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Original article

Constitutive arsenite oxidase expression detected in arsenic-hypertolerant *Pseudomonas xanthomarina* S11

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Abstract

Pseudomonas xanthomarina S11 is an arsenite-oxidizing bacterium isolated from an arsenic-contaminated former gold mine in Salsigne, France. This bacterium showed high resistance to arsenite and was able to oxidize arsenite to arsenate at concentrations up to 42.72 mM As[III]. The genome of this strain was sequenced and revealed the presence of three *ars* clusters. One of them is located on a plasmid and is organized as an "arsenic island" harbouring an *aio* operon and genes involved in phosphorous metabolism, in addition to the *ars* genes. Neither the *aioXRS* genes nor a specific sigma-54-dependent promoter located upstream of *aioBA* genes, both involved in regulation of arsenite oxidase expression in other arsenite-oxidizing bacteria, could be identified in the genome. This observation is in accordance with the fact that no difference was observed in expression of arsenite oxidase in *P. xanthomarina* S11, whether or not the strain was grown in the presence of As[III].

Keywords: Arsenic tolerance; Metal; Resistance; Genome

1. Introduction

Arsenic exerts a toxic effect on living organisms and is considered a major threat to human health [1]. However, a few microorganisms are not only resistant to high concentrations of arsenic, but have acquired various capacities to metabolize this element through arsenite oxidation, arsenate respiration and arsenite methylation [1]. In prokaryotes, arsenic resistance is mainly linked to the presence of the *ars* operon, which controls cytoplasmic reduction of arsenate (As[V]) to arsenite (As[III]) and active extrusion of As[III] [1]. In addition, some bacteria harbour the *aio* operon encoding periplasmic arsenite oxidase and conferring the capacity to oxidize As[III] to the less mobile As[V] form [1]. Bacterial oxidation of As[III] by arsenite oxidase AioBA has long been regarded as a detoxification process. However, in some microorganisms, As[III] is used as an electron donor for energy conversion [2]. Regulation of expression of *aioBA* genes has been studied in several bacteria and involves multiple control determinants that may differ from one strain to another [2]. In most studied microorganisms, arsenite oxidase expression is higher in the

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presence of arsenic, and several factors play a role in regulation of the *aio* operon transcription [2], for instance, periplasmic As[III]-binding protein AioX [3], two-component signal transduction AioRS [4] and the alternative sigma factor of RNA polymerase RpoN [5]. To date, the only exception described lies in *Polaromonas* sp. strain GM1, where *aioXRS* genes were not identified and expression of *aioBA* was shown to be constitutive [6].

In the present study, we isolated a *Pseudomonas xanthomarina* strain (strain S11) from the gold mine of Salsigne. This mining site, located near Carcassonne in the south of France, was the biggest gold mine in Europe during the 20th century. Gold deposits are hosted primarily by arsenic-rich pyrite [7] and are therefore of great interest for exploring and deciphering the mechanisms of arsenic metabolism. Generated wastes of the gold mine of Salsigne, representing a total amount of 15 million tons, still contain high concentrations of arsenic, and the mean concentration of arsenic in this polluted area was evaluated at 5.8 g of arsenic per kg of dry soil [8]. *P. xanthomarina* S11 showed high resistance to arsenic. Its genome was sequenced to identify the genetic determinants involved in arsenic metabolism. Three *ars* clusters and one *aioBA* operon are present in the *P. xanthomarina* S11 genome. One of these *ars* clusters is part of an arsenic island located on a plasmid that also encodes the *aio* genes. In *P. xanthomarina* S11, no difference in arsenite oxidase expression was detected when the strain was grown with or without As[III], in accordance with the fact that none of the genes coding for factors regulating arsenite oxidase transcription were identified in the genome. These results corroborate the constitutive expression of *aioBA* when *aioXRS* and the specific sigma-54 dependent promoter are absent from the genome sequence, as observed in *Polaromonas* sp. strain GM1, thus demonstrating that arsenic-dependent activation of *aio* operon transcription is not a general rule.

2. Materials and methods

2.1. Bacterial strains and growth conditions

P. xanthomarina S11 was isolated from arsenic-contaminated soil at the mining site of Salsigne in France and grown on chemically defined medium (CDM) [9] or in LB-Miller medium under aerobic conditions at 25 °C and 150 rpm. As[III] was added when needed from a 133.5 mM stock solution with a pH value adjusted to 7 with hydrochloric acid. *Herminiimonas arsenicoxydans* ULPAs1 [9] was grown in CDM medium at 25 °C and *Klebsiella pneumoniae ozenae* KIIIA [10] in LB-Miller medium at 30 °C.

2.2. DNA extraction

DNA was extracted from a 10 ml *P. xanthomarina* S11 culture grown in LB-Miller for 24 h. After centrifugation, pellets were treated with the Wizard® Genomic DNA purification kit (Promega) using the manufacturer's instructions. Primers fD1 and rD1 (Table S1) were used to amplify the 16S rRNA gene on the extracted DNA. The DNA fragment amplified from 16S rRNA was purified with the Qiagen PCR purification kit and sequenced by Millegen (France).

2.3. Genome sequencing and analysis

The extracted DNA concentration was determined with a QuBit® Fluorometer (Life Technologies) and quality was checked by electrophoregram analysis on a BioAnalyzser (Agilent). Genomic DNA libraries, 2 × 100 bp paired-end (500 bp inserts) and mate-pair (4 kb inserts), were sequenced on Illumina HiSeq 2000. Read pairs were assembled using the Assembly By Short Sequences (ABySS) software version 1.3.6 [11]. The whole-genome sequence has been deposited at the DDBL/EMBL/Genbank under the following accession numbers: contigs, CCYE01000001–CCYE01000073; scaffolds, LN614757–LN614826. The European Nucleotide Archive study accession number for the genome sequence of *P. xanthomarina* S11 is PRJEB7265. The nucleotide sequence was deposited in the Microscope annotation platform (http://www.genoscope.cns.fr/agc/microscope/home/index.php) [12], where manual annotation and comparative genomics were performed when needed. The *RGPfinder* tool was used to identify regions of genomic plasticity (RGP) in the whole genome sequence of *P. xanthomarina* S11 using a set of *Pseudomonas stutzeri* strains genome sequences as a reference.

2.4. Resistance to As[III] and oxidation

Ten microlitre bacterial suspensions grown in LB-Miller medium (OD_{600} = 0.1) were transferred in triplicate in 10 ml non-amended (controls) or amended with increasing concentrations of As[III] CDM into 15 ml tubes. Two series of As[III] concentrations were tested, i.e. 0, 5.34, 10.68, 21.36, 42.72 and 85.44 mM and 0, 6.67, 13.38, 26.69, 53.39 and 106.78 mM. The minimal inhibitory concentration (MIC), corresponding to the concentration of As[III] able to inhibit visible growth of *P. xanthomarina* S11, was determined at 24, 48 and 72 h.

P. xanthomarina S11 was transferred to CDM agar plates supplemented with 1.33 mM As[III] to elucidate its capacity to oxidize As[III] to As[V] as previously described [9].

2.5. Arsenic speciation

Bacteria were grown in liquid CDM in Erlenmeyer flasks supplemented with As[III] (0, 1.33, 10.68, 21.36 and 42.72 mM) for 72 h at 25 °C and 150 rpm. Two biological replicates, and controls without As[III] and without bacteria, were performed. Aliquots were taken immediately after adding As[III] in the cultures and after 24, 48 and 72 h culture for each As[III] concentration. Arsenic transformation was determined in culture supernatants obtained by filtration of the bacterial suspension through a sterile 0.22-µm filter. Arsenic species were separated and quantified by high performance liquid chromatography inductively coupled plasma-atomic emission spectrometry (HPLC-ICP-AES) as previously described [9].