TRABAJOS ORIGINALES

Autores

Robert Ccorahua 1,2,3
robertcsanto13@gmail.com
https://orcid.org/0000-0002-3771-5953

Anika Eca 1
anikaeca@gmail.com
https://orcid.org/0000-0003-4578-688X

Pablo Ramírez 1*
pramirezr@unmsm.edu.pe
https://orcid.org/0000-0001-9309-7021

Michel Abanto 4
michel.abanto@ufrontera.cl
https://orcid.org/0000-0001-9136-0041

Ruth Garcia-de-la-Guarda 1
rgarciad@unmsm.edu.pe
https://orcid.org/0000-0003-4801-5642

Tito Sánchez 1
tsanchezr@unmsm.edu.pe
https://orcid.org/0000-0003-3853-2128

Jaime Sánchez-Venegas 1
jaime.sanchez@unmsm.edu.pe
https://orcid.org/0000-0002-2160-2428

Correspondencia
***Corresponding author**

1 Universidad Nacional Mayor de San Marcos, Facultad de Ciencias Biologicas, Laboratory of Molecular Microbiology and Biotechnology, Lima, Peru.

2 Department of Materials Science and Engineering, Tokyo Institute of Technology, Tokyo, Japan

3 Centro de Investigación en Bioingeniería - BIO, Universidad de Ingenieria y Tecnologia - UTEC, Lima, Peru

4 Núcleo Científico y Tecnológico en Biorecursos – BIOREN, Universidad de La Frontera, Temuco, Chile.

Análisis genómico comparativo de dos nuevos plásmidos de la cepa PQ33 de *Acidithiobacillus ferrivorans*

Comparative genomic analysis of two novel plasmids from *Acidithiobacillus ferrivorans* **strain PQ33**

Abstract

Acidithiobacillus ferrivorans is a psychrotolerant acidophile capable of growing and oxidizing ferrous and sulphide substrates at low temperatures. To date, six genomes of this organism have been characterized; however, evidence of a plasmid in this species has been reported only once, whereby there is no conclusive role of the plasmids in the species. Herein, two novel plasmids of *A. ferrivorans* PQ33 were molecularly characterized and compared at a genomic scale. The genomes of two plasmids (12 kbp and 10 kbp) from *A. ferrivorans* PQ33 (NZ_LVZL01000000) were sequenced and annotated. The plasmids, named pAfPQ33-1 (NZ_CP021414.1) and pAfPQ33-2 (NZ_CP021415.1), presented 9 CDS and 13 CDS, respectively. *In silico* analysis showed proteins involved in conjugation (TraD, MobA, Eep and XerD), toxin-antitoxin systems (HicA and HicB), replication (RepA and DNA binding protein), transcription regulation (CopG), chaperone DnaJ, and a virulence gene (*vapD*). Furthermore, the plasmids contain sequences similar to phosphate-selective porins O and P and a diguanylate cyclase-phosphodiesterase protein. The presence of these genes suggests the possibility of horizontal transfer, a regulatory system of plasmid maintenance, and adhesion to substrates for *A. ferrivorans* species and PQ33. This is the first report of plasmids in this strain.

Resumen

Acidithiobacillus ferrivorans es un acidófilo psicrotolerante capaz de hacer crecer y oxidar sustratos ferrosos y sulfurosos a bajas temperaturas. Hasta la fecha se han caracterizado seis genomas de este organismo; sin embargo, la evidencia de un plásmido en esta especie ha sido informado solo una vez, por lo que no hay un rol concluyente de los plásmidos en la especie. Aquí, dos plásmidos novedosos de *A. ferrivorans* PQ33 se caracterizaron molecularmente y se compararon a escala genómica. Se secuenciaron y anotaron los genomas de dos plásmidos (12 kpb y 10 kpb) de *A. ferrivorans* PQ33 (NZ_LVZL01000000). Los plásmidos, denominados pAfPQ33-1 (NZ_CP021414.1) y pAfPQ33-2 (NZ_CP021415.1), presentaron 9 CDS y 13 CDS, respectivamente. El análisis *in silico* mostró proteínas involucradas en la conjugación (TraD, MobA, Eep y XerD), sistemas de toxina-antitoxina (HicA y HicB), replicación (RepA y proteína de unión al ADN), regulación de la transcripción (CopG), chaperona DnaJ y un gen de virulencia (*vapD*). Además, los plásmidos contienen secuencias similares a las porinas selectivas de fosfato O y P y una proteína diguanilato ciclasa-fosfodiesterasa. La presencia de estos genes sugiere la posibilidad de transferencia horizontal, un sistema regulador de mantenimiento de plásmidos y adhesión a sustratos para especies de *A. ferrivorans* y PQ33. Este es el primer informe de plásmidos en esta cepa.

Keywords:

Acidithiobacillus ferrivorans; plasmid; conjugation; replication; genomic comparative.

Palabras clave:

Acidithiobacillus ferrivorans; plásmido; conjugación; replicación; genómica comparativa.

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Introduction

Psychrotolerant bacterium *A. ferrivorans* has been successfully characterized due to its ability to oxidize Fe^{2+} more efficiently than *A. ferrooxidans* at 5 °C (Kupka et al. 2007; Escobar et al. 2010; Hallberg et al. 2010; Ccorahua-Santo et al. 2017). Compared to other *Acidithiobacillus* sp. strains, *A. ferrivorans* grows more quickly at 5 °C (t_d>50 h) in a standard 9K medium (33.3% Fe^{2+}) and in the presence of other substrates such as sulphides (Ccorahua-Santo et al. 2017) and pyrite (Barahona et al. 2014). Moreover, the microorganism's capacity to fix atmospheric carbon dioxide and its tolerance to heavy metals makes it the best candidate for industrial-scale bioleaching in extremely hostile environments, even at temperatures below 10°C. Recently, we reported the strain PQ33 of *A. ferrivorans*, which performs better at oxidizing ferrous iron at pH 1.6 and copper sulphide at low temperatures than other *A. ferrivorans* strains: *A. ferrivorans* SS3 (Kupka et al. 2007; Liljeqvist et al. 2011), CF27 (Talla et al. 2014) and ACH (Barahona et al. 2014). Additionally, subsequent genome and gene expression analyses have determined the principal traits of evolutionary fitness in *A. ferrivorans* for adaptation to low temperatures and oxidative capacity (Liljeqvist et al. 2015; Christel et al. 2016; Ccorahua-Santo et al. 2017). However, there is only one report of other genomic traits related to elements outside of the chromosomal genome (Tran et al. 2017).

On the other hand, other strains from the *Acidithiobacillus* genus, such as *A. caldus* and *A. ferrooxidans*, have shown traits related to functional plasmids, and some gene sequences involved in the conjugation mechanism have been found (Rohrer and Rawlings 1992; Gardner et al. 2001; Rawlings and Tietze 2001; You et al. 2011). Besides, *A. ferrivorans* has not been evaluated for any other traits than oxidative capacity or cold tolerance. Genome sequencing revealed that *A. caldus* SM-1 has one chromosome and four plasmids: pLAtc1, pLAtc2, pLAtc3 and pLAtcm (You et al. 2011). Preliminary analysis of these plasmids indicated that pLAtc1 is probably a member of the IncQ-like broad-host-range plasmid group. The IncQ-like plasmids have similar replicons comprising three essential replication genes (*repA*, *repB*, and *repC*) and an *oriV* region (Rawlings and Tietze 2001; Zhang et al. 2014).

Additionally, strains of *Acidithiobacillus* sp. have been shown to contain plasmids with complementary functions for adaptation in a bioleaching community. The main traits of these plasmids involve conjugation for *A. caldus*, and conjugation and additional heavy metal resistance genes for *A. ferrooxidans* and *A. thiooxidans*. Holmes et al. (Holmes et al. 1984) reported the mobile pTF1 plasmid (6.7 Kbp length) from *A. ferrooxidans*, with a 2.8 kbp region related to the mobilization function (Drolet et al. 1990). The coding sequence for the MobL protein is contained within this region and is essential for mobilization.

Furthermore, pTF-FC2 plasmid (12.2 Kbp length) from *A. ferrooxidans* was reported as a highly mobile plasmid (Rawlings and Kusano 1994). It comprises three regions: a replication region, a mobilization region, and a transpositional element (Rawlings 2005). Important roles have been described in relation to plasmids for species of *Acidithiobacillus*; however, only one plasmid has been previously isolated from *A. ferrivorans* (Tran et al. 2017). Despite the presence of characteristics related to plasmid stabilization in the genomes of the six *A. ferrivorans* strains, there are no experimental reports about the presence of plasmids in this species.

Despite the increased interest raised on *A. ferrivorans*, there are few studies on the role of the plasmids in the genomic context of this psychrotolerant specie. Here, we report the molecular characterization and comparative genomics of two novel plasmids (pAfPQ33-1 and pAfPQ33-2) of the recently reported *A. ferrivorans* PQ33 strain (Ccorahua-Santo et al. 2017). After gene annotation for those of genes encoded in two plasmids from this strain, we performed a genome-scale comparison with other plasmids from *Acidithiobacillus* genus. The comparison suggests that pAfPQ33-1 plasmid could be involved in the conjugation process and unveils details of its role in an evolutionary context. In addition, an origin of replication was found in both plasmids. The presence of a toxin-antitoxin system, as well as the detection of a putative VapD protein-encoding gene, suggest mechanisms for both positive and negative effects, respectively, on horizontal gene transfer in the PQ33 strain.

Materials and methods

Cell culture and harvesting.- A sample of *A. ferrivorans* PQ33 was obtained from the Laboratory of Microbiology and Molecular Biotechnology at Universidad Nacional Mayor de San Marcos, Lima, Peru (Ccorahua-Santo et al. 2017). The PQ33 strain was inoculated in 9K medium consisting of 3.33% w/v $FeSO_4.7H_2O$, 0.04% w/v $MgSO_4.7H_2O$, 0.01% w/v $(NH_4)_2SO_4$, and 0.004% w/v KH_2PO_4, pH 2.0 (Ramírez et al. 2004), and incubated at room temperature (~24 °C) in a shaker at 200 rpm. The cultures that reached the exponential phase (3 – 5 x 10^8 cell/mL) in 9k medium were inoculated again in the same medium until they reached the exponential phase. The cell count was verified with a Petroff-Housser counting chamber. After the second culture, the cells were centrifuged at 13000 rpm, and the pellet was washed with acid water (pH 2.0) and centrifuged at 13000 rpm. The process was repeated as many times as necessary until the jarosite precipitated was eliminated. Then, the pellet was washed five times with 10 mM sodium citrate at pH 7.0.

Plasmid isolation.- Plasmids of both 12 kbp and 10 kbp were isolated from *A. ferrivorans* PQ33 using the GeneJET™ Plasmid Miniprep kit following the manufacturer's instructions. To verify plasmid size, the isolated plasmids were run on a 1% agarose gel. DNA electrophoresis was performed at 50 V for 90 minutes with a Bio-Rad® PowerPac HC. Total purified plasmid DNA was used for Illumina HiSeq sequencing technology.

Sequencing, annotation and analysis.- For sequen-

cing, the library was prepared following the Illumina® TruSeq™ protocol, with an insert size of 400 bp and a size of 100 bp for the reads. The next-generation sequencing was performed using Illumina® HiSeq 2000 technology. The quality of sequencing reads was verified with FastQC software (Babraham Institute, Bioinformatics). Two *de-novo* assemblies were performed with Velvet 1.1 (Zerbino 2010) and SPAdes 3.07 softwares (Bankevich et al. 2012), and the best assembly was chosen. Preliminary gene annotations were performed with the Prokka tool (Seemann 2014) and ORFfinder. Within Prokka, Prodigal was run for coding sequence prediction (Hyatt et al. 2010), and the annotation was conducted by BLAST+ (Camacho et al. 2009), which was used locally against the UniProt database (Apweiler et al. 2004). For annotation, the best match was chosen. Additionally, Prokka was run with Markov Hidden Model on Hmmscan (Eddy 2011) against the Pfam (Finn et al. 2014) and TIGRFAMs databases (Haft et al. 2013). Moreover, the TRNAScan-SE (Lowe and Eddy 1997) and RNAmmer (Lagesen et al. 2007) tools were used for tRNA and rRNA prediction, respectively. For assignment of putative genes, an identity percentage greater than 30% and an E-value below e^{-05} were required. Final annotation was performed by NCBI's Prokaryotic Genome Automatic Annotation Pipeline (PGAP) service. The NCBI service determined 9 CDS and 13 CDS for the pAfPQ33-1 and pAfPQ33-2 plasmids, respectively. However, the cured annotation of this study was considered. The NCBI accession numbers for the pAfPQ33-1 and pAfPQ33-2 plasmids are NZ_CP021414.1 and NZ_CP021415.1, respectively (Table 1). Results of the identity and conserved pattern of the proteins related to other organisms are shown in Tables 2 and 4.

Table 1. General features of plasmid pAfPQ33-1 and pAfPQ33-2 from *Acidithiobacillus ferrivorans* PQ33

Characteristic	Data for plasmids	
	pAfPQ33-1	pAfPQ33-2
Bioproject no.	PRJNA317458	PRJNA317458
Biosample	SAMN04613415	SAMN04613415
Genome coverage	300X	300X
Sequencing technology	Illumina HiSeq	Illumina HiSeq
Genbank accession no.	NZ_CP021414.1	NZ_CP021415.1
Genome size (bp)	10218	12094
No. of contigs	1	1
G+C content (%)	60.3	57
No. of protein-coding genes:	10	15
Protein with assigned function	8	8
Conserved hypothetical proteins	2	2
Hypothetical proteins	-	5

the pAFPQ33-1 and pAFPQ33-2 plasmids were generated by DNAPlotter (Carver et al. 2009). The predicted domain organization of porins O and P and CDG-PDE was performed using Pfam.

Table 2. Identified ORFs in pAfPQ33-1 plasmid of *A. ferrivorans*.

ORF's designation	Location	Product size	% Coverage	E-Value	% Identity	Pfam*	ORF's description	Related organism
repA	(324-1211)c	299	94	0.0	98	RPA	Plasmid replication initiator protein	Acidithiobacillus thioxidans
Hypothetical protein	(1226-1549)c	108	88	1e-54	89	-	Hypothetical protein	Acidithiobacillus thioxidans
Hypothetical protein	(1527-1805)c	93	98	2e-56	96	-	Hypothetical protein	Acidithiobacillus thioxidans
eep	(1824-2228)c	136	99	4e-78	88	HTH_17	Entry exclusion protein	Providencia rustigianii
xerD	(2281-3171)c	300	70	1e-91	71	Phage_integrase	Tyrosine recombinase XerD.	Acidithiobacillus caldus
copG	3345-3599	85	80	8e-17	54	RHH_3	CopG family transcriptional regulator	Fischerella muscicola
gmr	3785-6429	837	99	0.0	78	EAL, GGDEF and Proto-globin	Cyclic di-GMP phosphodiesterase (CDG-PDE)	Acidithiobacillus ferrivorans
mobA	(6560-8401)c	623	80	2e-56	47	MobA_MobI and LPD7	Mobilization protein A	Acidithiobacillus caldus
traD	8582-8989	137	53	2e-05	41	TraD	Conjugal transfer protein TraD	Acidithiobacillus ferrivorans
Hypothetical protein	(8928-9491)c	191	90	9e-13	47	-	Hypothetical protein/ Plasmid replication region DNA-binding N-term	Acidithiobacillus caldus

*most significant match
cGene in complementary sequence

The origin of replication was verified by the deviation of GC skew in DNAPlotter (Carver et al. 2009). Additionally, the plasmids' origin of replication was compared with the origin of other available plasmids from *Acidithiobacillus* genus. The circular map and gene position in
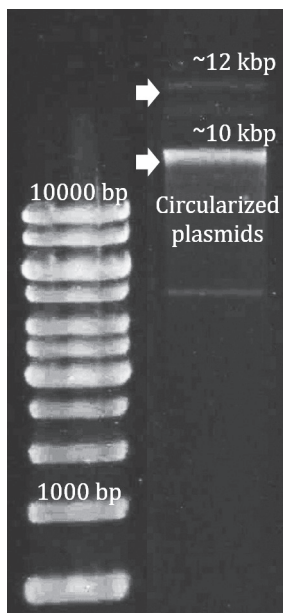
The comparative genomic analysis was carried out

using pTC-F14, pTcM1 plasmids, megaplasmid such as mpAca1.1, pACA1.1, pACA1.2, pLAtc1, pLAtc2 and pLAtc3 from *A. caldus*; pTF4 and pTF5 from *A. ferrooxidans*; and CF27 from *A. ferrivorans*. The program EasyFig was used to visualize the organization and comparison of *Acidithiobacillus* plasmids (Sullivan et al. 2011).
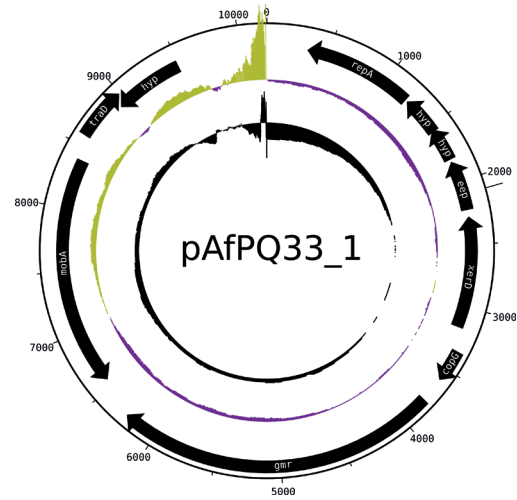
Results and discussion

General characterization.- The size of pAfPQ33-1 and pAfPQ33-2 plasmids were 10218 bp and 12094 bp, respectively (Fig. 1). The general features of both plasmids are shown in Table 1. The detailed characteristics of each ORF are summarized in Table 2 and Table 3. pAfPQ33-1 plasmid contained 10 predicted ORFs (Fig. 2), seven of which (70%) had a function assigned, while three (30%) were conserved hypothetical proteins. With regard to the second pAfPQ33-2 plasmid (Fig. 3), 15 ORFs were identified as putative proteins. From the genes predicted, eight (53%) were assigned a putative function, three (20%) were matched to some conserved hypothetical protein, and four (27%) were hypothetical proteins with no database match. The GC skew of the plasmids is shown in Figures 2 and 3. The change of GC skew indicated the plasmids' origin of replication.
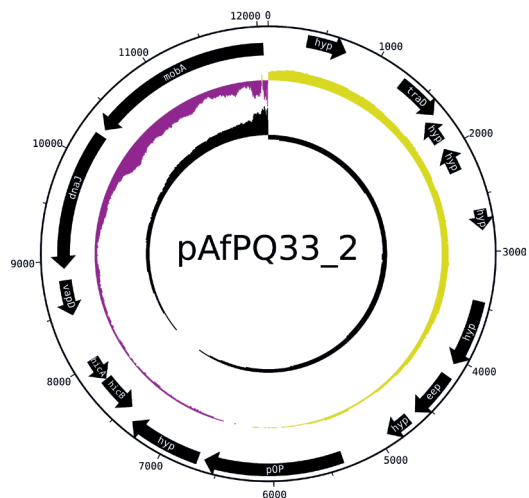
ria and is directly involved in DNA transfer in F⁺ bacteria (Panicker and Minkley, 1992).

Figure 2. pAfPQ33-1 plasmid from *A. ferrivorans* represented as a circular plasmid. The first ring (from outside to inside) shows the plasmid size. The internal black arrows indicate its 10 CDS. The second ring indicates the plasmid GC skew, where the origin of replication is indicated (abrupt change of GC skew). The third ring indicates GC content.

Figure 1. Agarose gel electrophoresis of isolated plasmids DNA from *A. ferrivorans* PQ33. First-line depicts the molecular size ladder in base pair (bp), and the second line shows the presence of ~10 Kb and ~12 Kb plasmids length.

Figure 3. Plasmid pAfPQ33-2 from *A. ferrivorans* represented as a circular plasmid. The first ring (from outside to inside) shows the plasmid size. The internal black arrows indicate its 15 CDS. The second ring indicates the plasmid GC skew, where the origin of replication is indicated (abrupt change of GC skew). The third ring shows GC content.

Proteins involved in conjugation.- To characterize the plasmid genomes, we describe the probable function of both identified and putative proteins. Among the genes encoding putative proteins involved in conjugation, we found the putative conjugation-related TraD protein in both plasmids, with more than 40% identity to the homologous proteins in *A. ferrooxidans* to pAfPQ33-1 plasmid. Protein TraD belongs to the type IV secretion system in the inner membrane of gram-negative bacte-

Moreover, plasmid pAfPQ33-2 contained the coding sequence for the entry exclusion protein (*eep*), which plays an essential role as a barrier to DNA transfer among bacteria sharing the same or a closely related F⁺ factor (Garcillán-Barcia and de la Cruz 2008). Additionally, we found putative *mobA* homologous genes in both plasmids with more than 40% of identity. This protein was found to be involved in plasmid mobilization in *Acidithiobacillus ferrooxidans* (Rohrer and Rawlings 1992). While

the presence of conjugative plasmids in *Acidithiobacillus* has been poorly described, the acquisition of *mob* and *oriT* sequences has been reported continually by mostly non-conjugative plasmids, such as our plasmids. This fact suggests that conjugative mobilization in *Acidithiobacillus* is frequent enough, so its plasmids have evolved to take advantage of it, making them potentially mobilizable. This was recently reported in *S. aureus* (Ramsay et al. 2016). Additionally, we predicted the presence of the tyrosine recombinase XerD in the pAfPQ33-1 plasmid with more than 70% identity to other tyrosine recombinases. This protein could be involved in disentangling chromosomal dimers after bacterial replication, or it could be acting to resolve multimeric forms generated from homologous recombination in a variety of multicopy plasmids (Hallet et al. 1999).

Toxin-antitoxin system.- We found a putative *hicAB* cassette comprising the *hicA* and *hicB* genes located in opposing strands in the pAfPQ33-2 plasmid. The *hicAB* cassette comprises a toxin-antitoxin system that constitutes a translation-independent RNA interference-like system in bacteria and archaea (Jørgensen et al. 2009). In this cassette, at least one *hicB* gene is always present, and the *hicA* gene is mostly located adjacent to the *hicB* gene in the same coding strand; however, sometimes it is positioned in the *hicB* upstream region in the non-coding strand (Makarova et al. 2006), as observed in plasmid pAfPQ33-2 (Fig. 3). Previous studies of the *hicAB* locus distribution in numerous bacterial genomes showed that *hicAB* is transferred horizontally (Makarova et al. 2006). Consistent with this transmission mode, these *hicAB* cassettes in PQ33 and *Sulfobacillus thermotolerans* were found to be encoded in plasmids that could serve as the vehicles for horizontal gene transfer (Deane and Rawlings 2011; Li et al. 2016). As previously reported by Makarova et al. (Makarova et al. 2009), HicB contains a helix-turn-helix DNA binding domain and a derivative RNase H fold, while HicA contains a double-stranded RNA binding domain.

Replication origin.- The RepA helicase protein was found in plasmid pAfPQ33-1. It shares a high percentage identity (98%) with RepA from *Acidithiobacillus thiooxidans* plasmid (Table 2). On the other hand, although no replication genes , such as *repA*, were found in pAf-PQ33-2 plasmid (Fig. 3), a putative DnaJ chaperone protein is reported, which has been not only associated with the function of protein re-folding in heat shock or stress conditions (Xiao et al. 2009). DnaJ chaperone protein has been demonstrated to play a role in the initiation of replication with the RepA plasmid protein, thereby forming a dimeric complex that allows the binding of RepA to the origin of replication (Wickner et al. 1991).

In plasmid pAfPQ33-1, the sequence was aligned with pACA1 and pACA2 plasmids from *A. caldus ATCC 51756* (Fig. 4). A homologous region (86%) of 456 bp that contained the replication origin of the plasmid was found. Additionally, an identity of 85% (175 bp) with pTF91 plasmid from *A. ferrooxidans* was found. This region is located close to the gene *repA*. For pAfPQ33-2 plasmid, no successful match with *Acidithiobacillus* plasmids was noted. However, a GC skew analysis helped identify the replication origin close to the gene *mobA* in pAfPQ33-2 plasmid, approximately 100 bp downstream to the coding region (Fig. 3). Following the report by Lopez et al. (1999), bacterial and archaeal extremophiles must show these GC skew patterns.

Evolutionary relationships among *Acidithiobacillus* plasmids.- As evidenced in Figure 4, there is an evolutionary relationship among plasmids pACA1 and pACA2 from *A. caldus* ATCC 51756 and pAfPQ33-1 plasmid. The particular identity of the diguanylate cyclase-phosphodiesterase (DGC-PDE) protein between the plasmids suggests a similar plasmid maintenance mechanism, either horizontal transfer or genome reorganization in bacteria belonging to the *Acidithiobacillus* genus (Fig. 4). Furthermore, the identity of genes *eep*, *xerD* and the two hypothetical genes with those in pACA2 plasmid suggests a common mechanism of genome reordering and plasticity between *A. caldus* and *A. ferrivorans*. Moreover, the similarity found among the plasmids of *A. caldus* M1, pTcM1, and those from the other strain, pACA1, pLAtc2 and pLAtc3, with pAfPQ33-2 plasmid, reinforce the hypothesis of shared evolutionary traits (Fig. 5). Liljeqvist et al. (2015) reported the metagenome of *Acidithiobacillus* from extremely cold environments and found an integrative community that could support the shared evolutionary traits among species. The exchanging of plasmids between species might confer this function.
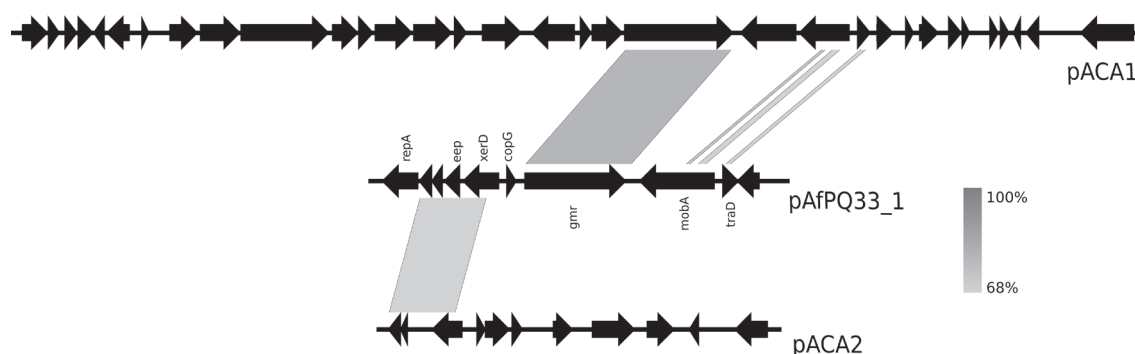
Figure 4. Comparative analysis of pAfPQ33-1 plasmid from *A. ferrivorans* PQ33 and pACA1 and pACA2 plasmids of *A. caldus* ATCC 51756.
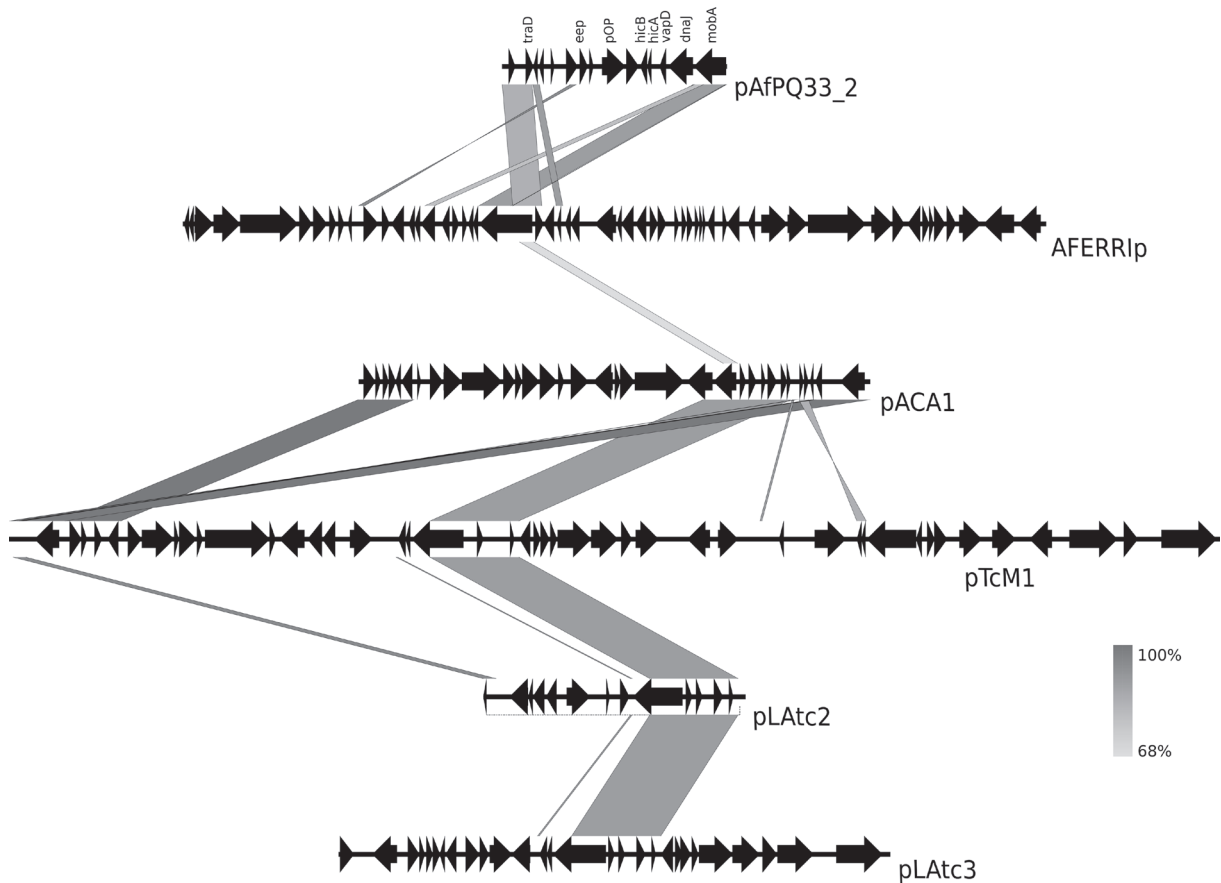
Figure 5. Comparative analysis of plasmids pAfPQ33-2 of *A. ferrivorans* PQ33, AFERRIp of *A. ferrivorans* CF27, pTcM1 of *A. caldus* MNG, pACA1 of *A. caldus* ATCC 51756, pLAtc2 and pLAtc3 of *A. caldus* SM-1.

Proteins involved in environmental adaptation

***Putative endoribonuclease* VapD.-** The coding sequence for the VapD protein (belonging to the CAS2 protein family) was found in plasmid pAfPQ33-2. It presented an identity of 65% compared with endoribonuclease from *Pseudomonas* sp. This protein acts as a protective element against extracellular genetic element invasion (Beloglazova et al. 2008), and it is likely to have an important role in the PQ33 strain because horizontal transfer capacity has become an advantage for *Pseudomonas* species. Since many proteins from the family CAS2 have been related to mRNA degradation, it is suggested that vapD could play a role as an endoribonuclease that is mRNA specific (Beloglazova et al. 2008).

Putative porins O and P and phosphate-selective proteins.- We identified the phosphate-selective porins O and P proteins in pAfPQ33-2 plasmid. These present an identity of 68% (417 aa) compared with the porin protein present in the chromosome of *A. ferrivorans*, which coincides with other phosphate porins found in the *Acidithiobacillus* genus. The predicted domain organization of porins O and P is shown in Figure 6. During bioleaching processes, phosphate starvation generates activation of genes encoding proteins that allow the recovery of environmental phosphate (Bobadilla Fazzini and Parada Valdecantos 2009), including selective phosphate porins O and P, which have a specific phosphate/orthophosphate

binding site and are overexpressed under conditions of starvation (Hancock et al. 1982; Poole et al. 1987). Thus, the specific location of the gene of this protein in the plasmid could be involved in the adaptation of *A. ferrivorans* to the environmental stress conditions. This suggests rapid adaptation of this specie, as evidenced in their ability to grow *in vitro* compared to *A. ferrooxidans*.
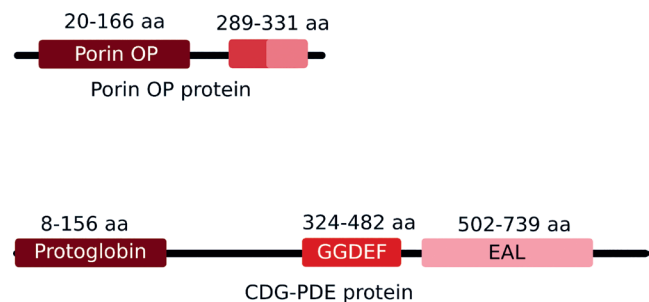
Figure 6. Domain organization of porins O and P and CDG-PDE proteins. a) Putative porins O and P aligned to positions 20-166 (E-value = 5.75e[-05]) and 289-331 (E-value = 4.6e[-05]) of a 359 residue-long Hidden Markov Model (HMM) defining a phosphate-selective porin O and P family. **b**) CDG-PDE aligned with high confidence to three HMMs defining a protoglobin family (E-value = 2.8e[-47]), and GDEF (E-value = 6.7e[-21]) and EAL domains (E-value = 6.3e[-67])

Table 3. Identified ORFs in pAfPQ33-2 plasmid of *A. ferrivorans*.

ORF's designation	Location	Product size	% Coverage	E-Value	% Identity	Pfam*	ORF's description	Related organism
Hypothetical protein	348-713	-	-	-	-	-	-	-
traD	1270-1680	135	53	1e^{-10}	51	TraD	Conjugal transfer protein TraD	Burkholderia cepacia
Hypothetical protein	(1684-1911)^c	76	98	7e^{-42}	92	-	Hypothetical protein	Acidithiobacillus ferrivorans
Hypothetical protein	(1988-2230)^c	81	-	-	-	-	-	-
Hypothetical protein	2624-2812	63	-	-	-	-	-	-
Hypothetical protein	3449-4057	203	-	-	-	-	-	-
Entry exclusion protein	4175-4609	143	96	9e^{-14}	31	HTH_17	Entry exclusion protein	Providencia rustigianii
Hypothetical protein	4689-4925	91	70	5e^{-33}	94	-	Hypothetical protein	Ferrovium myxofaciens
Porin O/P	(5374-6627)^c	423	99	6e^{-152}	68	Porin_O_P	Phosphate-selective porin O and P	Acidithiobacillus ferrivorans
Hypothetical protein	6682-7359	228	91	2e^{-09}	25	-	Hypothetical protein	Acidithiobacillus thiooxidans
hicB	(7445-7804)^c	120	95	3e^{-32}	50	HicB	HicB family protein/ Predicted nuclease of the RNAse H fold.	Desulfovibrio fructosivorans
hicA	(7807-8034)^c	76	94	4e^{-10}	42	HicA_toxin	YcfA-like protein.	Geobacter sp
vapD	(8483-8821)^c	113	83	1e^{-36}	65	CRISPR_Cas2	Endoribonuclease VapD	*Pseudomonas* sp.
dnaJ	(8929-10257)^c	401	86	7e^{-35}	34	DnaJ	DnaJ molecular chaperone.	Alicycliphilus denitrificans
mobA	(10318-12048)^c	845	47	8e^{-64}	44	LPD7	Mobilization protein A	Acidithiobacillus thiooxidans

*most significant match
^{c}Gene in complementary sequence

Diguanylate cyclase-phosphodiesterase (DGC-PDE) with a chemoreceptor zinc-binding domain.- Recently, diguanylate cyclase-phosphodiesterase (DGC-PDE) has been studied due to its role in adherence during the biomining process. In *A. caldus* and *A. ferrooxidans,* the protein has been expressed in bacteria growing over sulfide surfaces, and the efficiency of bioleaching was noted. Additionally, in *A. caldus* mutant of this protein, no evidence of adherence to sulfur surfaces was noted (Castro et al. 2015). Our plasmid pAfPQ33-1 has an ORF encoding the DGC-PDE protein that can hydrolyse and synthetize c-di-GMP with the presence of both GGDEF and EAL domains. It has an identity of 78% with GGDEF/EAL domains of *A. ferrivorans* SS3. However, we identified GGDEF, EAL and protoglobin domains in Pfam and Swiss-Model (Fig. 6). Surprisingly, a chemoreceptor zinc binding (CZB) domain adjacent to the GGDEF-EAL domains was predicted; however, it was found in a different open reading frame and is similar (63% of identity) to that present in *A. caldus*. The *A. ferrivorans* draft genome reports approximately 16 putative coding-genes for the enzyme DGC-PDE; many of them present GGDEF and EAL domains, as well as a series of sensor domains, and a CZB domain. Studies in *E. coli* have shown that biofilm formation was dependent on the DGC-CZB activity, which was modulated by the availability of zinc, since mutation of amino acids residues that coordinate this ion generated a decrease in exopolysaccharide formation (Zähringer et al. 2013).

Nucleotide sequence and accession number.- The NCBI accession numbers for the pAfPQ33-1 and pAfPQ33-2 plasmids are NZ_CP021414-1 and NZ_CP021415.1, respectively (Table 1). The cured annotation of this study was considered for the paper discussion (Tables 2 and 3).

Conclusions

In a genomic context, the analysis of plasmids from PQ33 strain suggests their key role in the horizontal transfer system of *A. ferrivorans*. The comparison between plasmids of *A. ferrivorans* and *A. caldus* shows that there are evolutionarily shared traits involving the gene exchange mechanism among species. These findings could be significant for a global understanding of the environmental role of the species. Additionally, the characterization of the plasmids offers new insights into the development of vectors for the psychrotolerant strains. The description of the metabolic diversity in psychrotolerant *Acidithiobacillus* spp. is becoming vital for the understanding of bioleaching operations in cold climate zones such as the South American Andes.

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The authors declare no conflict of interest.

Rol de los autores / Authors Roles:

RC, AE y PR: realizaron el diseño experimental, interpretación de resultados y corrección; AE: realizó los ensayos experimentales y procesó los datos; RC, AE y MA: analizaron los resultados computacionales. RC, AE y PR: redactaron el artículo. RC, PR, RGG, TS y JS revisaron el artículo antes de enviarlo.

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