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Data in Brief

Transcriptome profiling of *Lactococcus lactis* subsp. *cremoris* CECT 8666 in response to agmatine

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ABSTRACT

The dairy strain *Lactococcus lactis* subsp. *cremoris* CECT 8666 (formerly GE2-14) synthesizes the biogenic amine putrescine from agmatine via 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 co-transcribed as a single policistronic mRNA forming the catabolic operon *aguBDAC*, which encodes the proteins necessary for agmatine uptake and its conversion into putrescine [1,2]. The first gene of the cluster, *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]. The catabolic operon *aguBDAC* is transcriptionally activated by agmatine [2] and transcriptionally regulated by carbon catabolite repression (CCR) via glucose, but not by other sugars such as lactose or galactose [1,3]. On the contrary, the transcription of the *aguR* regulatory gene is not subject to CCR regulation [1,3] nor is regulated by agmatine [2]. In this study we report the transcriptional profiling of *L. lactis* subsp. *cremoris* CECT 8666 grown in M17 medium with galactose (GalM17) as carbon source and supplemented with agmatine, compared to that of the strain grown in the same culture medium without agmatine. The transcriptional profiling data of agmatine-regulated genes were deposited in the Gene Expression Omnibus (GEO) database under Accession no. GSE74808.

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 grown in GalM17 + 20 mM agmatine (test) versus *L. lactis* subsp. *cremoris* CECT 8666 grown in GalM17 (reference)
Experimental features	Microarray comparison was preformed to identify genes differentially expressed in *L. lactis* subsp. *cremoris* CECT 8666 grown in GalM17 medium supplemented with 20 mM agmatine compared to *L. lactis* subsp. *cremoris* CECT 8666 grown in GalM17 medium without 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=GSE74808.

2. Experimental design, materials and methods

2.1. Design of *Lactococcus 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 manufacturer's recommendations as described in [2]. Each microarray (8 × 15K) 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 accession no. AZSI00000000.1) [4].

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2.2. Bacterial strains and growth conditions

L. lactis subsp. *cremoris* CECT 8666 a putrescine producing strain [4] that was originally isolated from a traditional cheese [5] was used in this study. *L. lactis* CECT 8666 was grown in replicates (10 ml each) in M17 medium (Oxoid, Basingstoke, United Kingdom) supplemented with 1% galactose (*w*/*v*) (GalM17), with or without 20 mM agmatine (Sigma-Aldrich, Barcelona, Spain) for 6 h at 30 °C. Cells were harvested by 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]. 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 [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 were 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) were 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 previously described, 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 (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. GSE74808.

3. Results and discussion

In this study, we determined the effect of agmatine on the transcriptomic profile of *L. lactis* subsp. *cremoris* CECT 8666 grown in GalM17. The genes *aguB*, *aguD*, *aguA* and *aguC* coding for the proteins needed for the biosynthesis of putrescine through the AGDI pathway were highly upregulated in the *L. lactis* CECT 8666 strain grown with agmatine. This result corroborates the activator effect of agmatine on the transcription of the catalytic genes of the AGDI cluster, which was recently assessed by reverse transcription quantitative PCR (RT-qPCR) analysis [2]. The microarray analysis also revealed that the expression of the transcription regulator gene *aguR* was not affected by agmatine, as we previously demonstrated by RT-qPCR analysis [2]. In addition, other 85 genes were downregulated and 179 upregulated in the *L. lactis* CECT 8666 strain grown in GalM17 medium supplemented with agmatine compared to the strain grown without agmatine. A further analysis should be performed to identify key regulatory factors that may affect putrescine production and ultimately lead to adopt measures to reduce the presence in dairy products of this undesirable biogenic amine.

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