**agr System of Listeria monocytogenes EGD-e: Role in Adherence and Differential Expression Pattern**

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In this study, we investigated the agrBDCA operon in the pathogenic bacterium Listeria monocytogenes EGD-e. In-frame deletion of agrA and agrD resulted in an altered adherence and biofilm formation on abiotic surfaces, suggesting the involvement of the agr system of L. monocytogenes during the early stages of biofilm formation. Real-time PCR experiments indicated that the transcript levels of agrBDCA depended on the stage of biofilm development, since the levels were lower after the initial attachment period than during biofilm growth, whereas transcription during planktonic growth was not growth phase dependent. The mRNA quantification data also suggested that the agr system was autoinduced and pointed to a differential expression of the agr genes during sessile and planktonic growth. Although the reverse transcription-PCR experiments revealed that the four genes were transcribed as a single messenger, chemical half-life and 5’ RACE (rapid amplification of cDNA ends) experiments indicated that the full size transcript underwent cleavage followed by degradation of the agrC and agrA transcripts, which suggests a complex regulation of agr transcription.

**Listeria monocytogenes** is a gram-positive human pathogenic bacterium; it is the causative agent of listeriosis, a serious infection characterized by high mortality rates, in immunocompromised individuals and pregnant women (19). This pathogenic bacterium is widely spread in the environment (soil, vegetation, animals, farm environment, etc.). In connection with these extended reservoirs, L. monocytogenes is also a contaminant of the food industry. Its presence on working surfaces in food-processing plants is a major problem as a source of food contamination (1, 32). Like most bacteria, L. monocytogenes is able to colonize surfaces and form biofilms (sessile growth) while, in natural environments, free-floating cells (planktonic growth) are transitory (28). Several steps can be identified during biofilm development: after an initial step of reversible and then irreversible adherence, bacteria grow as microcolonies and spread on the surface. Finally, biofilms develop as complex, three-dimensional structures during the maturation step (17). Biofilm development and maturation requires complex cellular mechanisms in which cell-cell communication is involved (14, 30). To date, three major signaling systems have been identified to regulate these systems; bacterial extracellular signaling molecules called autoinducers are produced (8). The acylhomoserine lactones have been identified as autoinducers in gram-negative bacteria (3, 13, 27, 46). The autoinducer 2 is found in both gram-negative and gram-positive bacteria (5, 7, 11, 35, 36, 54). Finally, peptide-mediated signaling pathways have been characterized in gram-positive bacteria. Among these, the agr system has been described initially in Staphylococcus aureus (41); the production of many of its virulence factors (toxins, enzymes, and cell surface proteins) is regulated by this system (4). The role of the agr system during S. aureus biofilm development is complex (57). It depends on the hydrodynamic conditions of the experimental setup. Under static conditions agr expression reduces the attachment of the cells to the surface (52, 55), and under turbulent dynamic conditions agr expression may affect biofilm maturation (55). Orthologs of the agr system have also been described in Enterococcus faecalis (fsr) (45), Lactobacillus plantarum (lam) (48), and L. monocytogenes (agr) (6). In E. faecalis, expression of a gelatinase (GelE) is fsr dependent (45). In L. plantarum, the lam system plays a role during biofilm development (48).

In L. monocytogenes, the four genes (agrB, agrD, agrC, and agrA) of the agr locus are organized as an operon (Fig. 1A). They encode the two-component histidine kinase AgrC and response regulator AgrA, a precursor peptide AgrD and AgrB, a protein that is involved in the processing of AgrD into a mature autoinducing peptide. Limited data concerning the role of the agr locus on the physiology of L. monocytogenes are available. Williams et al. (53) showed that among 16 putative response regulator genes of L. monocytogenes EGD-e, in-frame deletion in agrA did not affect the growth in brain heart infusion (BHI) medium at various temperatures (20, 37, and 43°C), in the presence of 9% NaCl, 5% ethanol, or 0.025% H2O2. Swimming motility was also not affected. No alteration of virulence could be identified during in vitro infection of cell cultures nor in vivo after intravenous infection of BALB/c mice. In a previous study, Autret et al. (6) reported a moderate attenuation of the virulence in Swiss mice after insertion of Tn1545 in the L. monocytogenes EGD-e agrA gene.

To further elucidate the role of the agr system, we first of all examined its involvement during attachment to abiotic surfaces and biofilm growth. agrA and agrD in-frame deletion mutants were compared to the parental strain EGD-e during sessile growth. Second, we determined the expression pattern of the four genes of the agr operon during planktonic and sessile growth, and we evidenced posttranscriptional events during expression of this operon.
L. monocytogenes derivatives of genes EGD-e, isolated from a rabbit listeriosis outbreak (39), and two mutants, L. monocytogenes EGD-e Wild type of serotype 1/2a for which the genome sequence is available (39) and L. monocytogenes Match1 Cloning host Invitrogen E. coli were used in this study and their characteristics are shown in Table 1.

**TABLE 1. Bacterial strains and plasmids used in this study**

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a Km', kanamycin resistant; Am', ampicillin resistant; Cm', chloramphenicol resistant.

**FIG. 1.** (A) Schematic diagram of L. monocytogenes agr operon. The gray arrows indicate the orientation and the size in base pairs of the four genes. Numbers between parentheses indicate the size in base pairs of the two intergenic regions. Arrowheads indicate the positions of the oligonucleotides used for real-time PCR: b (BR2; BF2), d (DF2; DR2), c (CF2; CR2), and a (AF2; AR2). The black lines indicate the positions of the oligonucleotides used for real-time PCR: b (BR2; BF2), d (DF2; DR2), c (CF2; CR2), and a (AF2; AR2). The black lines indicate the probes used for Northern blotting (NB, NC, and NA). The position of the transcription initiation site and the transcription termination site are indicated, respectively, by the bent arrow and the gray dot. (B and C) DNA and deduced amino acid sequences of agrA gene containing a 106-bp deletion in DG125A (agrA) mutant (B) and of agrD gene containing a 49-bp deletion in DG119D (agrD) mutant (C). The position of the deletion is represented by an inverted black triangle, the nucleotides before and after the deletion are mentioned, the ribosome binding sites are underlined, the start codons are boxed, and the stop codon is represented by three asterisks.

**MATERIALS AND METHODS**

**Bacterial strains and growth media.** The bacterial strains and plasmids used in the present study and their characteristics are shown in Table 1. L. monocytogenes EGD-e, isolated from a rabbit listeriosis outbreak (39), and two mutants, derivatives of L. monocytogenes EGD-e, L. monocytogenes DG119D (agrD) and L. monocytogenes DG125A (agrA) were grown in tryptic soy broth (TSB; Biokar Diagnostics, Pantin, France) at 25°C for biofilm and planktonic cultures and in brain heart infusion broth (BHI; Biokar Diagnostics) at 40°C for mutant construction. Escherichia coli TOP10 and Match1 (Invitrogen, Cergy Pontoise, France) were grown aerobically in Luria-Bertani broth (LB; Biokar Diagnostics) at 37°C. When appropriate, antibiotics (Sigma, St. Quentin Fallavier, France) were added as follows: kanamycin, 50 μg ml⁻¹ (E. coli); ampicillin, 200 μg ml⁻¹ (E. coli); and chloramphenicol, 10 μg ml⁻¹ (L. monocytogenes) (Table 1).

**In-frame deletion of agrD and agrA genes.** The mutant strains listed in Table 1, carrying an in-frame deletion in the response regulator agrD or in the precursor peptide agrD genes, were constructed from the parental strain L. monocytogenes EGD-e by using a two-step integration/excision procedure (10) that is based on the mutagenesis plasmid pGF-EM (33).

First, for the construction of an agrA in-frame deletion mutant, primers C1 and A2 (Table 2) were used to amplify a 600-bp DNA fragment including the 3' end of agrC and the ATG of agrA. The PCR product was cloned into pGF-EM (33) after digesting this PCR product and vector with HindIII/XbaI. The resulting plasmid, pGID121 was transformed into chemically competent E. coli Match1 as recommended by the manufacturer (Invitrogen). Primers A15 and E2 (Table 2) were used to amplify a 500-bp internal fragment of agrA. The resulting fragment was cloned into pCR2.1 TOPO vector (Invitrogen) to obtain plasmid pGID118. This vector was transferred into E. coli TOP10. Plasmid pGID118 was digested with NheI/EcoRI and the resulting 500-bp fragment containing an internal part of agrA was ligated into pGID121 restricted with XbaI/EcoRI to obtain pGID125. This plasmid was electroporated into L. monocytogenes EGD-e. A, carrying an in-frame deletion in the response regulator agrA, and of agrD gene containing a 49-bp deletion in DG119D (agrD) mutant (C). The position of the deletion is represented by an inverted black triangle, the nucleotides before and after the deletion are mentioned, the ribosome binding sites are underlined, the start codons are boxed, and the stop codon is represented by three asterisks.
of 150 mM NaCl solution in order to remove loosely attached cells. The plates were then stained with a 0.05% (wt/vol) aqueous crystal violet solution for 45 min and washed three times. In order to quantitatively assess biofilm formation, 100 μl of 96% ethanol (vol/vol) were added to each well, and the optical density at 595 nm (OD595) was determined. For each experiment, 15 replicates resulting from three independent inocula were analyzed. Each microtiter plate included eight wells with sterile TSB as control.

### Biofilm formation on stainless steel chips
An overnight culture of the bacterium in TSB was used to inoculate (1/100, vol/vol) fresh TSB and was grown at 25°C to an optical density at 600 nm of 0.1. A portion (5 μl) of the culture to be tested was then deposited on a slide and, the glass slide was incubated for 2 h at 25°C. After incubation, the glass slide was washed twice in distilled water and the adhering cells were observed by using a Zeiss Axioscope 2 imaging microscope. For each experiment, 12 replicates resulting from three independent inocula were analyzed.

### RNA extraction and cDNA preparation
Cells were harvested by centrifugation (5 min), resuspended in 1 ml of Tri-reagent (Sigma), and disrupted with glass beads (10,000 rpm) in a FastPrep FP120 instrument (Thermo Savant-Bio). DNA was extracted with phenol/chloroform and ethyl alcohol precipitation. RNA was purified after digestion of DNA using RQ1 RNase-Free DNase (Promega). RNA concentrations were determined by spectrophotometry, and RNA integrity was assessed using a 1% denaturing agarose gel. cDNA was synthesized from 1 μg of RNA using Superscript III reverse transcriptase (Invitrogen) and random hexamer primers according to the manufacturer’s instructions.

### Northern blot
Cells were harvested by centrifugation (5 min), resuspended in 1 ml of Tri-reagent (Sigma), and disrupted with glass beads (10,000 rpm) in a FastPrep FP120 instrument (Thermo Savant-Bio). DNA was extracted with phenol/chloroform and ethyl alcohol precipitation. RNA was purified after digestion of DNA using RQ1 RNase-Free DNase (Promega). RNA concentrations were determined by spectrophotometry, and RNA integrity was assessed using a 1% denaturing agarose gel. cDNA was synthesized from 1 μg of RNA using Superscript III reverse transcriptase (Invitrogen) and random hexamer primers according to the manufacturer’s instructions.

### Primer extension
GACAAAAAGGACTTCTTGGCAGT
CTTCCTCACTCATCTTCTCCAGGC
CGGAATACCAACATCAGCGCAT
B18
B26
B34
FIG. 2. (A) \textit{L. monocytogenes} EGD-e (■), DG125A (ΔagrA) (□), and DG119D (ΔagrD) (□) cell adhesion after 2 h of incubation at 25°C on glass slides. Histograms represent the number of adhered cells counted per microscopic field. Each bar indicates the mean of three independent experiments with four microscopic fields per experiment. (B) Micrographs of microscopic fields showing \textit{L. monocytogenes} EGD-e, DG125A (ΔagrA), and DG119D (ΔagrD) cells adhering to glass slides after 2 h of incubation at 25°C. Magnification, ×63.

RT-PCR. RT-PCR were performed with cDNA synthesized with total RNA extracted from three different mid-exponential phase cultures (OD$_{600}$ of 0.4) as described above. Specific primers (Table 2) were designed to amplify transcripts of the four genes and the two intergenic regions. PCR products were separated by electrophoresis on a 1% agarose gel (Invitrogen). Samples were checked for DNA contamination by performing PCR prior to RT.

Northern blotting. Northern blot analysis were performed by fractionation of RNA samples on 1% (wt/vol) agarose gel containing 20% (wt/vol) formaldehyde. Then, 30 µg of total RNA was loaded into each well of the gel. Transfer to a nylon membrane (Hybond-N; Amersham, Orsay, France) was performed as recommended. Internal probes (Fig. 1A) of the agrB, agrC, and agrA genes were generated by PCR using primers described in Table 2. Portions (10 ng) of the PCR products were labeled with [β-32P]dATP (Amersham) by the random priming method according to the manufacturer’s instructions (Invitrogen) to produce specific RNA probes. Sizes were determined with a RNA ladder (Invitrogen) for the molecular weight standard.

mRNA chemical half-life determination. The chemical half-lives of mRNA of the four genes of the agr operon were determined from mid-exponential phase cultures (OD$_{600}$ of 0.4). Total RNA was isolated as described above from samples taken 1, 4, 7, and 10 min after rifampin treatment (250 µg ml$^{-1}$; Sigma). The half-life was determined for each gene by real-time PCR using the time at which rifampin was added as a calibrator and the 16S rRNA transcript levels as an internal standard.

Analysis of the 5’ end of agr mRNA. Primer extensions were performed by incubating 5 µg of RNA isolated from \textit{L. monocytogenes} cells in planktonic growth, 20 pmol of oligonucleotide, 92 GBq of [β-32P]dATP (Perkin-Elmer, Courtaboeuf, France), and 100 U of SuperScript II reverse transcriptase (Invitrogen). The oligonucleotides used in this experiment are described in Table 2. The corresponding DNA-sequencing reactions were carried out using the primers and Cycle Reader DNA sequencing kit as recommended by the manufacturer (Fermentas, Euromedex, Souffel Weyersheim, France).

A capping assay was used to distinguish 5’ triphosphate (indicating initiation
points) from 5’ monophosphate (indicating processed products) according to the method of Bensing et al. (9), using a 5’-RACE kit (Ambion; Applied Biosystems, Courtaboeuf, France). Only 5’ monophosphate are selectively ligated to an RNA oligonucleotide. The primary 5’ end may be ligated only after removal of a 5’ pyrophosphate through tobacco acid pyrophosphatase (TAP) activity. The oligonucleotides used in this experiment are described in Table 2. Sequencing of 5’-RACE products obtained from TAP-treated and nontreated RNA was performed to differentiate cleavage products from primary transcripts. Primers used for PCR amplification between adaptor and the gene of interest are described in Table 2. PCR products were sequenced by GENOME express.

**Statistical analysis.** A one-way analysis of variance was performed by using SigmaStat version 3.0.1 software (SPSS, Inc.) in order to test the significance of differences (i) in gene expression during planktonic growth and biofilm formation using the ΔC_{T} value and (ii) in biofilm quantity. When one-way analysis of variance was significant, the Holm-Sidak test (n = 3, P < 0.05) was used to locate significant differences.

**Nucleotide sequence accession numbers.** The DNA sequence data of the genes of the parental strain EGD-e and the mutant strains described in the present study have been deposited in GenBank/EMBL/DDJB under the accession numbers AM412557 and AM412558.

**RESULTS**

The **agr operon** is involved during the sessile growth of *L. monocytogenes* EGD-e. In order to study the involvement of the putative transcriptional regulator AgrA during sessile growth, an agrA in-frame deletion mutant of *L. monocytogenes* EGD-e was constructed. As expected, this mutation did not alter cell or colony morphology or planktonic growth (data not shown). In contrast, the glass slide adherence of mutant DG125A (ΔagrA) was affected. Indeed, the number of adhered cells of DG125A was significantly reduced (n = 3, P < 0.05) by 62% compared to the parental strain EGD-e (Fig. 2A). Since cell-cell communication affects biofilm formation in many bacterial species, an agrD in-frame deletion was designed to generate a mutant unable to produce the putative autoinducer peptide processed from AgrD. As demonstrated in mutant DG125A (ΔagrA), no pleiotropic effect was observed in mutant DG119D (ΔagrD) (data not shown). Moreover, the adhesion phenotype of DG119D (ΔagrD) was similar to that of DG125A (ΔagrA) (Fig. 2A). Micrographs of the microscopic field of adhering cells on glass slides confirmed that the quantity of adhering cells with *L. monocytogenes* DG119D (ΔagrD) and DG125A (ΔagrA) was less compared to the parental strain EGD-e (Fig. 2B). These results suggested the involvement of the agr system during adhesion of *L. monocytogenes*, the first step in biofilm development.

The ability of *L. monocytogenes* EGD-e to develop biofilms on polystyrene was also affected by the deletion of agrC and agrD (Fig. 3). Indeed, there was significantly less biofilm (n = 3, P < 0.05) produced within the first 24 h of incubation. The differences were no longer significant during the later stages of biofilm formation, namely, at 48 and 72 h.

In light of the sessile growth alteration observed in the mutants with deletion in the genes encoding the putative transcriptional regulator AgrA and the putative autoinducer peptide processed from AgrD, we therefore decided to investigate the expression of the agr operon during sessile and planktonic growth.

**Relative expression and transcriptional autoregulation of the genes of the agr operon.** The transcription of the four genes of the agr operon was studied during sessile and planktonic growth using real-time PCR experiments with each of the four primer sets (Fig. 1A, b [BF2-BR2], d [DF2-DR2], c [CF2-CR2], and a [AF2-AR2], and Table 2). Analysis of the relative expression levels indicated that, during sessile growth, the levels of transcripts of agrB, agrD, and agrC were significantly lower (n = 3, P < 0.05) after 2 h of adhesion than after 24 and 72 h of sessile growth (Fig. 4A). For each gene, the differences

![FIG. 3. Biofilm formation by *L. monocytogenes* EGD-e (■), DG125A (ΔagrA) (▲), and DG119D (ΔagrD) (▲) in batch conditions in polystyrene 96-well plates after 16, 24, 48, and 72 h of incubation at 25°C. Each histogram indicates the mean of three independent experiments, with five measurements for each point.](http://aem.asm.org/)

![FIG. 4. Semilogarithmic representations. (A) Relative expression levels of the agrB, agrD, agrC, and agrA genes of the parental strain *L. monocytogenes* EGD-e determined during biofilm formation (2, 24, and 72 h [the first three columns in each gene, respectively]) and planktonic growth (early exponential phase OD_{600} of 0.1, mid-exponential phase OD_{600} of 0.4, and stationary phase OD_{600} of 0.6 [the second three columns for each gene, respectively]). (B) Relative expression levels of the agrB, agrD, agrC, and agrA genes of *L. monocytogenes* determined during mid-exponential phase OD_{600} of 0.4: EGD-e strain (■), DG125A (ΔagrA) strain (▲), and DG119D (ΔagrD) strain (▲). For both graphs, gene expression was quantified by using real-time PCR and the comparative critical threshold (ΔΔC_{T}) method. The dmr gene was used as the internal standard, and expression of the agrA gene in the early exponential phase (OD_{600} of 0.1) was used as the calibrator. Three independent experiments were performed; histograms indicate standard deviations.](http://aem.asm.org/)
in the relative expression observed between 24 and 72 h of biofilm growth were not significant. The levels of agrA transcripts were similar at 2, 24, and 72 h. Furthermore, during sessile growth, for each condition, the levels of transcripts of agrB, agrD, and agrC were never significantly different. In contrast, the relative expression levels of agrA, for each condition, were significantly lower (n = 3, P < 0.05) than those of agrB, agrD, and agrC.

During planktonic growth, the relative expression levels of each gene was not affected by the phase of growth. The levels of transcripts of agrB and agrD, for each condition, were never significantly different. In contrast, the relative expression levels of agrC and agrA, for each condition, were significantly lower (n = 3, P < 0.05) than those of agrB and agrD. For example, under our experimental conditions, at mid-exponential growth phase (OD600 of 0.4) the agrC and agrA transcript levels were, respectively, 13- and 24-fold lower than the agrB transcript level (Fig. 4A).

The relative expression levels of the four genes of the agr operon were determined at mid-exponential phase during planktonic growth (OD600 of 0.4) in the parental and mutant (ΔagrA and ΔagrD) backgrounds. In mutant DG125A (ΔagrA), the relative expression levels of agrB, agrD, and agrC were significantly lower (n = 3, P < 0.05) than those of the parental strain EGD-e (Fig. 4B). Indeed, the relative transcript levels for agrB, agrD, and agrC were, respectively, of 56-, 68-, and 3-fold lower than those measured with the parental strain. A similar pattern of gene expression was observed for mutant DG119D (ΔagrD) (Fig. 4B). In terms of the relative expression levels of agrA, no significant differences were observed between the mutants (DG119D and DG125A) and parental strains. These results suggest an autoregulation of the transcription of agrB, agrD, and agrC and a low constitutive expression of the putative transcriptional regulator AgrA.

The mRNA quantification data suggested that the agr system was autoregulated and pointed to a differential expression of the agr genes during sessile and planktonic growth. Either posttranscriptional processing or transcription from another promoter region, not yet identified, could account for these results.

Processing of the mRNA and identification of the 5′ end of the mRNA agr operon. In order to further investigate the hypothesis of a posttranscriptional processing, RT-PCR, Northern blotting, and mRNA chemical half-life were carried out. The four genes and the two intergenic regions were detected by RT-PCR (Fig. 5), indicating cotranscription of the complete agrBDCA operon and the presence of a full-size transcript. PCR on the RNA samples before RT gave no amplification signals, confirming that there was no contamination by genomic DNA (data not shown). However, a polycistronic mRNA was never detected by Northern blotting; only small size products were detected (data not shown). The mRNA chemical half-life was determined by using real-time PCR experiments with the primer sets described above (Fig. 1A and Table 2). The results indicated that the chemical half-lives of agrB and agrD transcripts were 7.4 and 6.8 min, respectively, while they were lower for agrC and agrA (4.3 and 4.1 min, respectively) (Fig. 6). To pinpoint these differences in chemical half-life and to highlight the differential expression pattern observed by real-time PCR, 5′-RACE experiments were carried out to search for transcription initiation points or cleavages within agrC and agrA transcripts. Regardless of the treatment with TAP, multiple PCR products were detected, confirming degradation after cleavage through RNase activity. After amplification with adequate primers (Table 2), four 5′ ends were identified among the agrC fragments sequenced (Fig. 7). Similarly, five 5′ ends were observed among the agrA fragments sequenced (Fig. 7). From two hypotheses formulated, data analysis confirmed posttranscriptional cleavage and degradation.

5′-RACE was also used to characterize the 5′ end of the
mRNA agr operon. PCR amplification was observed in TAP-treated and untreated samples, suggesting a processing event at the 5' end, mapped by a “T” (Fig. 7). As expected, primer extension analysis with the three specific primers B34, B26, and B18 (Table 2) revealed two signals. They were separated by one nucleotide and corresponded to a “G” and the previously described “T.” This finding suggested that the 5’ end of the agr transcript was located 15 or 14 nucleotides upstream from the putative start codon (Fig. 7). Similar results were obtained in samples harvested at an OD600 of 0.1, 0.4, and 0.6. Moreover, two hexanucleotides (TGGTTA and TAAAAT) separated by mRNA growth phase (OD 600 of 0.4). #, 5’ extension and /H11032 end identified by primer extension; *, processing sites identified by 5’-RACE. The putative −10 with TGN extension and −35 sequences are double underlined, the ribosome binding sites are underlined, the start codons are boxed, and the stop codon of agrC is represented by three asterisks.

DISCUSSION

Orthologs of the agr system, initially described in S. aureus, have been reported in L. plantarum, E. faecalis, and L. monocytogenes (6, 44, 48, 56). Thus far, the role of the agr system has not been clearly described in the pathogenic bacterium L. monocytogenes. In the present study, we investigated the role of agrA and agrD in the sessile growth of L. monocytogenes, and we focused on the molecular characterization of the transcription of the agr operon. In-frame deletions of agrA, which encodes a transcriptional regulator, or in-frame deletions of agrD encoding a propeptide affected adhesion and the early stages of biofilm formation on glass and polystyrene surfaces within the first 24 h of incubation. No significant differences were observed afterward. These results are in accordance with those obtained by Sturme et al. (48) with a lamA mutant of L. plantarum WCF51. Indeed, the lamA mutant was impaired in its ability to adhere to glass surfaces. It showed 1.5- and 1.7-fold decreases in glass adherence compared to the parental strain after 24 and 48 h, respectively. In contrast, Vuong et al. (52), working with S. aureus RN6390 and 601055, demonstrated that their Δagr genotype led to a pronounced attachment to polystyrene, 1.8- and 2.5-fold increases, respectively, compared to that of the isogenic Δagr+ wild type after 24 h. Biofilm-associated infections have special clinical relevance, and in staphylococcal infections these diseases include endocarditis, osteomyelitis, implanted device-related infections, and even some skin infections (54). Indeed, the Δagr biofilm phenotype may have important consequences. For example, the dysfunction of agr is correlated with persistent bacteremia in S. aureus (49), and mutation of the S. aureus agr system increased bacterial persistence (52), suggesting that interference with cell-cell communication would enhance rather than suppress this important type of staphylococcal disease (42).

In our experimental conditions, growth-phase-dependent transcriptional regulation was not observed during planktonic growth. This is in agreement with a previous report showed that during exponential and stationary phases the amount of agrA transcripts was recorded by /H11002. In contrast, the transcription of the orthologous agr, lam, and fsr operons from S. aureus MN NJ, L. plantarum WCF51, and E. faecalis OG1RF, respectively, increased from /H9004 to /H11001, working with /H9004 S. aureus agr biofilm phenotype; this points out that developmental regulations in /H11002 the first 24 h of incubation. No significant differences were observed afterward. These results are in accordance with those obtained by Sturme et al. (48) with a lamA mutant of L. plantarum WCF51. Indeed, the lamA mutant was impaired in its ability to adhere to glass surfaces. It showed 1.5- and 1.7-fold decreases in glass adherence compared to the parental strain after 24 and 48 h, respectively. In contrast, Vuong et al.
ment but not in later stages; in contrast, the Rhl regulon plays a role in the maturation of the biofilm (14, 46). The agr system of \textit{L. monocytogenes} could also regulate the expression of proteins necessary for the ability to attach to abiotic surfaces without being involved once the cells attach to the surface. Indeed, in \textit{S. aureus} and \textit{S. epidermidis}, agr-dependent regulation of the expression of several adhesion proteins has been demonstrated (12, 34).

Significant differences were measured in the relative quantities of the transcripts of \textit{agrB}, \textit{agrD}, \textit{agrC}, and \textit{agrA}, while the differences between the transcripts of \textit{agrB} and \textit{agrD} were never significantly different. This observation suggested either posttranscriptional processing of the full-size \textit{agr} transcript of \textit{L. monocytogenes} or the presence of another promoter region between \textit{agrD} and \textit{agrC}. Determination of 5’ ends corresponding to cleavage, and not to initiation transcription points, supported the posttranscriptional events hypothesis. Indeed, several 5’ ends were detected within \textit{agrC} and \textit{agrA} transcripts. This suggested also that most of the transcripts quantified by real-time PCR were degradation products generated after cleavage of the full-length transcript, probably within the intergenic region or at the 5’ end of \textit{agrC} and \textit{agrA}. These posttranscriptional events may have regulatory functions resulting in a differential stability and a rapid processing of the mRNA. This could account for a fine tuning of the expression of the individual genes of the \textit{agr} operon, as has previously been suggested for the pattern of expression of other loci of gram-negative and gram-positive bacteria (2, 24, 25, 37, 40). Moreover, our work indicated that the \textit{agr} operon of \textit{L. monocytogenes} was autoregulated in a positive way since the deletion of \textit{agrA} or \textit{agrD} reduced the transcription of \textit{agrB}, \textit{agrD}, and \textit{agrC}, although \textit{agrA} transcription was not \textit{agr} dependent. A similar pattern has been described in the orthologous fsr system of \textit{E. faecalis} in which the expression of \textit{fsrB}, \textit{fsrD}, and \textit{fsrC} is \textit{fsr} dependent, whereas the expression of the \textit{fsrA} is weak, constitutive, and \textit{fsr} independent (29, 45). It is proposed, on the one hand, that the regulator is constitutively expressed in order to provide a basal amount of regulator ready to respond to the presence of the signal in the environment of the cell; on the other hand, when the signal is high in the environment of the cell, it would induce the expression of the transmembrane protein, the propeptide and the sensor. This, in turn, would enable the transfer of the signal to the neighboring cells and prepare the cell for the monitoring of an amplified signal (18, 26).

Two 5’ ends of the \textit{agr} mRNA were determined. One was mapped to a “G” and located at an appropriate distance downstream of the putative promoter region (43), leading us to consider it as the apparent start site for the promoter of the \textit{agr} operon. The second 5’ end identified was located one base downstream of the 5’ end of the primary transcript. Such cleavage of one nucleotide downstream of the initiation site may derive from a modification of the primary transcript by a phosphatase or pyrophosphatase or from endonucleolytic cleavage (31, 47, 50, 51). The significance of such commonly observed processing remains to be clarified.

In conclusion, this study is the first description of the involvement of cell-cell communication in the adherence of \textit{L. monocytogenes}. Our data show that impairment of the response regulator or the propeptide resulted in a similarly altered adhesion and biofilm phenotype. The \textit{agr} system of \textit{L. monocytogenes} differed from the homologous systems previously described since posttranscriptional processing occurred at the site of the initiation of transcription and within the full-length transcript. It will be of interest to investigate the significance of such a processing in the expression of the \textit{agr} system and in the physiology of \textit{L. monocytogenes}.

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