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Short Genome Communications

Complete genome sequence of *Bacillus amyloliquefaciens* subsp. *plantarum* S499, a rhizobacterium that triggers plant defences and inhibits fungal phytopathogens

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ABSTRACT

Bacillus amyloliquefaciens subsp. *plantarum* S499 is a plant beneficial rhizobacterium with a good antagonistic potential against phytopathogens through the release of active secondary metabolites. Moreover, it can induce systemic resistance in plants by producing considerable amounts of surfactins. The complete genome sequence of *B. amyloliquefaciens* subsp. *plantarum* S499 includes a circular chromosome of 3,927,922 bp and a plasmid of 8,008 bp. A remarkable abundance in genomic regions of putative horizontal origin emerged from the analysis. Furthermore, we highlighted the presence of genes involved in the establishment of interactions with the host plants at the root level and in the competition with other soil-borne microorganisms. More specifically, genes related to the synthesis of amylolysin, amylocyclicin, and butirosin were identified. These antimicrobials were not known before to be part of the antibiotic arsenal of the strain. The information embedded in the genome will support the upcoming studies regarding the application of *B. amyloliquefaciens* isolates as plant-growth promoters and biocontrol agents.

1. Introduction

Since its isolation from a cultivated soil in the Iturie region (Democratic Republic of Congo; Delcambe, 1965), the rhizobacterium *Bacillus amyloliquefaciens* subsp. *plantarum* S499 (available at BCCM/LMG Bacteria Collection, LMG 29676) has been extensively described due to its plant beneficial properties shown in greenhouse and field trials (Nihorimbere et al., 2010; Pertot et al., 2013). The protection provided by S499 against phytopathogens relies on its potential to produce multiple antimicrobial metabolites (Cawoy et al., 2015) and on its ability to induce systemic resistance in plants (Ongena et al., 2005a,b, 2007). This strain is an excellent producer of the surfactin-type lipopeptide, which not only contributes to the high rhizosphere competence of the bacterium but which also acts as the main elicitor of host immunity (Cawoy et al., 2014). It most probably explains why in a comparative study, which included 16 root-associated *Bacillus* isolates, S499 was the most efficient in disease reduction (Cawoy et al., 2014). For these reasons, S499 has often been used as model to investigate the impact of biotic and abiotic factors on rhizosphere fitness and antibiotic production in *B. amyloliquefaciens*, but also to study the molecular interactions established with the host plant (Debois et al., 2015; Henry et al., 2011; Nihorimbere et al., 2012; Pertot et al., 2013). Furthermore, expression of its biocontrol-related antibiome has been characterised in details, both *in vitro* and *in planta* (Debois et al., 2014). The availability of the S499 complete genome can provide an additional tool for in-depth investigation of the mechanisms involved in biocontrol of plant diseases and be the basis for the development of novel more effective biofungicides based on bacteria belonging to *B. amyloliquefaciens* subsp. *plantarum*.

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Table 1
Features of *Bacillus amyloliquefaciens* subsp. *plantarum* S499 genome.

Features	Chromosome	Plasmid
Genome size (bp)	3,927,922	8,008
G + C content (%)	46.6	40.40
Total predicted CDS[a]	3,974	7
rRNA operons	24	–
tRNA genes	81	–
Insertion sequence	1	–
Phage-associated genes	154	–

[a] CDS: Coding DNA Sequences.

2. Materials and methods

2.1. DNA extraction and genome sequencing

Genomic DNA was extracted from S499 cultures using a PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Invitrogen, USA) according to the manufacturer's instructions. A 10-kb PacBio® RS II single-molecule real-time (SMRT) cell (Chin et al., 2013) was used to sequence the whole genomic DNA of S499 through PacBio technology at Baseclear B.V. (Leiden, Netherlands).

2.2. Genome assembly and annotation

Assembly of subreads obtained with the PacBio® RS II SMRT was carried out using the RS hierarchical genome assembly process (HGAP) protocol version 3.0, as available in SMRT Portal v2.0 (https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/HGAP-in-SMRT-Analysis). The SMRT Portal was configured and used with a public machine image that Pacific Biosciences maintains and upgrades on Amazon Cloud (https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/%22Installing%22-SMRT-Portal-the-easy-way—Launching-A-SMRT-Portal-AMI). Whole genome assembly was achieved with a comparative method, which combined *de novo* assembly and mapping through the MAUVE aligner tool (Darling et al., 2010). Annotation was carried out using Rapid Annotation Subsystem Technology (RAST; Aziz et al., 2008). Tandem repeats were detected with Tandem Repeats Finder (Benson, 1999) and genomic islands were screened using IslandViewer (Dhillon et al., 2013; Langille and Brinkman, 2009). IS Finder (Siguier et al., 2006) and PHAST (Zhou et al., 2011) were used to identify insertion sequences and prophage regions. Genome mining for bioactive secondary metabolites was performed via antiSMASH 3.0 (Weber et al., 2015).

3. Results and discussion

The genetic equipment of S499 consists of a 3,927,922 bp circular chromosome and an 8,008 bp plasmid (Fig. 1). The plasmid contains only seven coding DNA sequences (CDS) while the circular chromosome has 3,974 CDS, and 106 predicted RNA genes (Table 1). The circular chromosome includes 99 tandem repeats and one insertion sequence element of 1,275 bp identified as "ISBsu1", encoding a transposase and a tail length tape measure protein. Furthermore, we detected four prophage regions, containing 154 putative phage-related genes. Screening for genomic islands revealed the presence of five additional regions of hypothetical horizontal origin, which include the CDS involved in antibiotic resistance, detoxification and stress responses (Table 1). This finding could reflect the S499 adaptation to its natural soil ecosystem. In such habitat, the evolution of bacterial populations continuously exposed to environmental stresses could largely depend on a high rate of horizontal gene exchanges (Aminov, 2011).

Through RAST classification into subsystems (Table 2), we highlighted the importance of the coding portion of the S499 genome that supports bacterial development on roots. Indeed, as much as 15% of the classified CDS were assigned to categories related to the ability of S499 to effectively colonize plant roots and produce secondary metabolites responsible for the control of phytopathogens ("Motility and Chemotaxis", "Membrane Transport", "Virulence, Disease and Defense", "Secondary Metabolism" and "Stress Responses"). Regarding the intrinsic plant growth promotion function of the strain (Nihorimbere et al., 2010), we assessed the presence of the genes necessary for synthesis of auxin, phytase, and volatile compounds (e.g. 2,3-butanediol and acetoin) known to be implicated in this activity (Idriss et al., 2002; Ryu et al., 2004; Table 2). In the context of phytopathogen biocontrol, the antiSMASH analysis allowed to identify gene clusters encoding the enzymatic machinery for synthesis of nonribosomal peptides (surfactins, iturins, fengycins, bacillibactin, and bacilysin) and polyketides (bacillaene, difficidin, and macrolactin, Table 3). The antiSMASH analysis identified additional genes related to the production of other antimicrobials, such as the lantibiotic amylolysin (Arguelles-Arias et al., 2013), the bacteriocin amylocyclicin (Scholz et al., 2014), and the aminoglycoside antibiotic butirosin (Llewellyn et al., 2007), which have not been detected using chemical analysis in culture media of the strain to date (Debois et al., 2014).

The genomic features of S499 thus clearly reflect its root-associated lifestyle and its biocontrol potential. Most importantly, the complete genome of this efficient and peculiar *Bacillus* strain is being used for developing transcriptomic studies with the aim of understanding how the antibiome expression profile in this bacterium is modulated upon interaction with a variety of host plants and with the numerous competitors present in the rhizosphere microbiome.

Table 2
Distribution of *Bacillus amyloliquefaciens* subsp. *plantarum* S499 coding DNA sequences (CDS) in subsystems according to RAST Server.

Subsystem	CDS
Cofactors, Vitamins, Prosthetic Groups, Pigments	229
Cell Wall and Capsule	138
Virulence, Disease and Defense	60
Potassium metabolism	9
Photosynthesis	0
Miscellaneous	47
Phages, Prophages, Transposable elements, Plasmids	25
Membrane Transport	69
Iron acquisition and metabolism	30
RNA Metabolism	155
Nucleosides and Nucleotides	114
Protein Metabolism	162
Cell Division and Cell Cycle	55
Motility and Chemotaxis	86
Regulation and Cell signaling	64
Secondary Metabolism	6
DNA Metabolism	105
Fatty Acids, Lipids, and Isoprenoids	138
Nitrogen Metabolism	31
Dormancy and Sporulation	117
Respiration	74
Stress Response	108
Metabolism of Aromatic Compounds	12
Amino Acids and Derivatives	444
Sulfur Metabolism	39
Phosphorus Metabolism	31
Carbohydrates	414

4. Nucleotide sequence accession number

The complete nucleotide genome sequence of *B. amyloliquefaciens* subsp. *plantarum* S499 has been deposited at