0851-5

Kim HU, Kim TY, Lee SY (2008) Metabolic flux analysis and metabolic engineering of microorganisms. Mol BioSyst 4:113–120. https://doi.org/10.1039/b712395g

Kim M, Sang Yi J, Kim J, Kim J-N, Kim MW, Kim B-G (2014) Reconstruction of a high-quality metabolic model enables the identification of gene overexpression targets for enhanced antibiotic production in *Streptomyces coelicolor* A3(2). Biotechnol J 9:1185–1194. https://doi.org/10.1002/biot.201300539

Konishi Y, Takasaka Y, Asai S (1994) Kinetics of growth and elemental sulfur oxidation in batch culture of *Thiobacillus ferrooxidans*. Biotechnol Bioeng 44:667–673. https://doi.org/10.1002/bit.260440602

Korehi H, Blöthe M, Sitnikova MA, Dold B, Schippers A (2013) Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile. Environ Sci Technol 47:2189–2196. https://doi.org/10.1021/es304056n

Latorre M, Cortés MP, Travisany D, Di Genova A, Budinich M, Reyes-Jara A, Hodar C, González M, Parada P, Bobadilla-Fazzini RA, Cambiazo V, Maass A (2016) The bioleaching potential of a bacterial consortium. Bioresour Technol 218:659–666. https://doi.org/10.1016/j.biortech.2016.07.012

Leirós M, Alonso E, Sanchez JA, Rateb ME, Ebel R, Houssen WE, Jaspars M, Alfonso A, Botana LM (2014) Mitigation of ROS insults by *Streptomyces* secondary metabolites in primary cortical neurons. ACS Chem Neurosci 5:71–80. https://doi.org/10.1021/cn4001878

Levicán G, Bruscella P, Guacunano M, Inostroza C, Bonnefoy V, Holmes DS, Jedlicki E (2002) Characterization of the *petI* and *res* operons of *Acidithiobacillus ferrooxidans*. J Bacteriol 184:1498–1501. https://doi.org/10.1128/JB.184.5.1498-1501.2002

Leyton YE, Letelier AS, Mata MT, Riquelme CE (2017) *Bacillus pumilus* marinos inhibidores de la fijación de microalgas a sustratos artificiales. Inf Tecnol 28:181–190. https://doi.org/10.4067/S0718-07642017000200019

Merino MP, Andrews BA, Asenjo JA (2010) Stoichiometric model and metabolic flux analysis for *Leptospirillum ferrooxidans*. Biotechnol Bioeng 107:696–706. https://doi.org/10.1002/bit.22851

Merino MP, Andrews BA, Asenjo JA (2015) Stoichiometric model and flux balance analysis for a mixed culture of *Leptospirillum ferriphilum* and *Ferroplasma acidiphilum*. Biotechnol Prog 31:307–315. https://doi.org/10.1002/btpr.2028

Merino MP, Andrews BA, Parada P, Asenjo JA (2016) Characterization of *Ferroplasma acidiphilum* growing in pure and mixed culture with *Leptospirillum ferriphilum*. Biotechnol Prog 32:1390–1396. https://doi.org/10.1002/btpr.2340

Mignone CF, Donati ER (2004) ATP requirements for growth and maintenance of iron-oxidizing bacteria. Biochem Eng J 18:211–216. https://doi.org/10.1016/j.bej.2003.08.010

Moreno ML, Piubeli F, Bonfá MRL, García MT, Durrant LR, Mellado E (2012) Analysis and characterization of cultivable extremophilic hydrolytic bacterial community in heavy-metal-contaminated soils from the Atacama Desert and their biotechnological potentials. J Appl Microbiol 113:550–559. https://doi.org/10.1111/j.1365-2672.2012.05366.x

Nachtigall J, Kulik A, Helaly S, Bull AT, Goodfellow M, Asenjo JA, Maier A, Wiese J, Imhoff JF, Süssmuth RD, Fiedler H-P (2011) Atacamycins A-C, 22-membered antitumor macrolactones produced by *Streptomyces* sp. C38*. J Antibiot 64:775. https://doi.org/10.1038/ja.2011.96

Norris PR, Murrell JC, Hinson D (1995) The potential for diazotrophy in iron- and sulfur-oxidizing acidophilic bacteria. Arch Microbiol 164:294–300. https://doi.org/10.1007/BF02529964

Ohata A, Manabe M, Parada PA (2013) Sulfur-oxidizing bacteria and their use in bioleaching processes for sulfured copper minerals. U. S. Patent No. 08372628

Orth JD, Thiele I, Palsson BØ (2010) What is flux balance analysis? Nat Biotechnol 28:245–248. https://doi.org/10.1038/nbt.1614

Parro V, Moreno-Paz M (2004) Nitrogen fixation in acidophile iron-oxidizing bacteria: the nif regulon of *Leptospirillum ferroxidans*. Res Microbiol 155:703–709. https://doi.org/10.1016/j.resmic.2004.05.010

Parro V, Moreno-Paz M, Gonzalez-Toril E (2007) Analysis of environmental transcriptomes by DNA microarrays. Environ Microbiol 9:453–464

Pivovarova TA, Kondrat'eva TF, Batrakov SG, Esipov SE, Sheichenko VI, Bykova SA, Lysenko AM, Karavaiko GI (2002) Phenotypic features of *Ferroplasma acidiphilum* strains Y^T and Y-2. Microbiology 71:698–706. https://doi.org/10.1023/a:1021436107979

Pronk JT, Meijer WM, Hazeu W, van Dijken JP, Bos P, Kuenen JG (1991) Growth of *Thiobacillus ferrooxidans* on formic acid. Appl Environ Microbiol 57:2057–2062

Rateb ME, Houssen WE, Arnold M, Abdelrahman MH, Deng H, Harrison WT, Okoro CK, Asenjo JA, Andrews BA, Ferguson G, Bull AT, Goodfellow M, Ebel R, Jaspars M (2011a) Chaxamycins A-D, bioactive ansamycins from a hyper-arid desert *Streptomyces* sp. J Nat Prod 74:1491–1499. https://doi.org/10.1021/np200320u

Rateb ME, Houssen WE, Harrison WT, Deng H, Okoro CK, Asenjo JA, Andrews BA, Bull AT, Goodfellow M, Ebel R, Jaspars M (2011b) Diverse metabolic profiles of a *Streptomyces* strain isolated from a hyper-arid environment. J Nat Prod 74:1965–1971. https://doi.org/10.1021/np200470u

Rateb ME, Hallyburton I, Houssen WE, Bull AT, Goodfellow M, Santhanam R, Jaspars M, Ebel R (2013) Induction of diverse secondary metabolites in *Aspergillus fumigatus* by microbial co-culture. RSC Adv 3:14444–14450. https://doi.org/10.1039/C3RA42378F

Rawlings DE, Johnson DB (2007) The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia. Microbiology 153:315–324. https://doi.org/10.1099/mic.0.2006/001206-0

Rawlings DE, Tributsch H, Hansford GS (1999) Reasons why '*Leptospirillum*'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. Microbiology 145:5–13. https://doi.org/10.1099/13500872-145-1-5

Razmilic V (2017) Metabolism analysis of *Streptomyces leeuwenhoekii* C34 with a genome scale model and identification of biosynthetic genes of specialised metabolites by genome mining. Doctoral thesis, University of Chile

Riquelme C, Araya R, Vergara N, Rojas A, Guaita M, Candia M (1997) Potential probiotic strains in the culture of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). Aquaculture 154:17–26. https://doi.org/10.1016/S0044-8486(97)00043-4

Rohwerder T, Sand W (2003) The sulfane sulfur of persulfides is the actual substrate of the sulfur-oxidizing enzymes from *Acidithiobacillus* and *Acidiphilium* spp. Microbiology 149:1699–1710. https://doi.org/10.1099/mic.0.26212-0

Sand W, Gehrke T (2006) Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron(III) ions and acidophilic bacteria. Res

Microbiol 157:49–56. https://doi.org/10.1016/j.resmic.2005.07.012

Schippers A, Hedrich S, Vasters J, Drobe M, Sand W, Willscher S (2014) Biomining: metal recovery from ores with microorganisms. Adv Biochem Eng Biotechnol 141:1–47. https://doi.org/10.1007/10_2013_216

Schulz D, Beese P, Ohlendorf B, Erhard A, Zinecker H, Dorador C, Imhoff JF (2011) Abenquines A-D: aminoquinone derivatives produced by *Streptomyces* sp. strain DB634. J Antibiot 64:763. https://doi.org/10.1038/ja.2011.87

Stephanopoulos GN, Aristidou AA, Nielsen J (1998) Metabolic flux analysis. In: Metabolic engineering: principles and methodologies. Academic Press, San Diego, pp 309–351. https://doi.org/10.1016/b978-012666260-3/50009-1

Sugio T, Wakabayashi M, Kanao T, Takeuchi F (2008) Isolation and characterization of *Acidithiobacillus ferrooxidans* strain D3-2 active in copper bioleaching from a copper mine in Chile. Biosci Biotechnol Biochem 72:998–1004. https://doi.org/10.1271/bbb.70743

Sugio T, Miura A, Parada P, Bobadilla R (2009) Bacteria strain Wenelen DSM 16786, use of said bacteria for leaching of ores or concentrates containing metallic sulfide mineral species and leaching processes based on the use of said bacteria or mixtures that contain said bacteria. U. S. Patent No. 7601530

Tano T, Kitaguchi H, Harada M, Nagasawa T, Sugio T (1996) Purification and some properties of a tetrathionate decomposing enzyme from *Thiobacillus thiooxidans*. Biosci Biotechnol Biochem 60:224–227. https://doi.org/10.1271/bbb.60.224

Travisany D, Di Genova A, Sepúlveda A, Bobadilla-Fazzini RA, Parada P, Maass A (2012) Draft genome sequence of the *Sulfobacillus thermosulfidooxidans* Cutipay strain, an indigenous bacterium isolated from a naturally extreme mining environment in northern Chile. J Bacteriol 194:6327–6328. https://doi.org/10.1128/jb.01622-12

Travisany D, Cortés MP, Latorre M, Di Genova A, Budinich M, Bobadilla-Fazzini RA, Parada P, González M, Maass A (2014) A new genome of *Acidithiobacillus thiooxidans* provides insights into adaptation to a bioleaching environment. Res Microbiol 165:743–752. https://doi.org/10.1016/j.resmic.2014.08.004

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM. Solovyev VV, Rubin EM, Rokhsar DS, Banfield JF (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428:37–43. https://doi.org/10.1038/nature02340

Valdés N, Rivera-Araya J, Bijman J, Escudero L, Demergasso C, Fernández S, Ferrer A, Chávez R, Levicán G (2014) Draft genome sequence of *Nitrincola* sp. strain A-D6, an arsenic-resistant Gammaproteobacterium isolated from a salt flat. Genome Announc 2:e01144. https://doi.org/10.1128/genomeA.01144-14

van Scherpenzeel DA, Boon M, Ras C, Hansford GS, Heijnen JJ (1998) Kinetics of ferrous iron oxidation by *Leptospirillum* bacteria in continuous cultures. Biotechnol Prog 14:425–433. https://doi.org/10.1021/bp980016h

Wichner D, Idris H, Houssen WE, McEwan AR, Bull AT, Asenjo JA, Goodfellow M, Jaspars M, Ebel R, Rateb ME (2016) Isolation and anti-HIV-1 integrase activity of lentzeosides A-F from extremotolerant *Lentzea* sp. H45, a strain isolated from a high-altitude Atacama Desert soil. J Antibiot 70:448. https://doi.org/10.1038/ja.2016.78