Title: N-acyl-homoserine lactone quorum sensing switching from acidogenesis to solventogenesis during the fermentation process in Serratia marcescens MG1

Article Type: Research article

Keywords: Serratia marcescens MG1, N-acyl-homoserine lactone quorum sensing, acidogenesis, solventogenesis, switch
Full Title: N-acyl-homoserine lactone quorum sensing switching from acidogenesis to solventogenesis during the fermentation process in *Serratia marcescens* MG1

Authors: Wensong Jin¹,² †, Hui Lin¹,² †, Huifang Gao¹,², Zewang Guo¹, Jiahuan Li¹,², Quanming Xu¹, Shujing Sun¹,², Kaihui Hu¹,², Jung-Kul Lee³*, Liaoyuan Zhang¹,²*

¹College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, People’s Republic of China.

²Gutian Edible Fungi Research Institute, Fujian Agriculture and Forestry University, Gutian 352200, People's Republic of China.

³Department of Chemical Engineering, Konkuk University, Seoul 05029, Republic of Korea.

†These authors contributed equally to this work.

* To whom correspondence should be addressed:

Liaoyuan Zhang, PhD

Phone: 86-591-83789492

Fax: 86-591-83789121

E-mail: zliaoyuan@126.com

Jung-Kul Lee, PhD

Phone: 82-2-450-3505

Fax: 82-2-458-3504
Running title: \textit{N}-acyl-homoserine lactone quorum sensing switching from acidogenesis to solventogenesis in \textit{Serratia marcescens} MG1
Abstract

N-acyl-homoserine lactone quorum sensing (AHL-QS) has been shown to regulate many physiological behaviors in *Serratia marcescens* MG1. In the current study, the effects of AHL-QS on the biosynthesis of acid and neutral products by *S. marcescens* MG1 and its isogenic ΔswrI with or without supplementing exogenous N-hexanoyl-L-homoserine lactone (C₆-HSL) were systematically investigated. The results showed that swrI disruption resulted in rapid pH drops from 7.0 to 4.8, which could be restored to wild type by supplementing C₆-HSL. Furthermore, fermentation product analysis indicated that ΔswrI could lead to obvious accumulation for acidogenesis products such as lactic acid and succinic acid, especially excess acetic acid (2.27 g/L) produced at the early stage of fermentation, whereas solventogenesis products by ΔswrI appeared noticeable decrease by approximate 30% for acetoin during 32-48 h and by approximate 20% for 2,3-butanediol during 24-40 h, when compared to those by wild type. Interestingly, the excess acetic acid produced could be removed in an AHL-QS-independent manner. Subsequently, quantitative real-time PCR was used to determine the mRNA expression levels of genes responsible for acidogenesis and solventogenesis and showed consistent results with those of product synthesis. Finally, by close examination of promoter regions of the analyzed genes, four putative luxI box-like motifs were found upstream of genes encoding acetyl-CoA synthase, lactate dehydrogenase, α-acetolactate decarboxylase, and Lys-like regulator. The information from this study provides a novel insight into the roles played by AHL-QS in switching from acidogenesis to solventogenesis in *S. marcescens* MG1.
Keywords:
Serratia marcescens MG1, N-acyl-homoserine lactone quorum sensing, acidogenesis, solventogenesis, switch

Introduction
Quorum sensing (QS) is a communication system that allows bacteria to monitor their population density through a process of chemical cell-to-cell signaling mediated by diffusible signal molecules [1-3]. Previous studies showed that a considerable number of bacteria employ a QS system to regulate their physiological behaviors, including bioluminescence, biofilm development, virulence factor secretion, and other cellular processes [4, 5]. Serratia liquefaciens MG1 was isolated from a softened cucumber [6] and was later renamed Serratia marcescens MG1 in 2005 [7]. S. marcescens MG1 can secrete a variety of hydrolytic enzymes with potential applications, including nuclease, lipase, protease, and phospholipase [6, 8]. In addition, S. marcescens MG1 also produces the biosurfactant serrawettin [9], which exhibits antimicrobial, antitumor and plant protecting properties. These hydrolytic enzymes and serrawettin production have been shown to be controlled by QS. The N-acyl-homoserine lactone (AHL) QS system in S. marcescens MG1 consists of swrI and swrR genes, which belong to the luxIR type of QS system. swrI, a homolog of luxI, is responsible for directing the synthesis of two AHL signal molecules, N-butanoyl-L-homoserine lactone (C₄-HSL) and N-hexanoyl-L-homoserine lactone (C₆-HSL); swrR, a homolog of luxR, is a transcriptional factor that senses and
interacts with C₄-HSL and C₆-HSL autoinducers in the cytoplasm and then binds to its
target gene promoters, functioning as either a transcriptional activator or repressor.

Another important property of *S. marcescens* MG1 is that it is capable of producing
a large number of neutral compounds (instead of mixed acids), such as acetoin and
2,3-butanediol (two important biobased bulk chemicals [10, 11]), when cultured under
fermentation conditions using carbohydrates as a carbon source. To date, the
physiological function of 2,3-butanediol has not been clarified. In many cases, the
*Enterobacteriaceae* members utilize sugar to produce organic acids mainly including
acetate, lactate, succinate, and formate, resulting in excessive acidification and growth
inhibition during the culture process. However, some microorganisms, such as
*Klebsiella* sp., *Clostridium acetobutylicum*, and *Serratia* sp., seem to possess the
capability of switching their metabolism to the production of neutral products such as
acetone, butanol, alcohols, 1,3-propanediol, or 2,3-butanediol [12-15]. It was reported
that in *Aerobacter aerogenes*, acetate, among all of the acid and neutral products
tested, was found to be the most effective inducer for α-acetolactate synthase,
α-acetolactate decarboxylase, and butanediol dehydrogenase activities, all of which
are involved in the conversion of pyruvate to 2,3-butanediol [16]. In *S. marcescens*
MG1 acetate at 40 mM dramatically promotes the mRNA expression levels of
Lys-like regulator (*slaR*), α-acetolactate decarboxylase (*slaA*), and α-acetolactate
synthase (*slaB*), but not butanediol dehydrogenase (*slaC*) [14]. Moreover,
fermentation media supplemented with various amounts of sodium acetate increased
the productivity of acetoin, and acetate boosted the α-acetolactate synthase gene
expression in the tested Serratia sp. [17]. Therefore, Serratia sp. and perhaps those microorganisms that have developed defensive strategies against excessive acidification in the culture medium might employ acetate to switch the fermentation process from acidogenesis to solventogenesis.

Interestingly, acetoin and 2,3-butanediol production regulated by the AHL-QS system were uncovered by Rob et al. in the S. plymuthica RVH1 and S. marcescens MG1 strains [18]. Inactivation of splI (a homolog of luxI) in the AHL-QS system showed a sharp decrease in pH and less acetoin and 2,3-butanediol production, which could be restored by supplementation with exogenous 3-oxo-C6-HSL [18]. Moreover, splR (a homolog of luxR) was found to be a negative regulator of acetoin production [18]. In addition, swrR negatively regulated slaR mRNA expression in S. marcescens MG1; however, ΔswrR produced a comparable amount of 2,3-butanediol as the wild-type strain did [14]. These findings clearly demonstrated that the AHL-QS system is associated with the mixed-acid fermentation pathway and the 2,3-butanediol synthesis pathway. However, how the AHL-QS system in S. marcescens MG1 exerts effects on these pathways remains to be elucidated. A previous finding indicated that regulation of a total of 28 genes was dependent on the C4-HSL-mediated QS system [19], but only swrA, lipB, bsmA, and bamB gene functions were uncovered, the gene products of which are responsible for swarming motility, protein transport and biofilm development in S. marcescens MG1, respectively [9, 20, 21]. Functions of the remaining 24 genes remain unknown. In other words, these genes might be involved in biological processes other than those mentioned above. In light of AHL-QS
associated with both the pH value change and the 2,3-butanediol synthesis pathway during *S. marcescens* MG1 fermentation, we speculated that it might be involved in switching from acidogenesis to solventogenesis. Therefore, we systematically determined the main acid product and neutral product contents generated by *S. marcescens* MG1 during the fermentation process (Fig. 1). Moreover, by applying a quantitative real-time PCR approach, the mRNA expression levels of genes responsible for producing corresponding acids and solvents were analyzed.

**Materials and Methods**

**Strains, plasmids, and culture conditions**

The strains and plasmids used in this study are listed in Table 1. All strains were routinely maintained in Luria-Bertani (LB) media at 30°C for *S. marcescens* MG1, ΔswrI, and *Chromobacterium violaceum* CV026, at 37°C for *E. coli* DH5α and *E. coli* S17-1 λpir. Antibiotics were added in the following amounts: 50 μg/mL kanamycin for *E. coli* S17-1 λpir, 20 μg/mL kanamycin for *Chromobacterium violaceum* CV026, and 100 μg/mL kanamycin for ΔswrI. For flask fermentation experiments, seed culture was prepared in LB media. After overnight cultivation, 5% seed culture of *S. marcescens* MG1 and ΔswrI were inoculated into the fermentation medium (50 mL in 250 mL shake flask). Fermentation medium for *S. marcescens* MG1 and ΔswrI consisted of 7% glucose, 1.5% yeast extract, 0.1% NH₄H₂PO₄, 0.03% MgSO₄, and 0.01% MnSO₄, pH 7.0. For the rescue experiments, in parallel with the wild-type strain fermentation, a final concentration of 10 μM C₆-HSL (Sigma, Saint Louis, USA) was added to the ΔswrI medium at the initial stage of fermentation. Samples were
collected at 8 h intervals and were used for the analysis of the culture pH values, dry cell weight, residual sugar, acid products, neutral products, and target gene mRNA expression levels. All flask experiments were performed in triplicate.

Construction of the ΔswrI mutant strain

swrI-KO-S and swrI-KO-AS primers were designed according to the released swrI gene sequence in GenBank (GenBank accession number AY168877.1) and were subsequently used to amplify partial swrI fragments using the S. marcescens MG1 genome as a PCR template. All primers used in this study are provided in Supplementary Table 1. The amplified DNA fragment was digested with KpnI and Scal, purified, and ligated to the suicide vector pUTkm1, which was treated with the same enzymes as the swrI fragment. Thus, the marker-exchange plasmid pUT-swrl vector was created. The pUT-swrl vector insertion was verified by double enzyme digestion and commercial DNA sequencing. Then, the pUT-swrl vector was transformed into E. coli S17-1 λpir for mating with S. marcescens MG1. Transconjugants were screened on LB agar plates supplemented with 100 μg/mL ampicillin and 100 μg/mL kanamycin. A PCR-based approach was used to confirm disruption of the swrI locus with KR-S and KR-AS primers, which were designed to complement the KmR cassette and the endogenous swrI locus outside of the insert in pUT-swrl (data not shown).

Analytic methods

The biomass concentration and glucose concentration in the collected samples were determined by the methods described previously in our laboratory [22]. Gas
chromatography (Agilent 7890A, Waldblum, Germany) was used to analyze and quantify ethanol, acetoin, and 2,3-butanediol as described previously [22]. The method for acid product analysis was described as follows. Samples were centrifuged at 10 000 g for 10 min to remove strain pellets, and then, acidic ethanol (80 mL ethanol plus 20 mL deionized water, adjusted to pH 3.0 by H$_3$PO$_4$) was added to the supernatant and mixed and then centrifuged again to remove any residual proteins. The obtained supernatant was filtered through a 0.22 μm filter membrane (Millipore, Darmstadt, Germany). Finally, the lactic acid, acetic acid, citric acid, and succinic acid contents were determined by high-performance liquid chromatography machine (Agilent 1100, Waldblum, Germany) equipped with a Diode Array Detector (DAD). Gradient elution was conducted using a mobile phase starting with 95% 0.01 M KH$_2$PO$_4$ (pH 2.05) and 5% organic (100% methanol) solvent on an SB-Aq C18 column at 30°C. The flow rate was set at 0.6 mL/min, with a total run time of 30 min. The signal was monitored at 210 nm. Data were collected and calculated according to the strand curve generated by the corresponding standard samples.

Quantitative real-time PCR analysis

Total RNA from the samples collected was extracted using Trizol reagent (Invitrogen, Carlsbad, USA) individually, according to the manufacturer’s protocols. cDNA was synthesized from the extracted total RNA with PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Beijing, China). All real-time primers with the exception of slaC were designed with Beacon Designer 7 software, based on the available S. marcescens WW4 genomic sequence data. slaC real-time primers were
used as designed by Rao et al. [14]. Relevant gene expression profiles were determined by StepOne Real-Time PCR (Applied Biosystems, Singapore) with TB Green™ Premix Ex Taq™ (Tli RNase H Plus) (Takara, Beijing, China). The relative target gene RNA expression levels were analyzed. Data were normalized to 16S RNA expression levels and calculated as $2^{-\Delta\Delta CT}$. Experiments were performed in triplicate.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 7 (GraphPad Software, Inc., San Diego, USA). The results were expressed as the mean values with SDs and were conducted with two-way ANOVA. A $P$-value of $<0.05$ was considered to be significant.

Results and discussion

Effect of swrI disruption on the biomass concentration, pH profile, and sugar consumption of S. marcescens MG1

To test whether $N$-acyl-homoserine lactone quorum sensing (AHL-QS) functions in switching from acidogenesis to solventogenesis, we first used a marker-exchange strategy to construct a swrI disruption mutant. As shown in Fig. 2A, the wild-type strain (WT) efficiently elicited the indicative strain C. violaceum CV026 to produce violacein, which could not be induced by $\Delta swrI$ under the same tested conditions. This demonstrates that $\Delta swrI$ lost the capability to synthesize AHL autoinducers and indicates that the endogenous swrI locus was destroyed completely in this mutant strain. Subsequently, disruption of the swrI locus was confirmed by PCR-based analysis (data not shown). The cell growth profile, culture pH, and sugar consumption...
of WT and ΔswrI were evaluated and compared. The cell growth profiles determined by dry cell weight assays showed that WT produced more biomass than ΔswrI, but slightly less than ΔswrI complemented with exogenous C₆-HSL, during the whole fermentation process (Fig. 2B). The data presented in Fig. 2B demonstrate that the AHL-QS system positively contributes to the growth rate of S. marcescens MG1 to a certain degree, but it is dispensable for its growth. Our finding is consistent with results discovered by Rob et al. [18] but is in contrast to the data found from Aeromonas hydrophila AH-1N and V. fischeri [23, 24], both of which showed that AHL-QS and AinS (specifically for synthesizing autoinducer-2) QS systems are essential for their growth. In agreement with the strain growth profiles, ΔswrI exhibited the lowest sugar consumption kinetics, but the defective phenotype of ΔswrI in sugar consumption could be rescued by the addition of exogenous C₆-HSL (Fig. 2D). After analyzing the data generated by examining the culture pH values at different fermentation time points, the corresponding results showed that the pH values recorded for WT and ΔswrI supplemented with or without exogenous C₆-HSL represented inversely typical parabolic curves that reached the baseline at 24 h for WT and ΔswrI supplemented with exogenous C₆-HSL and at 32 h for ΔswrI (Fig. 2C). The lowest pH value for WT was approximately 5.5, while that for ΔswrI was 4.8 during the whole fermentation process. As culture acidifiers gradually accumulate, the growth of the fermentation strain gradually ceases [13]. In the case of S. marcescens MG1, the more culture acidifiers accumulated in the spent medium might account for the lower biomass.
Effect of \textit{swrI} disruption on acidogenesis in \textit{S. marcescens} MG1

The pH values of \textit{AswrI} culture were dramatically lower compared with those of WT culture during the fermentation process (Fig. 2C) \((P<0.05, \text{ data not shown})\). To explore the possible factor(s) contributing to this phenomenon, we systematically analyzed the contents of the mixed acid products (Fig. 1), including lactic acid, acetic acid, citric acid and succinic acid, which are the main \textit{S. marcescens} MG1 fermentation acid products secreted to the medium, according to our preliminary experiment results (data not shown). As the fermentation began, WT quickly produced lactic acid and succinic acid. Subsequently, lactic acid contents were maintained at approximate 2 g/L during the whole fermentation process (Fig. 3A), while succinic acid contents gradually decreased after 8 h fermenting (Fig. 3D), implying that succinic acid must be reused in some way. In contrast, WT produced a slight amount of citric acid and did not produce acetic acid at all during the fermentation period of 0-24 h. After that period, these two acids were concomitantly accumulated. The kinetics of lactic acid, citric acid and succinic acid produced by \textit{AswrI} exhibited the similar pattern to those of WT or \textit{AswrI} complemented with exogenous C\textsubscript{6}-HSL (Figs. 3A, 3C, and 3D). However, \textit{AswrI} produced large amounts of acetic acid as early as the fermentation period of 0-8 h which was then gradually removed from the media (Fig. 3B). It was reported that \textit{V. fischeri} cells initially excrete acetic acid, and the excess acetic acid can be removed from the media in an AHL-QS-dependent manner [24]. However, our data clearly demonstrate that \textit{AswrI} can remove excess acetic acid in the medium with an unknown strategy separate from the AHL-QS system, although
it was also obvious that the AHL-QS is involved in the process of acetic acid production (Fig. 3B). According to the conclusion addressed by Sarah et al., adding the same amount of exogenous acetic acid as generated by the AinS strain to uninoculated medium is sufficient to account for the pH drop [24]. The large amount of acetic acid produced by ΔswrI during the fermentation period of 0-24 h might account for the sharp pH drop (Figs. 2C and 3B). Since WT generated more citric acid than ΔswrI (Fig. 3C), the combined contribution of the other three acids analyzed in the culture to acidification possibly leads to the phenomenon that the pH values of ΔswrI culture were accordingly lower than that of WT culture during the whole fermentation process (Figs. 3 and 2C).

Effect of swrI disruption on solventogenesis in S. marcescens MG1

S. marcescens MG1 produced a small amount of ethanol, and the maximal records for WT, ΔswrI, and ΔswrI complemented with exogenous C₆-HSL were approximately 1.2, 3, and 1.3 g/L, respectively. ΔswrI produced more ethanol content than WT (Fig. 4A). The QS regulatory system involved in playing a positive role in acetoin synthesis has been well documented [18, 23]. Our results collected from acetoin content determination assays are in agreement with those previous findings. ΔswrI produced fewer amounts of acetoin compared with WT at the end of fermentation (Fig. 4B). For 2,3-butanediol production, in the fermentation period of 0-40 h, WT produced more 2,3-butanediol content than ΔswrI, which correlates well with previous findings described by Rob et al. [18]. However, at the end of fermentation, all strains tested here showed comparable 2,3-butanediol production
According to our data, AHL-QS in *S. marcescens* MG1 negatively contributes to ethanol production but positively promotes acetoin and 2,3-butanediol generation. In addition, *S. marcescens* MG1 seems to reuse its neutral products, with the exception of acetoin [25].

**Metabolic flux analysis**

As shown in Table 2, ΔswrI generated more acid products than WT during the fermentation period of 0-8 h. To be more specific, the amount of lactic acid, acetic acid, and succinic acid generated by ΔswrI were increased by up to 5.1%, 18.0%, and 8.0% compared with that produced by WT, respectively. As the fermentation process progressed, both WT and ΔswrI reduced the generation of their acid products but increased the generation of their neutral products. However, the acid products generated by ΔswrI accordingly were much more than those produced by WT. Importantly, by the addition of exogenous C6-HSL to the ΔswrI medium, the fermentation products generated by ΔswrI could be restored to those produced by WT.

The metabolic flux analysis results showed that AHL-QS exerts a role in switching from acidogenesis to solventogenesis in *S. marcescens* MG1 during the fermentation process.

**Effect of swrI disruption on relevant enzyme gene expression in *S. marcescens* MG1**

In light of the AHL-QS involved in switching from acidogenesis to solventogenesis, it is intriguing for us to test the hypothesis that AHL-QS might directly exert effects on the expression of relevant enzyme genes, which are responsible for synthesizing
the acid and neutral products we determined. It has been demonstrated that exogenous autoinducer turnover was approximately 20 h when added to the media [26]. Glucose was completely exhausted at approximately 32 h during the *S. marcescens* MG1 fermentation process (Fig. 2D). Therefore, samples used for gene expression analysis were prepared at fermentation time points of 8, 16, and 24 h. The mRNA expression levels of *ldh* encoding lactate dehydrogenase in all tested strains exhibited the same pattern at the analyzed fermentation time points, which showed that the mRNA levels of *ldh* expressed by ΔswrI were approximately four times those generated by WT; the overexpressed *ldh* mRNA levels in ΔswrI could be restored by the addition of exogenous C₆-HSL (Figs. 5A, 5B and 5C). It was reported that *acs*, encoding acetyl-CoA synthase, was under the control of AinS-QS regulatory system in *V. fischeri* [24]. Our data clearly demonstrate that the AHL-QS regulatory system negatively regulated *acs* mRNA expression levels. However, AHL-QS seems to be unrelated to regulating the mRNA expression profiles of *adh*, *cs*, and *sdh*, which encodes alcohol dehydrogenase, citrate synthase, and succinate dehydrogenase, respectively (Figs. 5A, 5B and 5C). Unexpectedly, *sdh* mRNA levels were dramatically reduced to baseline levels in ΔswrI supplemented with or without exogenous C₆-HSL compared to WT. Although the succinic acid contents produced by WT and ΔswrI supplemented with or without exogenous C₆-HSL were comparable during the fermentation time period of 0-32 h (Fig. 3D), the exact cause for the resulting conflicting results is currently unknown. The genes encoding α-acetolactate synthase, α-acetolactate decarboxylase, and 2,3-butanediol dehydrogenase in some
microorganisms such as Klebsiella terrigena, Enterobacter aerogenes, and Vibrio cholera cluster together to form an operon [27, 28]. However, in S. marcescens MG1, slaA and slaB were found in one operon apart from slaC, and slaR was divergently transcribed with slaA [14]. QS was found to exert a positive effect on the expression of slaR and slaA [14, 29]. Our data presented here clearly demonstrate that slaA, slaB, and slaR expression levels were positively influenced by AHL-QS (Figs. 5A, 5B and 5C). It is of note that AHL-QS seems to exert more profound effects on the slaA and slaB rather than slaR expression levels, especially at the later fermentation stage (Fig. 5C). This implies that slaR expression might also be controlled by other regulatory circuits. Unexpectedly, slaC expression levels in ΔswrI, complemented with or without exogenous C6-HSL, were accordingly higher than in WT (Figs. 5B and 5C), which is in contrast to the conclusion drawn by Rao et al. [14]. The different fermentation media used for assays might account for these conflicting results.

To further uncover how the AHL-QS exerts its effects on the expression of the genes analyzed above, we attempted to determine whether these gene promoter regions contain luxI box-like elements by in silico analysis. We cloned and sequenced the promoter regions (approximately -350 to the translation start site) of the genes we analyzed. All primers used to amplify the target promoter regions are provided in Supplementary Table 1, and the corresponding sequences are provided in Supplementary Table 2. Based on the known luxI box-like elements [30, 31], we aligned these elements and created a consensus motif (Fig. 5D). Four bases are conserved in all elements, including C3, T4, A17, and G18, implying that these four
bases might be very important for luxI box-like elements recognized and bound by LuxR type protein. We scanned target gene promoter regions using the created consensus motif with two criteria: 1) with the best similarity only, and 2) the candidate motif including C3, T4, A17, and G18 bases. Scanning results showed that there are four candidate motifs localized on the slaR, slaA, acs, and ldh promoter regions (Fig. 5E), implying that these four genes are the true targets of AHL-QS in S. marcescens MG1, although it requires further experiments to validate.

Based on our results, we propose a SwrR working model during the S. marcescens MG1 fermentation process that, in the absence of autoinducers, SwrR directly binds to the acs, ldh, slaR and slaA promoter and activates acs and ldh but represses slaR and slaA. In the presence of autoinducers, SwrR interacts with autoinducers and then detaches from the target gene promoters, resulting in the repression of acs and ldh and removing the repression of slaR and slaA. This model allows S. marcescens MG1 to produce more acid products at low cell density, but to generate more neutral products at high cell density. However, this model seems to be a contradiction where SwrR exerts two opposite functions when binding to its different targets. However, it is reminiscent of us that SpnR represses its target genes but activates its own expression [30] and that Lys-like regulator activates α-acetolactate decarboxylase gene expression but represses its own expression [29].

In conclusion, according to the data presented in this study, three conclusions can be drawn. First, AHL-QS is required, but not essential, for S. marcescens MG1 growth. Second, AHL-QS facilitates S. marcescens MG1 to produce acid products
during the early phase of fermentation and is susceptible to generating more neutral products during the later phase of fermentation. However, the excess acetic acid is removed in an AHL-QS-independent manner. Third, AHL-QS in *S. marcescens* MG1 exerts its negative effects on regulating the expression of genes responsible for acidogenesis and plays an active role in promoting the expression of genes involved in solventogenesis. By close examination of these analyzed gene promoter regions, *slaR, slaA, acs,* and *ldh* promoter regions contain the putative *luxI* box-like motifs, implying that these four genes are the true targets of AHL-QS in *S. marcescens* MG1. However, it should be noted that there may be some unknown regulatory circuits involved in switching from acidogenesis to solventogenesis, other than the AHL-QS regulatory system, in *S. marcescens* MG1.

**Supplementary information**

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (No. 81673542 and 31870481), New Century Excellent Talents Supporting Plan of the Provincial Education Department of Fujian Province of China (No. K8015056A), Key project of Fujian province fund (No. 2019J01070602), and the Science Fund of the Provincial Education Department of Fujian Province of China (No. KLA17041A).

**Competing financial interests:** The authors declare no competing financial interests.

**References**

1. Papenfort K, Bassler BL. 2016. Quorum sensing signal-response systems in


127-140.

an Erwinia carotovora quorum-sensing signaling molecule. J. Bacteriol. 184:
1163-1171.

27. Blomqvist K, Nikkola M, Lehtovaara P, Suihko ML, Airaksinen U, Straby KB,
et al. 1993. Characterization of the genes of the 2,3-butanediol operons from
Klebsiella terrigena and Enterobacter aerogenes. J. Bacteriol. 175:
1392-1404.

in acetoin biosynthesis and motility/biofilm formation by the virulence
activator AphA and the acetate-responsive LysR-type regulator AlsR in Vibrio

regulation of acetoin fermentation by quorum sensing and pH in Serratia

LuxR family protein SpnR functions as a negative regulator of N-acyl
homoserine lactone-dependent quorum sensing in Serratia marcescens. Mol.


---

**Figure legends**

Fig. 1. Fermentation pathways in *S. marcescens* MG1.

Abbreviations: LDH, lactate dehydrogenase; ACS, acetyl-CoA synthase; CS, citrate synthase; SDH, succinate dehydrogenase; ALS, α-acetolactate synthase; ALDC, α-acetolactate decarboxylase; BDH, 2,3-butanediol dehydrogenase; ADH, alcohol dehydrogenase.

Fig. 2. Effects of *swrI* disruption on biomass concentration, pH profile, and sugar consumption of *S. marcescens* MG1.

Construction of *S. marcescens* MG1 *swrI* disruption mutant (A). Samples were collected at the indicated fermentation time points and biomass concentration (B), pH
profile (C), and sugar consumption (D) determination assays for *S. marcescens* MG1 WT and ΔswrI complemented with or without C₆-HSL were performed. Values are means, and bars indicate standard deviations (SDs) (*n*=3).

Fig. 3. Effect of *swrI* disruption on acidogenesis in *S. marcescens* MG1.

Samples were collected at the indicated fermentation time points and lactic acid content (A), acetic acid content (B), citric acid content (C), and succinic acid content (D) produced by *S. marcescens* MG1 WT and ΔswrI complemented with or without C₆-HSL were determined. Values are means, and bars indicate standard deviations (SDs) (*n*=3).

Fig. 4. Effect of *swrI* disruption on solventogenesis in *S. marcescens* MG1.

Samples were collected at the indicated fermentation time points. Ethanol content (A), acetoin content (B), and 2,3-butanediol content (C) produced by *S. marcescens* WT and ΔswrI complemented with or without C₆-HSL were determined. Values are means, and bars indicate standard deviations (SDs) (*n*=3).

Fig. 5. Effect of *swrI* disruption on relevant enzyme gene expression in *S. marcescens* MG1.

Quantitative real-time PCR was used to determine mRNA expression levels of samples collected at fermentation time points of 8 h (A), 16 h (B), and 24 h (C). Relative quantification is reported as fold expression against the WT sample in each
set of data. Values are means, and bars indicate standard deviations (SDs) \((n=3)\).

Single and double asterisks represent \(P<0.05\) and \(P<0.01\), respectively. The consensus motif was created by aligning \(V. \text{fischeri} \ luxI\) with: \(Pseudomonas \text{aeruginosa} \ lasI\) and \(rhII\); \(Ralstonia \text{solanacearum} \ solI\); \(Acidithiobacillus \text{ferrooxidans} \ afeI\); \(Burkholderia \text{cepacia} \ cepI\); \(S. \text{marcescens} \ SS-1 \ spnR\) and \(recA\); \(Pantoea \text{stewartii} \ esaR\), \(luxIQ_{SS-1}\), \(luxIQ_{QS10-1}\), and \(luxR_{QS10-2}\) as described elsewhere previously \([30, 31]\). The consensus sequence is shown below the alignment, N=A, T, C or G; V=A, C, or G; R=A or G; H=A, T, or C; D=A, T, or G; Y=C or T; B=T, C, or G (D). Four putative \(luxI\) box-like motifs existing upstream of \(S. \text{marcescens} \ MG1 \ slaR, \ slaA, \ acs\), and \(ldh\) (E).

Tables

Table 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strains or plasmids</th>
<th>Characteristics</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S. \text{marcescens} \ MG1)</td>
<td>Wild Type, Tc'Ap'</td>
<td>CICC 25567 and laboratory stock</td>
</tr>
<tr>
<td>(\Delta \text{swrI})</td>
<td>(S. \text{marcescens} \ MG1) harboring (\text{swrI}) deletion mutant, TcR ApR KmR</td>
<td>This study</td>
</tr>
<tr>
<td>(E. \text{coli} \ S17-1 \ \lambda\pir)</td>
<td>(\text{recA pro hsdR RP4-2-Tc::Mu-Km::Tn7})</td>
<td>Laboratory stock and [32]</td>
</tr>
<tr>
<td>(C. \text{violaceum} \ CV026)</td>
<td>a mutant strain deficient in production of autoinducers used as quorum sensing reporter organism</td>
<td>Laboratory stock and [33]</td>
</tr>
<tr>
<td>pUTKm1</td>
<td>ApR KmR (\text{oriR6K oriTRP4})</td>
<td>Laboratory stock and [32]</td>
</tr>
<tr>
<td>pUT-(\text{swrI})</td>
<td>pUTKm1 harboring (\text{swrI}) fragment, KmR</td>
<td>This study</td>
</tr>
</tbody>
</table>

Table 2. Metabolic flux analysis for the products generated by WT and \(\Delta \text{swrI}\) with or without exogenous \(C_6\)-HSL.

<table>
<thead>
<tr>
<th>(S. \text{marcescens})</th>
<th>WT(^*)</th>
<th>(\Delta \text{swrI})</th>
<th>WT(^*)</th>
<th>(\Delta \text{swrI})</th>
<th>WT(^*)</th>
<th>(\Delta \text{swrI})</th>
<th>WT(^*)</th>
<th>(\Delta \text{swrI})</th>
<th>WT(^*)</th>
<th>(\Delta \text{swrI})</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG1</td>
<td>C(_6)-HSL(^*)</td>
<td>C(_6)-HSL(^*)</td>
<td>C(_6)-HSL(^*)</td>
<td>C(_6)-HSL(^*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.82</td>
<td>9.38</td>
<td>10.45</td>
<td>7.19</td>
<td>6.37</td>
<td>7.23</td>
<td>7.25</td>
<td>7.00</td>
<td>2.34</td>
<td>6.47</td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>9.25</td>
<td>14.32</td>
<td>10.52</td>
<td>0.13</td>
<td>0.36</td>
<td>0.11</td>
<td>1.48</td>
<td>5.44</td>
<td>1.53</td>
<td>-3.54</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.00</td>
<td>18.03</td>
<td>0.00</td>
<td>0.00</td>
<td>-15.24</td>
<td>0.00</td>
<td>0.00</td>
<td>2.70</td>
<td>0.00</td>
<td>3.83</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>34.02</td>
<td>42.06</td>
<td>33.76</td>
<td>-3.11</td>
<td>-0.04</td>
<td>-4.79</td>
<td>-3.96</td>
<td>5.04</td>
<td>-2.95</td>
<td>-4.04</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.83</td>
<td>0.94</td>
<td>1.64</td>
<td>-2.09</td>
<td>0.73</td>
<td>-1.68</td>
<td>-0.14</td>
<td>0.39</td>
<td>0.01</td>
<td>9.49</td>
</tr>
<tr>
<td>Ethanol</td>
<td>7.64</td>
<td>5.24</td>
<td>9.28</td>
<td>4.80</td>
<td>7.09</td>
<td>3.83</td>
<td>-4.88</td>
<td>14.58</td>
<td>-0.73</td>
<td>2.85</td>
</tr>
<tr>
<td>Acetoin</td>
<td>2.63</td>
<td>0.68</td>
<td>2.47</td>
<td>-3.21</td>
<td>7.09</td>
<td>-2.67</td>
<td>0.37</td>
<td>2.50</td>
<td>0.64</td>
<td>45.96</td>
</tr>
<tr>
<td>2,3-butanediol</td>
<td>34.82</td>
<td>9.35</td>
<td>31.88</td>
<td>96.30</td>
<td>93.64</td>
<td>97.96</td>
<td>104.38</td>
<td>67.95</td>
<td>99.16</td>
<td>38.98</td>
</tr>
</tbody>
</table>

Note: Metabolic flux was calculated based on the assumption that acid and neutral end products were generated by bacteria consuming 100 g glucose. Superscript letters a, b, c, and d represent the fermentation periods of 0-8 h, 8-16 h, 16-24 h, and 24-32 h, respectively.
Fig. 1. Fermentation pathways in S. marcescens MG1.
Abbreviations: LDH, lactate dehydrogenase; ACS, acetyl-CoA synthase; CS, citrate synthase; SDH, succinate dehydrogenase; ALS, α-acetolactate synthase; ALDC, α-acetolactate decarboxylase; BDH, 2,3-butanediol dehydrogenase; ADH, alcohol dehydrogenase.
Fig. 2. Effects of swrI disruption on biomass concentration, pH profile, and sugar consumption of S. marcescens MG1. Construction of S. marcescens MG1 swrI disruption mutant (A). Samples were collected at the indicated fermentation time points and biomass concentration (B), pH profile (C), and sugar consumption (D) determination assays for S. marcescens MG1 WT and ΔswrI complemented with or without C6-HSL were performed. Values are means, and bars indicate standard deviations (SDs) (n=3).
Fig. 3. Effect of swrI disruption on acidogenesis in S. marcescens MG1. Samples were collected at the indicated fermentation time points and lactic acid content (A), acetic acid content (B), citric acid content (C), and succinic acid content (D) produced by S. marcescens MG1 WT and ΔswrI complemented with or without C6-HSL were determined. Values are means, and bars indicate standard deviations (SDs) (n=3).
Fig. 4. Effect of swrI disruption on solventogenesis in S. marcescens MG1. Samples were collected at the indicated fermentation time points. Ethanol content (A), acetoin content (B), and 2,3-butanediol content (C) produced by S. marcescens WT and ΔswrI complemented with or without C6-HSL were determined. Values are means, and bars indicate standard deviations (SDs) (n=3).
Fig. 5. Effect of swrI disruption on relevant enzyme gene expression in S. marcescens MG1. Quantitative real-time PCR was used to determine mRNA expression levels of samples collected at fermentation time points of 8 h (A), 16 h (B), and 24 h (C). Relative quantification is reported as fold expression against the WT sample in each set of data. Values are means, and bars indicate standard deviations (SDs) (n=3). Single and double asterisks represent P<0.05 and P<0.01, respectively. The consensus motif was created by aligning V. fischeri luxI with: Pseudomonas aeruginosa lasI and rhlI; Ralstonia solanacearum solI; Acidithiobacillus ferrooxidans afeI; Burkholderia cepacia cepI; S. marcescens SS-1 spnR and recA; Pantoea stewartii esaR, luxIQS6-1, luxIQS10-1, and luxRQS10-2 as described elsewhere previously [30, 31]. The consensus sequence is shown below the alignment, N=A, T, C, or G; V=A, C, or G; R=A or G; H=A, T, or C; D=A, T, or G; Y=C or T; B=T, C, or G (D). Four putative luxI box-like motifs existing upstream of S. marcescens MG1 slaR, slaA, acs, and ldh (E).