行政院國家科學委員會專題研究計畫　成果報告

結核菌感染快速血清及側流向技術診斷技術之研究

計畫類別：個別型計畫
計畫編號：NSC92-2314-B-002-362-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：國立臺灣大學醫學院醫事技術學系暨研究所

計畫主持人：何憲武

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 12 月 22 日

Activation of *ygfF*$_{Sm}$ expression by long chain fatty acids and 3-oxo-C12 quorum sensing signal is antagonized by RssA, a two-component system sensor kinase involved in regulation of *Serratia marcescens* swarming

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Running title: Swarming regulation in *Serratia marcescens*.
Keywords: *Serratia marcescens*, two-component system, swarming motility, fatty acids, quorum sensing signals.

Abstract

Through mini-Tn5 transposon mutagenesis for screening swarming mutants that swarmed independent of temperature regulation in *Serratia marcescens*, a mutant named WC100 was isolated. Analysis of the DNA region flanking mini-Tn5 transposon identified a chromosomal DNA fragment comprising three genes encoding a pair of two-component system RssA-RssB, and a potential oxidoreductase protein YgfF$_{Sm}$. While long chain saturated fatty acids (SFAs) and long chain acyl homoserine lactone (AHL) quorum sensing signals are observed to inhibit swarming of the parent strain *S. marcescens* CH-1, swarming of WC100 and the *rssA* re-knock-out mutant strain CH-1ΔA was not inhibited. *S. marcescens* CH-1 and CH-1ΔA were used to host a recombinant plasmid pYS401 in which the *ygfF*$_{Sm}$ promoter from *S. marcescens* CH-1 was fused to a promoterless *Vibrio harveyi luxCDABE* operon. Expression of *ygfF*$_{Sm}$ in CH-1 was observed to reach its peak at about one hour after inoculation on MGM-casamino acids agar surface at 37°C, when cells are grown at the transitional stage between lag and log phase. Such a basal expression activity was rapidly reduced as cells are grown into log phase. Compare with the basal level activity of CH-1, long chain SFAs and 3-oxo-C12 quorum sensing signal activated *ygfF*$_{Sm}$ expression. Furthermore, the *ygfF*$_{Sm}$ expression level in CH-1ΔA was observed to be increased for 2.25-fold compared with that in CH-1 grown on MGM-casamino acids agar, and was further activated when long chain SFAs or 3-oxo-C12 AHL signal were added. These data showed that while long chain SFAs and AHL signals inhibit swarming, expression of *ygfF*$_{Sm}$ is activated by these compounds in both *S. marcescens* CH-1 and CH-1ΔA. The *ygfF*$_{Sm}$ expression is down-regulated by RssA as in *rssA* mutant CH-1ΔA, not only its expression activity is higher but is also maintained at a higher level for a longer time.

More and more bacteria are identified to exhibit a form of cell differentiation and multicellular behaviour termed swarming migration. Swarming involves differentiation of vegetative cells into hyperflagellated swarm cells that undergo rapid and coordinated population migration across solid surfaces [1-3]. We are interested in unraveling the underlying mechanism of *S. marcescens* swarming. Previous works have shown that when inoculated on LB medium solidified with 0.8% agar (LB swarming plate) at 30°C, *S. marcescens* shows a swarming phenomenon where a colony of short motile vegetative rods differentiate at the colony margin into elongated, aseptate and hyperflagellate swarm cells which migrate rapidly and coordinately away from the colony[4-6]. Phenotypically, the process of swarmer cell differentiation and swarming migration behavior is divided into two separate phases in *S. marcescens*: (i) the lag period prior to onset of swarming behavior and the induction of swarmer cell differentiation at the colonial edge and (ii) active motile swarming migration (or translocation) from colonial edge. Individual swarmer cells by themselves do not have the ability to swarm. Rather, swarming behavior in *S. marcescens* is the result of a coordinated, multicellular effort of groups of differentiated swarmer cells functioning through close cell-cell interactions[6].

Development of a *Serratia* surface expansion colony requires the sensing and integration of a variety of environmental, cell-to-cell, as well as intracellular signals involving surface contact and local high population density[4,6-8]. The flagellar master operon and the quorum-sensing system are global regulators which control two separated regulons involved in swarming regulation[8]. An additional layer of regulation of surface migration may also be exerted through *S. marcescens* RsmA (an *E. coli* CsrA homologue and a repressor), which functions as a global regulator and when over-expressed, also produces a non-spreading colony[9].

Although previous works have unraveled many critical components involved in *Serratia* spp. swarming behaviour itself, many questions remain elusive. For example, it is not explained why swarming of *S. marcescens* is strictly temperature-dependent, i.e., when inoculated on a 0.8% LB swarming plate, *S. marcescens* swarms at 30°C, but this behavior was completely inhibited at 37°C. Furthermore, the potential physiological signals providing critical stimuli for initiation of swarming and the signal transduction system are also not characterized. Through mini-Tn5 mutagenesis, we have screened a group of *S. marcescens* strains that swarmed well at 37°C. One of the mutants WC100 was selected for further physiological characterization. We identified a gene that, when mutated, lead to defect in haemolysin production and biofilm formation, and showed a "super-swarming" behaviour: decreased the length of the lag phase prior to swarming migration and swarmed not only faster, but also at higher agar concentration surface[10]. In the process of characterization of this mutated gene and its flanking DNA sequences, we have identified an *rssA-rssB* two-component regulatory system and an open reading frame $ygfF_{Sm}$ predicted to encode a potential oxidoreductase protein predicted to be involved in fatty acid synthesis[10]. Interestingly, this mutant appears to function to coordinate the initiation of swarming migration independent of *flhDC* flagellar swimming motility, AHL quorum-sensing system, and production of biosurfactant-- factors already characterized to be involved in the regulation of population surface migration behaviours in many bacterial species including *S. marcescens*[4,7,10].

Our previous works have shown that swarming behaviour of *Proteus mirabilis* was inhibited by long chain saturated fatty acids (SFAs), and that the inhibitory effect of

lauric acid, myristic acid and palmitic acid was via a sensor kinase RsbA [11]. Whether swarming of *S. marcescens* CH-1 was also inhibited by the long chain SFAs remained undetermined. Previously we have also observed that the flagellum-independent populational surface spreading behavior was inhibited by some long chain N-acyl homoserine lactone (AHL) quorum sensing signals (autoinducer-1, AI-1) in *S. marcescens* [7]. In this communication we assayed whether swarming of the AI-1 negative *S. marcescens* strain CH-1 was affected by the long chain SFAs and AHL signals. In this communication, we reported that swarming of CH-1 was inhibited by long chain SFAs and some long chain AHL signals. Expression of $ygfF_{Sm}$ was activated by some long chain SFAs or 3-oxo-C12 AHL signal in both CH-1 and the *rssA* reknock-out strain CH-1ΔA. Expression of $ygfF_{Sm}$ was found to be rapidly antagonized by RssA sensor kinase of the two-component system as $ygfF_{Sm}$ promoter activity was activated and maintained at a much higher level for a longer time in CH-1ΔA than in CH-1. These observations suggest existence of a common regulatory pathway of long chain SFAs and long chain AHLs on swarming of *S. marcescens* CH-1. Such regulation is closely related to expression of $ygfF_{Sm}$ which was negatively regulated by RssA.

Materials and Methods

Bacterial strains, plasmids and culture conditions. S. marcescens CH-1 is a clinical isolate and is functionally wild-type for swimming motility and swarming migration behavior. No AHL quorum sensing signals were detected from CH-1 cells. *S. marcescens* SS-1 which is without flagellum and produces AHL signals is isolated from environmental soil sample [7]. *S. marcescens* WC100 is a mini-Tn5 transposon inserted mutant strain derived from CH-1 and swarmed vigorously at 37°C. *S. marcescens* CH-1ΔA is an *rssA* insertion-deletion re-knockout strain by homologous recombination [10]. *S. marcescens* was routinely cultured at 30^{o}C on either LB or MGM-casamino acids agar plates [12] which contain 0.8% Bacto agar, unless specifically mentioned in the text. The pYS401(P*ygfF*$_{Sm}$::*luxCDABE*) used as a bioreporter for monitoring the promoter activity of P*ygfF*$_{Sm}$ was generated by insertion of a PCR amplified promoter region upstream of *luxCDABE* derived from pSB1075, into the pACYC184 (Tc^{r};Cm^{r}) vector [13]. The PCR primer pair was: 5'GATATCCAGCCTCAGGCGGAGGG3'/5'GGATCCATTCCCCATCCCGACAGAC TA3'. DNA sequence was confirmed by bi-directional sequencing reactions. Bacterial colonies were examined under light microscopy at a magnification of 1X or 30X using an Olympus SZH microscope. For determining bacterial growth rates, hourly increases in the optical density of broth cultures at 600nm were measured.

Northern blot hybridization. Basically the experimental procedures followed the protocol of Sambrook et al., (1989) [12]. Total cellular RNA was prepared by hot phenol method[12] and transferred to nylon filters and hybridized with DNA probes labeled with DIG (Roche). The *ygfF*$_{Sm}$ probe was a 200 bp partial *ygfF*$_{Sm}$ DNA fragment amplified by PCR/ DIG-labeling kit (Roche) using the primer pair 5'-CTTCGCCTGCAGCAGGC-3'/5'-AAGTCGACGGTA GCAA AAGTAGTG-3'.

Recombinant DNA techniques. Unless otherwise indicated, standard protocols were used for DNA/DNA hybridization, plasmid and chromosomal DNA preparation, transformation, electroporation, PCR, restriction digestion, agarose gel electrophoresis, DNA recovery from agarose gels, DNA ligation [12], and conjugation [14]. PCR DNA amplicons were cloned by pCR^{TM}II$^{®}$ and the TA Cloning Kit (Invitrogen). DNA sequencing and analysis was performed using a Perkin-Elmer Autosequencer model 377 with a Taq DyeDeoxyTM terminator cycle sequencing kit (Applied Biosystems). The DNA sequences of PCR products were confirmed by sequencing both strands from two or three independent reactions.

Enzymes and chemicals. Restriction enzymes were purchased from Roche (Germany). *Taq* polymerase and PCR-related products were obtained from either Perkin Elmer (USA) or Takara Biomedicals (Japan). Other laboratory grade chemicals were purchased from Sigma (USA), Merck (Germany) or BDH (UK).

N-Acylhomoserine lactones. The AHLs used in this study, *N*-butanoyl-L-homoserine lactone, (C4-HSL); *N*-hexanoyl-L-homoserine lactone, (C6-HSL); *N*-octanoyl-L-homoserine lactone, (C8-HSL); *N*-decanoyl-L-homoserine lactone, (C10-HSL); *N*-dodecanoyl-L-homoserine lactone, (C12-HSL); *N*-(3-oxobutanoyl)-L-homoserine lactone, (3-oxo-C4-HSL); *N*-(3-oxohexanoyl)-L-homoserine lactone, (3-oxo-C6-HSL); *N*-(3-oxooctanoyl)-L-homoserine lactone, (3-oxo-C8-HSL);

N-(3-oxodecanoyl)-L-homoserine lactone, (3-oxo-C10-HSL); *N*-(3-oxododecanoyl)-L-homoserine lactone, (3-oxo-C12-HSL) and *N*-(3-oxotetradecanoyl)-L-homoserine lactone (3-oxo-C14-HSL) were synthesized as described by Chhabra *et al*., (1993)[15]. Stock solutions at 10 mM in acetonitrile (far-UV grade) were diluted into the growth medium to give the stated concentrations.

Detection of luciferase activity. The Autolumat LB 953 luminometer (EG&G, Germany) with the 'replicates' program was used for bioluminescence measurement. All procedures followed the protocols supplied by the manufacturer.

Results

Long chain saturated fatty acids inhibit swarming of S. marcescens CH-1 but not the rssA mutant

Although *S. marcescens* CH-1 cells do not swarm on 0.8% LB swarming plates (0.8% bacto agar) at 37°C, we observed that CH-1 did exhibit ample migratory swarming on defined MGM-casamino acids swarming plates (M9 minimal growth medium containing 1% (w/v) casamino acids solidified with 0.8% bacto agar) at 37°C (Fig. 1), suggesting that a higher environmental temperature is not inhibitory to *S. marcescens* CH-1 swarming in MGM-casamino acids media. These observations suggested that some components (or their metabolic derivatives) of yeast extracts contained in LB medium inhibited CH-1 cell swarming at 37°C. Changes in culture conditions including twenty individual amino acid concentrations, glucose concentration, addition of mono- or disaccharides, iron starvation and pH, all did not lead to inhibition of CH-1 swarming at 37°C (data not shown). Exogenous saturated or unsaturated fatty acids of different chain lengths were then added to see whether there is any effect on CH-1 swarming. Long chain saturated fatty acids (SFAs) such as lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) and unsaturated fatty acids (UFAs) such as myristoleic acid (14:1 Δ9), palmitoleic acid (16:1 Δ9) and oleic acid (C18:1 Δ9) at a final concentration of 0.01% (w/v) were added separately to MGM-casamino acids plates followed by swarming assays at 37°C. We found that whereas there were no significantly different swarming-inhibition effects of UFAs, CH-1 cell swarming was completely inhibited by addition of lauric acid and myristic acid, and slightly inhibited by the presence of palmitic acid and stearic acid. None of the fatty acids tested showed significant inhibitory effect on swarming of *S. marcescens* WC100 and CH-1ΔA. Effect of myristic acid on swarming of *S. marcescens* CH-1 and the *rssA* mutant CH-1ΔA was shown in figure 1.

Long chain AHL quorum sensing signals inhibit swarming of S. marcescens CH-1, but not CH-1ΔA

The N-acyl homoserine lactone (AHLs) quorum sensing signals in *S. marcescens* SS-1 are synthesized by SpnI, a homologue of LuxI synthetase [7]. To study the role of quorum sensing in the regulation of swarming motility in *S. marcescens*, we conducted studies to identify AI-1 quorum sensing signals in *S. marcescens* CH-1 cells. Unexpectedly, we found that the CH-1 was unable to produce AHLs capable of activating biosensors based upon LuxR, CviR, AhyR and LasR in T-streak assays and in multi-well assays following dichloromethane extraction of spent culture supernatants (data not shown). To exclude the possibility that inhibitory compounds were present in the crude dichloromethane extracts we performed fractionation by both HPLC and TLC and subsequently assayed these crude preparations using multi-well assays and biosensor overlays, respectively. In both instances, no AHL activity was detected (data not shown). Southern blotting failed to identify any homologs of *spnTIR* genes from *S. marcescens*

SS-1[7]. Further experiments using PCR failed to detect a LuxRI/AHL-based quorum sensing system in *S. marcescens* CH-1 (data not shown).

Previously we have found that long-chain AHLs have inhibitory effects upon the sliding behavior of *S. marcescens* SS-1 cells [7]. Although *S. marcescens* CH-1 cells do not produce detectable AHLs, we hypothesized the existence of long chain AHL signals produced from other sources inhibits CH-1 swarming. To confirm this possibility, CH-1 swarming assays at 37°C were undertaken on MGM-casamino acids swarming plates supplemented with a range of synthetic AHLs including C4, C6, C8, C10, C12, 3-oxo-C4, 3-oxo-C6, 3-oxo-C8, 3-oxo-C10, 3-oxo-C12, and 3-oxo-C14 at a final concentration of 10 μM (Fig. 2A). The data indicate that whereas none of the short chain AHLs tested in the experiment inhibited swarming, a clear and inhibitory effect of some specific long chain AHLs upon swarming plate motility was detectable. Among the long chain AHLs tested, 3-oxo-C10, 3-oxo-C12 and 3-oxo-C14 in particular, significantly inhibited CH-1 swarming. Similar long chain AHLs effect was not observed on inhibition of CH-1ΔA swarming and effect of 3-oxo-C12 on CH-1ΔA swarming was shown in figure 2B. These data suggested that the inhibitory effect may be closely related to the RssA-RssB two component system.

Expression of $ygfF_{Sm}$ is activated by long chain saturated fatty acids

The effect of long chain SFAs on $ygfF_{Sm}$ promoter activity was evaluated. A recombinant plasmid pYS401 (P$ygfF_{Sm}$::*luxCDABE*) was constructed in which expression of a *luxCDABE* was driven by $ygfF_{Sm}$ promoter. The pYS401 was electroporated into CH-1 cells followed by monitoring of bioluminescent activity on MGM-casamino acids plate culture with or without addition of 0.01% (w/v) long chain SFAs at 37°C. The $ygfF_{Sm}$ promoter activity in CH-1 cells reached its peak at one hour after transfer onto fresh MGM-casamino acids plates whilst cells were still growing in lag-log transition phase. Reporter activity decreased rapidly thereafter as cells entered the log phase and was maintained at a very low level in the stationary phase (Fig. 3A). The light intensity of CH-1(pYS401) detected at one hour after inoculation was used as the basal level for subsequent comparisons. Addition of long chain SFAs including C12, C14, C16 and C18 at the final concentration of 0.01% (w/v) was tested to see whether level of $ygfF_{Sm}$ promoter activity was affected. Except C12 which does not show significant activation effect, $ygfF_{Sm}$ promoter activity was all significantly activated by the other long chain SFAs tested (Fig. 3 A and B), with the average induction ratio of 1.5-fold by C14, 1.75-fold by C16 and 2-fold by C18, respectively. These data showed that the peak $ygfF_{Sm}$ promoter activity occurred at the stage of lag-log transition growth phase and was activated by long chain SFAs except C12.

The bioluminescence activities of CH-1(pYS401) and CH-1ΔA(pYS401) were compared following the growth on MGM-casamino acids plates at 37°C. Expression level of $ygfF_{Sm}$ was increased for 2.25-fold at about one hour after inoculation followed by significantly slower rate of reduction in CH-1ΔA compared with in CH-1 (Fig. 3A). Activation effect by long chain SFAs was also observed in CH-1ΔA, where $ygfF_{Sm}$ expression was further activated in the presence of C14, C16 and C18 fatty acids, with a 3.75-fold increase by C18 activation (Fig. 3 A and B). In brief, $ygfF_{Sm}$ promoter activity is activated by some long chain SFAs in both CH-1 and CH-1ΔA.

Expression of $ygfF_{Sm}$ is activated by 3-oxo-C12

The effect of 3-oxo-C12 on expression of $ygfF_{Sm}$ was determined. Light emission patterns of CH-1(pYS401) seeded on MGM-casamino acids plates with or without supplementation of 10 μM 3-oxo-C12 at 37°C were measured, and was shown in figure 4A. While there was no significant difference in the growth rates, $ygfF_{Sm}$ promoter activity was found to be 1.5-fold higher through activation of 3-oxo-C12 in CH-1 (Fig. 4B). These data suggested that similar to the long chain SFA effect, the $ygfF_{Sm}$ promoter activity was also activated by 3-oxo-C12. To see whether activation of $ygfF_{Sm}$ promoter activity was also observed in CH-1ΔA, 3-oxo-C12 at the concentration of 10 μM was tested. A 3-fold activation by 3-oxo-C12 in CH-1ΔA was detected compared with the basal level in CH-1. Briefly, $ygfF_{Sm}$ promoter activity was also activated by 3-oxo-C12 in both CH-1 and CH-1ΔA.

Expression of $ygfF_{Sm}$ is down-regulated by RssA

Compared with the pattern of $ygfF_{Sm}$ promoter activity in CH-1, not only was the increase of $ygfF_{Sm}$ peak promoter activity, but this activity was also found to be maintained at a higher level and for a longer time period of up to 4 hours in CH-1ΔA (Fig. 3A and Fig. 4A). For confirmation of the RssA effect, total cellular RNA was extracted from *S. marcescens* CH-1 and CH-1ΔA seeded on MGM-casamino acids plates containing myristic acid (0.01% w/v) at 37°C. Northern blot hybridization using partial $ygfF_{Sm}$ as the probe was performed. The result was shown in figure 3C. Level of $ygfF_{Sm}$ mRNA was observed to be higher and last for a longer time before degradation in CH-1ΔA than in CH-1. These data suggested that RssA plays a negative regulatory role on $ygfF_{Sm}$ expression. In the absence of wild type *rssA*, the $ygfF_{Sm}$ promoter activity was not only increased, but also maintained at a higher level for a longer time.

Discussion

Although a large body of information concerning the swarming mechanisms has been accumulated in *S. marcescens* and also many other Gram-positive and Gram-negative swarming bacteria, the molecular mechanism(s) of swarming is far from understood. This may be because the wide spectrum effects of physiology in swarming bacteria making it difficult to study the mechanisms directly. Furthermore, a potential specific and conserved regulator governing initiation of swarming has not been clearly identified, albeit a regulator of swarming behaviour of *P. mirabilis*, RsbA, has been reported by us [11] and Belas et al., (1998)[16]. Identification of such a swarming regulator gene is thus an essential step in understanding the underlying mechanism of swarming. In this paper we present strong evidences to show that the sensor kinase RssA is involved in regulation of *S. marcescens* swarming, which is influenced by environmental factors including temperature and the provision of long chain SFAs and AHLs compounds. Although kinase-response regulator pairs of this type were frequently reported as governors of a wide variety of pathways in response to a myriad of environmental signals [17,18], here we showed that long chain SFAs and AHLs inhibit swarming and we have added more information showing the SFAs- and 3-oxo-C12-dependent regulation of swarming is closely related with two component system.

In this study we describe an *ygfF*$_{Sm}$*-rssA-rssB* genetic locus involved in regulation of *S. marcescens* CH-1 swarming. We showed that phenotypically, similar to environmental temperature upshift, addition of long chain SFAs and AHLs at 37°C inhibits CH-1 swarming. Long chain SFAs and AHLs activate expression of *ygfF*$_{Sm}$, which was down-regulated by the RssA, the sensor kinase of two component signal transduction system. Activation of *ygfF*$_{Sm}$ by these long acyl chain derived compounds was independent of RssA, as *ygfF*$_{Sm}$ promoter was all activated in both CH-1 and CH-1ΔA by these compounds. While swarming of CH-1 was not inhibited by changes of other growth conditions such as change of twenty individual amino acid concentrations, glucose concentration, addition of mono- or disaccharides, iron starvation and pH, these observations suggested long chain SFAs and AHLs may specifically regulate swarming of *S. marcescens*. Thus expression level of *ygfF*$_{Sm}$ may either directly or indirectly indicate the nutritious state of the cells. Activation of *ygfF*$_{Sm}$ expression may suggest to the cells there are plenty of fatty-acid derived nutrients in the surrounding medium, which might be a signal encouraging cells to swarm away to obtain more nutrients. However, RssA the two-component sensor kinase rapidly antagonized such an effect. The negative regulation by RssA may mean to the cells that there are still plenty of nutrients within cells so that it is not necessary to swarm away at the current status, as swarming is a energy-consuming behavior [1]. Although we still do not understand whether long chain SFAs or AHLs act as a signaling molecule or as nutrients for *S. marcescens* in this aspect, addition of these compounds lead to activation of *ygfF*$_{Sm}$ expression. Although the YgfF$_{Sm}$ is a potential oxido-reductase protein whose function is not confirmed, it is possible that YgfF$_{Sm}$ is involved in fatty acid synthesis as YgfF$_{Sm}$ shows high homology to members of the NADPH-dependent 3-ketoacyl-ACP reductase family, which is involved in fatty acid synthesis. Possibility exists that activation of *ygfF*$_{Sm}$ expression leads to change of cellular fatty acid synthesis pattern, which affects the composition of membrane fatty acids. This will subsequently affect the conformation of RssA and lead to

activation of RssA-RssB signal transduction pathway. The activated RssB response regulator might act as a repressor which then inhibits *ygfF*$_{Sm}$ expression.

It is a common phenomenon that bacterial two-component systems are proved to be important for regulation of bacterial physiological behaviours under the regulation of physical or chemical environmental signals. Examples include the PhoP/PhoQ system of *Salmonella enterica* governing transcription of some 25 loci in response to the extracellular concentration of Mg^{2+} [19], the regulation of he *arc* system of *E. coli* by redox Quinone signals [20], the interaction between two autoinducers (AI-1 and AI-2) and two cognate membrane-bound two-component hybrid sensor kinases called LuxN and LuxQ respectively and regulation of light emission in *Vibrio harveyi* [21]. Furthermore, mostly regulation of swarming, although the signals that interact with bacterial two-component systems and thus the mechanism remain uncharacterized, regulation of *P. mirabilis* swarming by the putative RsbA sensor kinase [11,16], *P. aeruginosa* swarming, virulence factor expression and biofilm formation by GacA-GacS [22], *E. coli* swarming and capsule synthesis by RcsC-YojN-RcsB [23], and the *S. enterica* swarming by the putative two-component signaling components are also reported [24].

Although we have established a clear evidence that *S. marcescens* swarming is closely related to culture conditions (especially to the existence of long chain SFAs and AHLs) and RssA-RssB signal transduction system, due to the fact that the *rssA* mutant does not change in any identified swarming-related factors such as flagellar swimming motility and production of biosurfactant in *Serratia* spp. [10], our finding may open a novel research direction that focuses on understanding the underlying mechanism of cell-cell and cell-environment communication. More and more evidences gathered indicate that bacterial mutants defective in genes involved in synthesis of either fatty acids or lipopolysaccharide (LPS) are defective in swarming regulation, recognition function or regulation of virulence factors. Although the main function of LPS for bacterium is probably to provide an impermeable barrier against entry of harmful substances, it also plays various roles in interaction with other bacteria or eukaryotic cells [25]. In animals, LPS fractions are powerful endotoxins and contribute to disease symptoms. Recognition of LPS, which is an indispensable component of the Gram–negative bacterial cell surface, can thus sensitize surrounding bacterial cells to the presence of high or low-density bacteria. McCoy [26] demonstrate that LPS modifications is not only involved in the antimicrobial resistance, but also play a role in *P. mirabilis* swaming due to surface charge alterations. Using transposon mutagenesis,[24] indicated a critical role of LPS for swarming regulation in *S. typhimurium* [24]. Besides, it is reported that *rpfB* and *rpfF* are involved in a novel type of regulation mediated by a small diffusible molecule. The factor responsible for these effects has been named DSF (for Diffusible Signal Factor), which is probably a fatty acid derivative [27]. It is speculated that *rpfF* and *rpfB* are involved in diverting intermediates of lipid metabolism to DSF production. Production of DSF is suggested to be under control of two–component system including RpfC, RpfG and RpfH network [27].

In summary, we provide evidence that a histidine sensor kinase may act as a molecular switch for the regulated transcription of a potential fatty acid synthesis gene and regulates swarming behaviour of *S. marcescens*. The level of *ygfF*$_{Sm}$ expression was affected by long chain fatty acids and 3-oxo-C12. Effect of these compounds seems not

to directly via RssA signaling activity, but the induced *ygfF*$_{Sm}$ activity may somehow activate RssA signaling, which antagonize *ygfF*$_{Sm}$ expression activity. Further studies on the relationship between ygfF$_{Sm}$ and RssA-RssB signalling activity are being performed, which will help further on understanding the underlying mechanism of regulation of *S. marcescens* swarming.

Acknowledgements

This work was supported by grants from National Science Council (NSC, grants number NSC-91-2314-B-002-258, NSC-92-2314-B-002-356), Technology Development Program for Academia, Ministry of Economical Affairs (grant number 91-EC-17-A-10-S1-0013) and Environmental Protection Administration (EPA, grant number EPA-91-NSC-01-B003). We thank Sunny Ang for construction of plasmid pYS401.

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Figure legends

Fig. 1. Swarming of *S. marcescens* CH-1 and CH-1ΔA on LB, MGM-casamino acids plates and MGM-casamino acids plates containing 0.1% (w/v) myristic acid at 37°C. Bacterial cells were over night cultured in either LB broth or MGM-casamino acids broth respectively at 37°C. A 5μl bacterial suspension was inoculated onto the center of the plates followed by incubation at 37°C. Photos taken were after 10 hours and 16 hours of incubation for LB and MGM-casamino acids plates, respectively.

Fig. 2. Effect of AHLs on the swarming of *S. marcescens* CH-1 and 3-oxo-C12 inhibits swarming of CH-1 but not CH-1ΔA. The extent of CH-1 swarming on MGM-casamino acids swarming plates supplemented with 10μM synthetic AHLs C4-HSL, C6-HSL, C8-HSL, C10-HSL, C12-HSL, 3-oxo-C4-HSL, 3-oxo-C6-HSL, 3-oxo-C8-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL and 3-oxo-C14-HSL is shown after incubation at 37^{o}C for 16 hours (A). Effect of 10μM 3-oxo-C12 on swarming of CH-1 and CH-1ΔA after 16 hours of incubation was shown in B.

Fig. 3. Effect of long chain saturated fatty acids on *ygfF*$_{Sm}$ promoter activity in *S. marcescens* CH-1(pYS401) and CH-1ΔA(pYS401). (A) Bacterial cells were seeded on MGM-casamino acids plates containing either without or with lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) or stearic acid (C18:0) at a final concentration of 0.01% (w/v) at 37°C. The promoter activity of *ygfF*$_{Sm}$ expressed as specific light emission intensity [relative light units/O.D. (A_{600nm})] was measured hourly following the growth. CH-1 (◇); CH-1+C12 (□); CH-1+C14 (△); CH-1+C16 (×); CH-1+C18 (○); CH-1ΔA (♦); CH-1ΔA+C12 (■); CH-1ΔA+C14 (▲); CH-1ΔA+C16 (×); CH-1ΔA+C18 (•). Effect of long chain SFAs on *ygfF*$_{Sm}$ promoter activity of CH-1(pYS401) (white bar) and CH-1ΔA(pYS401) (grey bar) detected at one hour after inoculation were compared and was shown in (B). Results of Northern blot hybridization using partial *ygfF*$_{Sm}$ 200 bp DNA fragment as the probe for detection of *ygfF*$_{Sm}$ mRNA expression level following the growth of CH-1 and CH-1ΔA on MGM-casamino acids plates at 37°C were shown in (C). Numbers mean hours after inoculation.

Fig. 4. Effect of 3-oxo-C12 on *ygfF*$_{Sm}$ promoter activity in *S. marcescens* CH-1(pYS401) and CH-1ΔA(pYS401). (A) Bacterial cells were seeded on MGM-casamino acids plates containing without or with 10 μM 3-oxo-C12. The promoter activity of *ygfF*$_{Sm}$ expressed as specific light emission intensity [relative light units/O.D. (A_{600nm})] was measured hourly following the growth. CH-1 (□); CH-1+3-oxo-C12 (△); CH-1ΔA (■); CH-1ΔA+3-oxo-C12 (▲). Effect of 3-oxo-C12 on *ygfF*$_{Sm}$ promoter activities of CH-1(pYS401) and CH-1ΔA(pYS401) detected at one hour after inoculation were compared and was shown in (B).

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Fig. 1

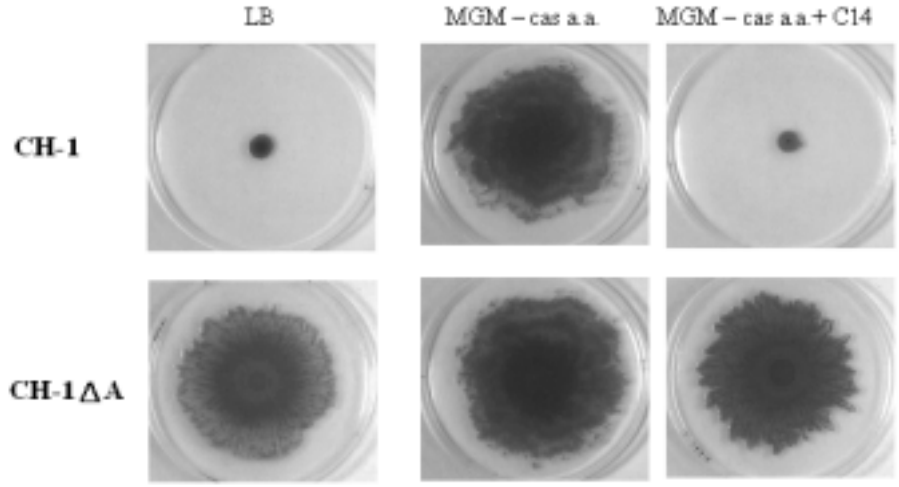

Fig. 2

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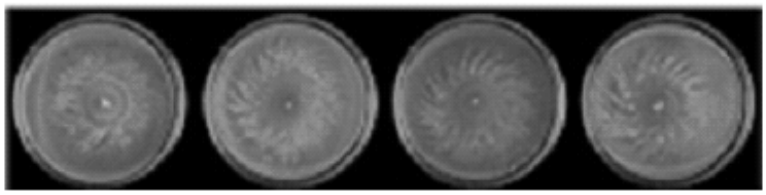

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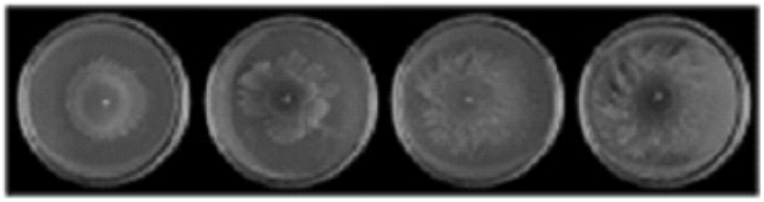

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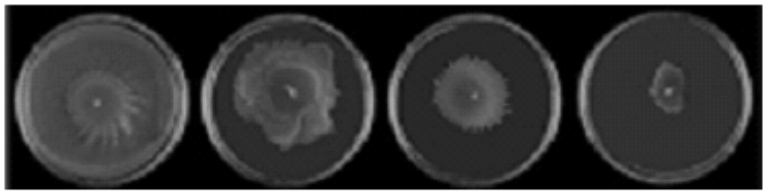

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B

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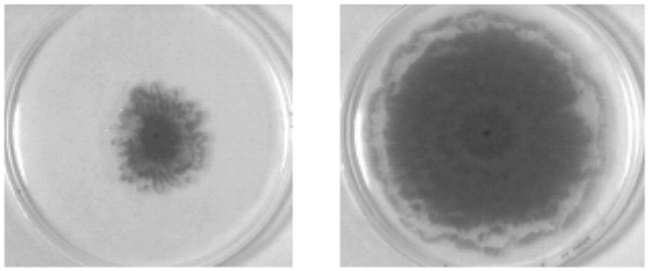

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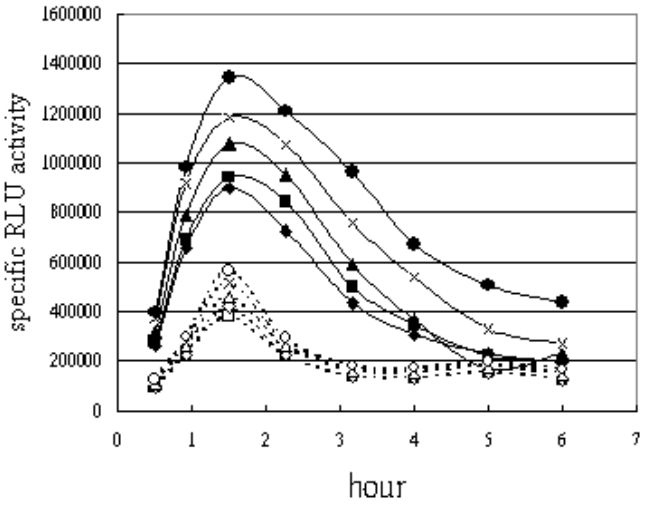
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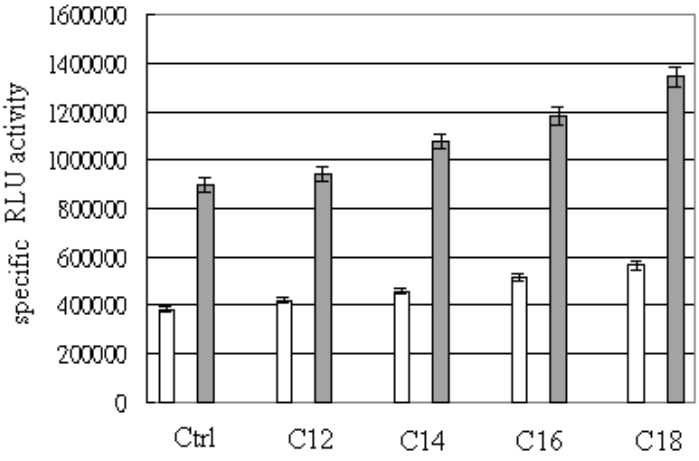
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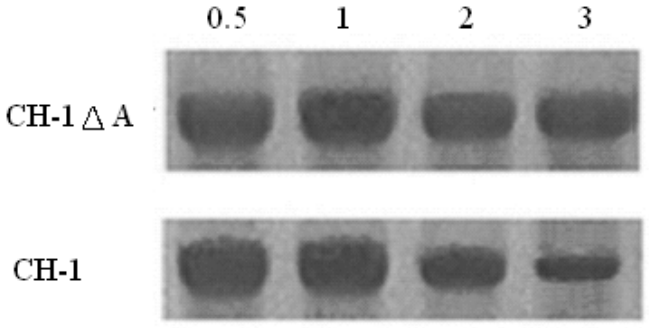
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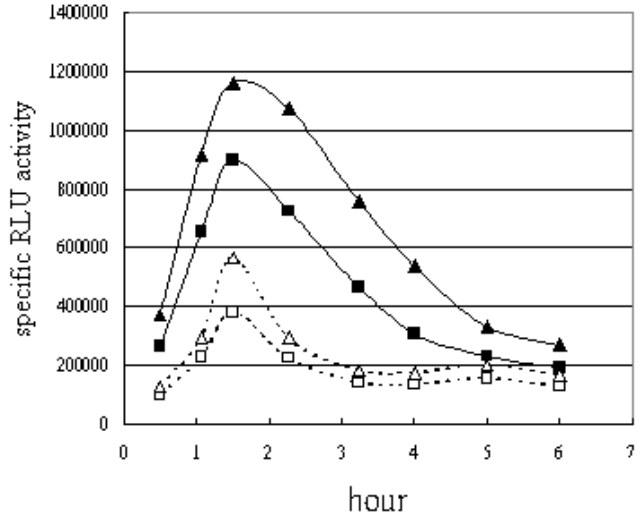
Fig. 4

A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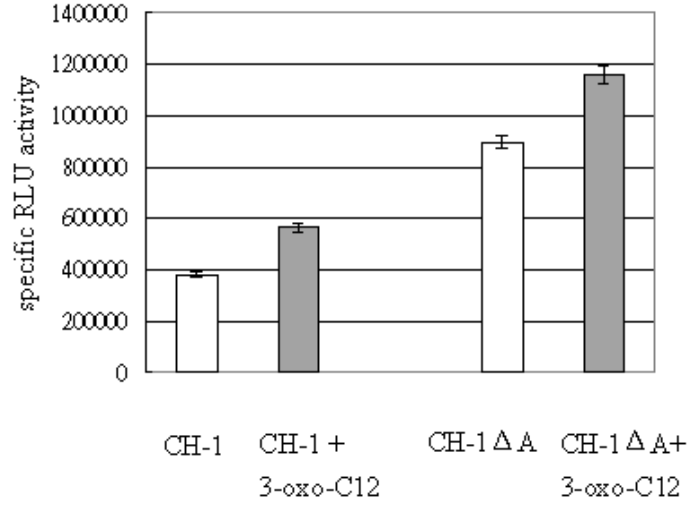
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