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Data in Brief

Transcriptomic profile of *aguR* deletion mutant of *Lactococcus lactis* subsp. *cremoris* CECT 8666

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ABSTRACT

Lactococcus lactis subsp. *cremoris* CECT 8666 (formerly GE2-14) is a dairy strain that catabolizes agmatine (a decarboxylated derivative of arginine) into the biogenic amine putrescine by the agmatine deiminase (AGDI) pathway [1]. The AGDI cluster of *L. lactis* is composed by five genes *aguR*, *aguB*, *aguD*, *aguA* and *aguC*. The last four genes are responsible for the deamination of agmatine to putrescine and are co-transcribed as a single policistronic mRNA forming the catabolic operon *aguBDAC* [1]. *aguR* encodes a transmembrane protein that functions as a one-component signal transduction system that senses the agmatine concentration of the medium and accordingly regulates the transcription of *aguBDAC* [2], which is also transcriptionally regulated by carbon catabolic repression (CCR) via glucose, but not by other sugars such as lactose and galactose [1,3]. Here we report the transcriptional profiling of the *aguR* gene deletion mutant (*L. lactis* subsp. *cremoris* CECT 8666 Δ*aguR*) [2] compared to the wild type strain, both grown in M17 medium with galactose as carbon source and supplemented with agmatine. The transcriptional profiling data of AguR-regulated genes were deposited in the Gene Expression Omnibus (GEO) database under accession no. GSE59514.

Specifications	
Organism/cell line/tissue	*L. lactis* subsp. *cremoris* CECT 8666 (formerly GE2-14)
Sex	N/A
Sequencer or array type	Oligo-based DNA microarray
Data format	Raw and normalized
Experimental factors	*L. lactis* subsp. *cremoris* CECT 8666 Δ*aguR* (test) versus *L. lactis* subsp. *cremoris* CECT 8666 (reference)
Experimental features	Microarray comparison was preformed to identify genes differentially expressed in *L. lactis* subsp. *cremoris* CECT 8666 Δ*aguR* compared to *L. lactis* subsp. *cremoris* CECT 8666 grown in M17 medium supplemented with 1% galactose (*w*/*v*) and 20 mM agmatine.
Consent	N/A
Sample source location	Villaviciosa, Spain

1. Direct link to deposited data

Microarray data are accessible in the following link: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59514.

2. Experimental design, materials and methods

2.1. Design of L. lactis subsp. cremoris CECT 8666 DNA microarrays

L. lactis subsp. *cremoris* CECT 8666 DNA microarrays (Agilent Technologies, Santa Clara, CA) were designed using the Agilent eArray (v5.0) program according to the manufacturers' recommendations as described in Linares et al. (2015) [2]. Each microarray (8 × 15 K) was designed to contain spots of two different 60-mer oligonucleotide probes (in duplicate) specific for each of the 2635 coding DNA sequences (CDSs) representing the protein-coding genes of the *L. lactis* subsp. *cremoris* CECT 8666 genome (GenBank accesion no. AZSI00000000.1) [4].

2.2. Bacterial strains and growth conditions

Table 1 shows the strains used in this study. *L. lactis* subsp. *cremoris* CECT 8666 was originally isolated from a traditional cheese [5]. The mutant strain *L. lactis* subsp. *cremoris* CECT 8666 Δ*aguR* was constructed by homologous recombination [2]. Both strains were grown in replicates (10 ml each) in M17 medium (Oxoid, Basingstoke, United Kingdom) supplemented with 1% galactose (*w*/*v*) and 20 mM agmatine (Sigma-Aldrich, Barcelona, Spain) for 6 h at 30 °C. Cells were harvested by

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Table 1
Strains used in this study.

Strain	Description	Reference/source
L. lactis subsp. *cremoris* CECT 8666[a]	Putrescine producer	[3], CECT[b]
L. lactis subsp. *cremoris* CECT 8666 Δ*aguR*	Non-putrescine producer	[5]

[a] Formerly *L. lactis* subsp. *cremoris* GE2-14.
[b] CECT: Colección Española de Cultivos Tipo.

centrifugation at 8000 ×*g* for 5 min at 4 °C. The supernatants were removed and cell pellets were frozen in liquid nitrogen and stored at −80 °C.

2.3. RNA extraction

RNA extraction was performed as previously described [6] with minor modifications. Briefly, cell pellets were thaw on ice and resuspended in 500 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA pH 8.0) and transferred to screw-capped tubes containing 50 μl of 10% SDS, 500 μl of phenol:chloroform:isoamyl alcohol (25:24:1) (Sigma-Aldrich), 500 mg of glass beads (75–150 μm) (Sigma-Aldrich), and 175 μl of Macaloid suspension (Bentone MA, Rheox Inc., Scotland, United Kingdom). Cells were mechanically disrupted in a bead beater at 4 °C. The samples were shaken two times for 45 s. During the shaking intervals the cells were kept on ice for 1 min. The samples were then centrifuged at 8000 ×*g* for 10 min at 4 °C. The upper phase was transferred to fresh tubes containing 500 μl chloroform:isoamyl alcohol (24:1) and centrifuged for 5 min at 4 °C. 500 μl of the upper phase was transferred to fresh tubes containing 1 ml of lysis/binding buffer of the High Pure RNA Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany). All subsequent steps including the DNaseI treatment were performed following the instructions provided by the manufacturer. The concentration and quality of the RNA were checked on a NanoDrop spectrophotometer (Thermo Scientific, Landsmeer, The Netherlands).

2.4. Synthesis of cDNA

The synthesis of cDNA was performed using 20 μg of total RNA and the SuperScript® III Reverse Transcriptase kit (Life Technologies, Bleiswijk, Netherlands), as described in Shafeeq et al. (2015) [6]. After the cDNA was synthesized, the mRNA of the reverse transcription mixture was denaturalized by adding 3 μl of 2.5 mM NaOH for 15 min at 37 °C. The NaOH was neutralized by adding 15 μl of 2 M HEPES free acid. The cDNA was purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nager, Landsmeer, The Netherlands). Briefly, 200 μl of NTC buffer was mixed with the unpurified cDNA, added to a column and centrifuged for 1 min at 11,000 ×*g*. The column was washed first with 600 μl of buffer NT3 and then with 500 μl 80% ethanol. The residual ethanol was completely removed by centrifugation for 2 min at 11,000 ×*g*. To elute the cDNA, 60 μl of 0.1 M sodium bicarbonate pH 9.0 was added to the column and incubated for 1 min at room temperature. Purified cDNA was collected by centrifugation for 1 min at 11,000 ×*g* and was immediately labeled.

2.5. Labeling of cDNA

DyLight 550 NHS ester and DyLight 650 NHS ester (Thermo Scientific) were used to label the cDNAs. Dyes were dissolved in 200 μl of DMSO (dimethyl sulfoxide) (Sigma-Aldrich). 60 μl of purified cDNA (in 0.1 M sodium bicarbonate pH 9.0, see above) labeled with 5 μl DyLight 550 or DyLight 650 in the dark for 90 min at room temperature. Labeled cDNA was purified using NucleoSpin Gel and PCR Clean-up columns as described in the previous section, with the exception that cDNA was eluted with 50 μl of elution buffer NE of the NucleoSpin Gel and PCR Clean-up kit.

2.6. Hybridization and washing

Nine hundred nanograms of DyLight 550- and DyLight 650-labeled cDNA was mixed and hybridized for 17 h at 60 °C in the *L. lactis* subsp. *cremoris* CECT 8666 DNA microarray using the In situ Hybridization Kit Plus, the Hybridization Gasket Slide and the Agilent G2534A Microarray Hybridization Chamber (Agilent Technologies). After hybridization, slides were washed using appropriate washing buffers as recommended by the manufacturer.

2.7. Microarray data analysis

Slides were scanned using a GenePix 4200A Microarray Scanner (Molecular Devices, Sunnyvale, CA) and the images analyzed using GenePix Pro v.6.0 software. Background subtraction and LOWESS (locally weighted scatterplot smoothing) normalization were performed using the standard routines provided by GENOME2D software available at http://server.molgenrug.nl/index.php/analysis-pipeline. DNA microarray data were obtained from three independent biological replicates and two technical replicates (including a dye swap). Expression ratios were calculated from the comparison of four spots per gene per microarray (a total of 20 measurements per gene). A gene was considered differentially expressed when a p value of at least <0.05 was obtained and the expression fold-change was at least $>|0.5|$. The microarray data were deposited in Gene Expression Omnibus (GEO) database under the accession no. GSE59514.

3. Discussion

In this study, we determined the effect of *aguR* deletion on the transcriptomic profile of *L. lactis* subsp. *cremoris* CECT 8666 grown in M17 supplemented with 1% galactose and 20 mM agmatine. The genes *aguB*, *aguD*, *aguA* and *aguC* coding for the proteins needed for the biosynthesis of putrescine through the AGDI pathway are highly downregulated in the Δ*aguR* mutant strain, indicating the role of AguR as transcriptional activator of the catalytic AGDI genes that results essential for putrescine biosynthesis [2]. The microarray analysis also reveals the low expression of *aguR* in the CECT 8666 wild-type strain. In addition, other 49 genes were downregulated and 41 upregulated in the Δ*aguR* mutant strain compared to the CECT 8666 wild-type strain. Further investigations are required to elucidate the role of AguR in the regulation of these genes.

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