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The genomic and transcriptomic basis of the potential of *Lactobacillus plantarum* A6 to improve the nutritional quality of a cereal based fermented food

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ABSTRACT

The objective of this work was to investigate the nutritional potential of *Lactobacillus plantarum* A6 in a food matrix using next generation sequencing. To this end, we characterized the genome of the A6 strain for a complete overview of its potential. We then compared its transcriptome when grown in a food matrix made from pearl millet to and its transcriptome when cultivated in a laboratory medium.

Genomic comparison of the strain *L. plantarum* A6 with the strains WCFS1, ST-III, JDM1 and ATCC14917 led to the identification of five regions of genomic plasticity. More specifically, 362 coding sequences, mostly annotated as coding for proteins of unknown functions, were specific to *L. plantarum* A6. A total of 1201 genes were significantly differentially expressed in laboratory medium and food matrix. Among them, 821 genes were upregulated in the food matrix compared to the laboratory medium, representing 23% of whole genomic objects. In the laboratory medium, the expression of 380 genes, representing 11% of the all genomic objects was at least double than in the food matrix.

Genes encoding important functions for the nutritional quality of the food were identified. Considering its efficiency as an amylolytic strain, we investigated all genes involved in carbohydrate metabolism, paying particular attention to starch metabolism. An extracellular alpha amylase, a neopullulanase and maltodextrin transporters were identified, all of which were highly expressed in the food matrix. In addition, genes involved in alpha-galactoside metabolism were identified but only two of them were induced in food matrix than in laboratory medium. This may be because alpha galactosides were already eliminated during soaking. Different biosynthetic pathways involved in the synthesis of vitamin B (folate, riboflavin, and cobalamin) were identified. They allowed the identification of a potential of vitamin synthesis, which should be confirmed through biochemical analysis in further work. Surprisingly, some genes involved in metabolism and bioaccessibility of iron were identified. They were related directly to the use of transport of iron, or indirectly to metabolism of polyphenols, responsible of iron chelation in the food.

The combination of genomics and transcriptomics not only revealed previously undocumented nutritional properties of *L. plantarum* A6, but also documented the behaviour of this bacterium in food.

1. Introduction

Lactic acid bacteria (LAB) are widely used as probiotics thanks to their effects, which include mitigation of lactose intolerance and stimulation of the immune system. They are also used in food processing to develop flavour components, to contribute to the food structure, and to improve nutritional quality and health aspects (Corsetti and Settanni, 2007; Rijkers et al., 2010; Turpin et al., 2010). The genus *Lactobacillus* is found in different environments including breast milk, the digestive and urogenital tracts, fermented meat, dairy products and cereal based foods such as sourdough (Martino et al., 2016). In the *Lactobacillus* genus, the *Lactobacillus plantarum* species is extremely well adapted to different niches due to a highly flexible genome with life-style islands mainly related to the utilization of carbohydrates (Siezen and van

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Hylckama Vlieg, 2011). Extensive molecular and post-genomic analysis has been performed on *L. plantarum* species in recent years, and many strains have been used as a model. Several strains of *L. plantarum* have been sequenced and the strain WCFS1, which was isolated from human saliva, was the first *Lactobacillus* genome to be sequenced (Kleerebezem et al., 2003). Other sequenced *L. plantarum* have been isolated from human vagina (KCA1) (Anukam et al., 2013), from grass silage (NC8) (Axelsson et al., 2012), from new-born infant faecal samples ZJ316 (Li et al., 2013), from kimchi (ST-III) (Wang et al., 2011), from pickled cabbage (ATCC® 14917™) (NCBI Accession Code NZ_ACGZ00000000) or used as commercial probiotics in China (JDM1) (Zhang et al., 2009). The sequenced strains isolated from foods were all of plant origin but, to the best of our knowledge, no strain isolated from a starchy rich matrix has been sequenced to date. We hypothesize that the genetic equipment of *L. plantarum* A6, isolated from retted cassava and highly efficient in hydrolysing starch (Giraud et al., 1991) would present interesting features, especially in the repertoire of enzymes involved in starch metabolism.

The efficiency of *L. plantarum* A6 in breaking down starch led to its use to make high energy density fermented gruels made from pearl millet or rice (Nguyen et al., 2007; Songre-Ouattara et al., 2010). Its efficiency in pearl millet porridge has been linked to the expression of genes encoding different enzymes involved in starch hydrolysis at transcriptional and enzymatic levels (Humblot et al., 2014). The most active enzymes were shown to be an extracellular alpha-amylase (*E.C.* 3.2.1.1) encoded by *amyA* and a neopullulanase (*E.C.* 3.2.1.135) encoded by *dexC*. In addition to its interest as an amylolytic bacterium, this strain also has nutritional potential. Indeed, using PCR, we detected several targeted genes encoding enzymes involved in folate and riboflavin synthesis (Turpin et al., 2011). However, the whole gene pool involved in these pathways and needed for vitamin B synthesis remained to be detected. In addition, for a complete view of other nutritional potential, the sequencing of the entire genome would be of great help. By nutritional potential, we include all elements related to the improvement of digestion, absorption and availability of nutrient through action of bacteria in the food matrix or in the digestive tract (Turpin et al., 2010).

Even if the availability of genomic data allows a better comprehension of the adaptation of bacteria to their environment, it nevertheless only provides a static view of their genetic potential. Transcriptome analysis enables a better comprehension of bacterial physiology, and provides new insights into genomic elements and gene expression (Siezen et al., 2010b). Although there have been many studies of bacterial genome expression in their natural habitat, publications on analysis of bacterial transcriptomes in food matrices are rare (Jung et al., 2013), and of those, most were performed using microarrays (Siezen et al., 2010b), which provided insights into transcriptomes, but have several limitations. They often have biased genome coverage since they are based on known or expected genes in sequenced genomes, and they rarely contain intergenic regions (Siezen et al., 2010b). Next generation sequencing technologies applied to high-depth sequencing of whole transcriptomes, now referred to as RNA-seq, offer new perspectives for a better understanding of bacterial physiology in their natural habitats. For example, during growth of *Lactococcus lactis* KF147 in plant leaf tissue lysate, 853 genes were induced in comparison with GM17 laboratory medium (Golomb and Marco, 2015).

The general objective of this work was to investigate the genetic potential of *L. plantarum* A6 to improve the nutritional quality of a cereal-based food matrix. To this end, we first sequenced the genome of the strain to obtain the most complete overview possible of its potential. We focused on genes already demonstrated through functional analysis as involved in functions of nutritional interest. We compared the transcriptome of the strain *L. plantarum* A6 grown in a food matrix made from pearl millet to the transcriptome of *L. plantarum* A6 grown in a laboratory medium.

2. Materials and methods

2.1. Chemicals and raw material

De Man Rogosa and Sharpe (MRS) medium and agar were purchased from Difco (Le Pont de Claix, France). All other chemicals were purchased from Sigma-Aldrich (St-Quentin-Fallavier, France).

Pearl millet was purchased in Ouagadougou (Burkina Faso) for the preparation of gelatinized starchy batter. The same batch of pearl millet grains was used for all the experiments.

2.2. Experimental design

The amylolytic strain *L. plantarum* A6 (LMG 18053) (Giraud et al., 1991) was inoculated (1% v/w) on a MRS broth or on a gelatinized pearl millet batter using the modified method described in Tou et al. (2007) with one volume of pearl millet for 1.5 volumes of water (w/v) for the soaking step and one volume of pearl millet for 5 volumes of water (w/v) for the fermentation step (Tou et al., 2007). Gelatinization of the pearl millet batter was achieved by cooking at 80 °C for 10 min under stirring, which also leads to a major reduction of endogenous microbiota (< 10 cfu/g of porridge) to enable the growth of only the inoculated bacteria. To inoculate the gelatinized pearl millet porridge previously cooled at 30 °C, an overnight culture on MRS was centrifuged at 8000 × *g* for 10 min at 4 °C, and the cells obtained were washed in sterile NaCl 0.9 g/L, centrifuged in the same conditions and suspended in sterile NaCl 0.9 g/L to form a cell suspension containing approximately 10^9 cfu/mL. All the fermentations were incubated at 30 °C under static conditions and sampled at hourly intervals for 5 h. All the samples were then stored at − 80 °C until analysis.

Viable *L. plantarum* A6 bacteria were counted at the beginning and at the end of the fermentation by plating on MRS agar and varied between $9.7 \pm 1.2 \cdot 10^6$ cfu/g to $3.2 \pm 2.1 \cdot 10^7$ cfu/g of dough, respectively.

The fermentations on MRS broth and on pearl millet were performed as three independent experiments.

2.3. DNA extraction

DNA was extracted from the bacterial pellet of overnight pure cultures of *L. plantarum* A6 grown in MRS broth using the Wizard genomic DNA purification kit (Promega, Charbonnières, France) with an additional lysis step using an amalgamator with zirconium beads (VWR, Fontenay-sous-Bois, France). First, the cells were lysed with 0.1 mm zirconium beads for 30 s, and then incubated for 1 h at 37 °C with lysozyme (Eurobio, 40 kU) and mutanolysine (Promega, 10 U). Following to the manufacturer's instructions, cell lysis was completed with the Nuclei lysis solution® (Promega). RNA was removed by the RNase solution® (Promega), and proteins with the Protein Precipitation Solution® (Promega). DNA was precipitated with isopropanol and washed with 70% ethanol. DNA quality was checked by separation on agarose gel followed by ethidium bromide staining and by capillary electrophoresis (MultiNA microchip electrophoresis system, Shimadzu system, Vertis Biotechnologies, AG).

2.4. Total RNA extraction, quality evaluation and reverse transcription

RNA extraction were performed after 5 h incubation since it was the time where all genes encoding enzymes involved in starch metabolism were expressed (data not shown).

To limit RNA degradation, RNA extraction was performed on samples kept at − 80 °C for less than 3 days, and the extraction steps were performed at 4 °C unless otherwise indicated. For extraction from food matrices, as the batters were very sticky, they were diluted 10 times in NaCl 0.9 g/L and centrifuged for 10 min at 1000*g* (4 °C) twice to