Regulation of *Serratia marcescens* *ompF* and *ompC* porin genes in response to osmotic stress, salicylate, temperature and pH

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Serratia marcescens is a Gram-negative enterobacterium that has become an important opportunistic pathogen, largely due to its high degree of natural antibiotic resistance. One factor contributing to this natural antibiotic resistance is reduced outer membrane permeability, which is controlled in part by OmpC and OmpF porin proteins. OmpF expression is regulated by *micF*, an RNA transcript encoded upstream of the *ompC* gene, which hybridizes with the *ompF* transcript to inhibit its translation. Regulation of *S. marcescens* porin gene expression, as well as that of *micF*, was investigated using β -galactosidase reporter gene fusions in response to 5, 8 and 10 % sucrose, 1, 5 and 8 mM salicylate, and different pH and temperature values. β -Galactosidase activity assays revealed that a lower growth temperature (28 °C), a more basic pH (pH 8), and an absence of sucrose and salicylate induce the transcription of the *ompF* gene, whereas the induction of *ompC* is stimulated at a higher growth temperature (42 °C), acidic pH (pH 6), and maximum concentrations of sucrose (10 %) and salicylate (8 mM). In addition, when multiple conditions were tested, temperature had the predominant effect, followed by pH. In this study, it was found that the MicF regulatory mechanism does not play a role in the osmoregulation of the *ompF* and *ompC* genes, whereas MicF does repress OmpF expression in the presence of salicylate and high growth temperature, and under low pH conditions.

INTRODUCTION

Serratia marcescens is a Gram-negative enteric bacterium that has become an important opportunistic pathogen associated with a number of life-threatening diseases and nosocomial infections. In the last two decades, *S. marcescens* has gained much attention due to a high incidence of antibiotic resistance. β -Lactam resistance in this organism is due in part to β -lactamase enzymes (Sanders & Sanders, 1992); however, a reduction in the levels of outer membrane porins is also responsible for altering resistance levels by decreasing the outer membrane permeability (Gutmann *et al.*, 1984).

Our group has characterized two *S. marcescens* porins, OmpC and OmpF (Hutsul & Worobec, 1997), with molecular masses of 40 and 41 kDa, respectively. These porins are non-specific protein channels that serve to take in nutrients and antibiotics, such as β -lactams, and export waste products. OmpF is believed to have a slightly larger pore diameter, resulting in a faster rate of diffusion through OmpF than through OmpC. The structural genes for both OmpF and OmpC have been cloned and sequenced. *S. marcescens* OmpC and OmpF are 71 % and 68 % similar to *Escherichia*

coli OmpC and OmpF, respectively, at the amino acid level (Hutsul & Worobec, 1997).

The production of *E. coli* OmpF and OmpC is regulated by many environmental factors, such as osmotic pressure, temperature and pH. Production of the appropriate outer membrane porin is required for survival of the organism under widely differing conditions (Csonka, 1989). For example, in the human gut, where concentrations of both nutrients and toxic products, such as bile salts, are relatively high, OmpC is the predominant porin. In a nutrient-poor environment, such as fresh water, OmpF is the major porin species (Mizuno & Mizushima, 1990; Pratt & Silhavy, 1995). High osmotic pressure, high temperature, low pH and higher concentrations of salicylate favour the expression of *E. coli* OmpC over OmpF (Pratt *et al.*, 1996).

The most extensively studied mechanism of porin regulation in *E. coli* is a two-component regulatory system composed of an inner membrane sensor protein called EnvZ, and a response regulator, OmpR (Batchelor *et al.*, 2004; Cai & Inouye, 2002; Pratt & Silhavy, 1995; Mizuno & Mizushima, 1990). EnvZ monitors the external osmolarity, and communicates this information to OmpR by phosphorylation and dephosphorylation. In its phosphorylated state, OmpR-P regulates transcription from the *ompF* and

Abbreviations: IHF, integration host factor; OBS, OmpR-binding site.

ompC genes. Under conditions of high osmolarity, OmpR-P represses transcription of *ompF*, and activates *ompC* transcription, while under conditions of low osmolarity, OmpR-P activates transcription of *ompF* (Aiba *et al.*, 1989). The difference in transcriptional activation is a result of the differential organization of the OmpR-binding sites (OBSs) upstream of the two porin genes, with *ompF* having an additional OBS (Harlocker *et al.*, 1995). The OBSs also have different affinities, such that at low levels of OmpR-P, the higher-affinity sites of *ompF* are preferentially bound by OmpR, and transcription is activated, while at higher levels of OmpR-P, the lower-affinity sites of *ompC* are bound, resulting in increased transcription (Liu & Ferenci, 2001; Harlocker *et al.*, 1995). In addition, when the low-affinity upstream site of *ompF* is bound by OmpR-P, the integration host factor (IHF) helps to form a DNA loop that stalls the transcription of the *ompF* gene (Ramani *et al.*, 1992). As a result, as external osmolarity increases, the levels of OmpC increase, while those of OmpF decrease.

In *E. coli*, OmpF levels also decrease with increased growth temperature (Andersen *et al.*, 1989), and in the presence of salicylate (Nikaido, 2003; Rosner *et al.*, 1991). These conditions involve the antisense RNA, *micF* (mRNA interfering complementary RNA). The *micF* gene is located immediately upstream of *ompC*, and is transcribed in the opposite direction (Mizuno *et al.*, 1984). Two species of RNA exist due to two promoters upstream of *micF*, which result in a 93 nt 4·5S RNA, and a 174 nt 6S RNA. The 4·5S RNA product of the *micF* gene forms a stable duplex with the RBS of *ompF* mRNA, thus inhibiting *ompF* translation, and destabilizing the mRNA (Schmidt *et al.*, 1995; Andersen & Delihis, 1990). Transcription of *micF* is activated by OmpR at the same regulatory sites as those involved in *ompC* transcription (Coyer *et al.*, 1990). In *E. coli*, *micF* transcription has been shown to decrease under conditions of low osmolarity (Takayanagi *et al.*, 1991), lower temperature (Forst & Inouye, 1988; Delihis & Forst, 2001), and decreased weak acid concentrations (Pratt *et al.*, 1996).

Based on the inherent similarities between *S. marcescens* and *E. coli* OmpF and OmpC porins, we decided to investigate the expression of *S. marcescens ompF* and *ompC* genes in the presence of different environmental factors, including osmotic pressure, temperature, pH and salicylate. In addition, we addressed the possibility that the MicF regulatory system might play a role in controlling the expression of *S. marcescens ompC* and *ompF* genes.

METHODS

Bacterial strains, plasmids and growth conditions. Plasmids used are described in Table 1. pOYL338W, pOY012 and pOY009 plasmid electroporations were performed using *E. coli* NM522 competent cells at 1·4 V, whereas pOY9micF, pOY9ompF and pOY9ompC plasmid electroporations were performed using *S. marcescens* UOC-67 competent cells at 1·5 V. M9 minimal medium containing M9 salts, 0·1 mM calcium chloride, 0·002 M magnesium sulfate, 20 µg vitamin B₁ ml⁻¹, 0·4 % (w/v) glucose, and 100 µg ampicillin ml⁻¹ was used for all strains. M9 salts, vitamin B₁, ampicillin, salicylate, glucose, sucrose and ONPG were all obtained from Sigma-Aldrich.

***lacZ* fusions and β-galactosidase assays.** DNA preparation and cloning were performed as described by Sambrook *et al.* (1989). Fig. 1 shows the promoter region sequences of *ompC* and *ompF* genes in both *S. marcescens* and *E. coli*. The promoter region of the *S. marcescens ompF* gene (GenBank accession no. U81967) was cloned as a 0·5 kb *Xba*I, genomic DNA, PCR product into the broad-host-range *lacZ* transcriptional fusion vector pOY009, which harbours the promoterless *E. coli lacZ* gene (Ozawa *et al.*, 1987). The promoter region of the *S. marcescens ompC* gene (GenBank accession no. L24960) was cloned as a 0·25 kb *Xba*I, genomic DNA, PCR product upstream of the promoterless *lacZ* gene of pOY009, and the *micF* gene of *S. marcescens* was cloned as a 0·45 kb *Xba*I, genomic DNA, PCR product. The orientation of all cloned promoters was confirmed by sequencing and restriction digestion, followed by agarose gel electrophoresis. The induction of the *ompC*, *ompF* and *micF* genes under varying environmental conditions was monitored by assaying for β-galactosidase, according to the method of Miller (1972). *E. coli* NM522, containing pOYL338W, pOY009 and pOY012 *lacZ* fusion plasmids, and *S. marcescens* UOC-67, containing pOY9micF, pOY9ompF and pOY9ompC *lacZ* fusion plasmids, were grown overnight in 3 ml M9 medium containing 0·4 % (w/v)

Table 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or reference
<i>S. marcescens</i> UOC-67	Type strain	ATCC 13880
<i>E. coli</i> NM522	<i>rec+</i> [<i>supE thi Δ(lac-proAB) hsd5</i> (F' <i>proAB lacI^r lacZ ΔM15</i>)]	Promega
pOY009	Ap ^r , promoter-deleted <i>lacZ-lacY</i> operon, copy number is six per genome, vector domain of the plasmid derived from pSC101	Ozawa <i>et al.</i> (1987)
pOYL338W	Ap ^r , <i>E. coli ompC</i> promoter- <i>lacZ</i> hybrid	Ozawa <i>et al.</i> (1987)
pOY012	Ap ^r , <i>E. coli ompF</i> promoter- <i>lacZ</i> hybrid	Ozawa <i>et al.</i> (1987)
pOY9ompF	Ap ^r , <i>S. marcescens ompF</i> promoter- <i>lacZ</i> hybrid	This study
pOY9ompC	Ap ^r , <i>S. marcescens ompC</i> promoter- <i>lacZ</i> hybrid	This study
pOY9micF	Ap ^r , <i>S. marcescens micF</i> promoter- <i>lacZ</i> hybrid	This study

Fig. 1. Nucleotide sequences of the *S. marcescens* (upper sequence) and *E. coli* (lower sequence) *ompC* (a) and *ompF* (b) porin genes. (a) and (b) Promoters (–10 and –35) are in bold and underlined; possible OmpR-binding sites are underlined, with the OBSs involved in repression of *E. coli ompF* transcription also underlined in (b) (this recognition sequence is absent in *S. marcescens ompF*); the integration host factor binding region is in bold (absent in *S. marcescens ompF*); deletions are represented by a double dashed line.

glucose, supplemented with 100 µg ampicillin ml⁻¹, at 37 °C with aeration. Overnight cultures were diluted into fresh M9 medium plus different appropriate experimental additives (sucrose and/or salicylate), and grown to mid-exponential phase, before growth was monitored by measuring OD₆₀₀. Environmental conditions studied included salicylate concentration (1, 5 and 8 mM), osmotic pressure (5, 8 and 10 % sucrose), pH (pH 6, 7 and 8), and temperature (28, 37 and 42 °C). We decided to experiment at these particular temperatures because *S. marcescens* strains grow at a minimum temperature of 27 °C, and a maximum temperature of 44 °C (Williams *et al.*, 1971); similar temperature parameters have been used with other enteric bacteria (Dedieu *et al.*, 2002). External pH was manipulated by adjusting the pH of M9 medium containing 0.4 % (w/v) glucose with HCl

(pH 6) or 1 M NaOH (pH 8), prior to monitoring the cell growth at OD₆₀₀. Assays were conducted for each of the single conditions, followed by combining two of the conditions, and then combinations of three conditions. When OD₆₀₀ reached 0.50, 0.1 ml of each culture was diluted to 1.0 ml with Z buffer (0.06 M Na₂HPO₄·7H₂O, 0.04 M NaH₂PO₄·H₂O, 0.01 M KCl, 0.001 M MgSO₄·7H₂O, 0.05 M β-mercaptoethanol, pH 7.0). Bacterial membranes were disrupted by the addition of one drop of toluene to release β-galactosidase into the medium. The β-galactosidase reaction was initiated by adding 0.2 ml ONPG (4 mg ml⁻¹), and terminated by adding 0.5 ml 1 M Na₂CO₃. Enzyme activity was measured by determining absorbance of the *o*-nitrophenol product (420 nm). Units of β-galactosidase activity were calculated according to Miller (1972).

RESULTS

Influence of osmotic pressure on *ompF*, *ompC* and *micF* expression

Minimal concentrations of sucrose (5 %, w/v) in the medium were found to affect the expression of the *S. marcescens* porin genes by reducing the expression of *ompF* (pOY9ompF) by 10 %, and increasing the expression of *ompC* (pOY9ompC) by 28 %, as compared with expression in the absence of sucrose (Fig. 2a). The highest concentration of sucrose (10 %, w/v) led to a 34 % decrease in expression of the *ompF*, and a 50 % increase in *ompC* expression. These findings are consistent with the results of an earlier study on osmoregulation of *S. marcescens* porins (Hutsul, 1996), and the same pattern holds true for *E. coli ompF* (pOY012) and *ompC* (pOYL338W). Considering that bacteria are faced with more than one type of a cellular stressor at a time, the maximum concentration of sucrose was tested in combination with low (28 °C) and high (42 °C) growth temperatures (Table 2). Temperature was found to have a predominant effect over sucrose. When the highest concentration of sucrose (10 %, w/v) was tested in conjunction with pH, it was found that the effect of pH was predominant. In combinations of sucrose, salicylate and temperature, and sucrose, pH and temperature, the temperature effect predominated (Table 2).

With respect to *micF* transcription, *micF* expression levels remained constant at all sucrose concentrations tested (Fig. 3a); this is in sharp contrast with the porin studies in *E. coli*, where the MicF system plays a major role in the negative osmoregulation of *ompF* in cells grown in media of low-to-intermediate osmolarity (Ramani & Boakye, 2001).

Influence of salicylate on *ompF*, *ompC* and *micF* expression

The highest concentration of salicylate tested (8 mM) resulted in a 40 % decrease in expression of *S. marcescens ompF* (pOY9ompF), and approximately a 50 % increase in *S. marcescens ompC* (pOY9ompC) expression (Fig. 2b). At 5 mM salicylate, the expression of *ompC* increased by approximately 34 %, and the *ompF* expression was reduced by 25 %. There was a 45 % decrease in the expression of *E. coli ompF* (pOY012). The addition of 1 mM salicylate, which

Fig. 2. Effect of (a) osmotic pressure, (b) salicylate, (c) temperature and (d) pH on the expression of *lacZ* under the influence of *S. marcescens* *ompF* (pOY9ompF) and *ompC* (pOY9ompC) porin gene promoters. *E. coli* *ompF* (pOY012) and *ompC* (pOYL338W) porin gene promoter-*lacZ* fusions were used as controls. All assays were performed in triplicate. The controls in (a) and (b) did not contain sucrose and salicylate, respectively.

is the therapeutic concentration most often used (Berlanga & Vinas, 2000), resulted in a 10 % decrease in *S. marcescens* *ompF* expression, and a 20 % increase in *ompC* expression. When salicylate + temperature combinations were examined, temperature was the predominant effect (Table 2). When the highest concentration of salicylate (8 mM) was tested in conjunction with pH, pH was the predominant effect (medium pH was adjusted after the addition of salicylate). Temperature was the prevalent condition when

salicylate, temperature and pH combinations were tested, and this was also the case with salicylate, temperature and sucrose (Table 2).

When the effect of salicylate on the *S. marcescens* *micF* expression was examined, 1 mM increased the transcription of the *micF* gene by 10 %, whereas 8 mM salicylate induced the gene by 30 % (Fig. 3b).

Influence of temperature on *ompF*, *ompC* and *micF* expression

When compared with expression of *S. marcescens* *ompF* (pOY9ompF) at 28 °C, there was approximately a 55 % decrease in expression at 42 °C. *S. marcescens* *ompC* (pOY9ompC) expression was increased by more than 75 % at 42 °C, as compared with 28 °C (Fig. 2c). In the case of *E. coli*, *ompF* expression at 42 °C was reduced by 50 % (pOY012), and the expression of *ompC* increased by 80 % at 42 °C (pOYL338W). In all two- and three-condition assays, the effect of temperature was predominant (Table 2). With respect to *micF*, transcription increased by approximately 64 % at 42 °C, as compared with 28 °C (Fig. 3c).

Influence of pH on *ompF*, *ompC* and *micF* expression

There was a 30 % increase in the *S. marcescens* *ompC* (pOY9ompC) expression in response to acidification of the medium (pH 6), while expression decreased by more than 55 % at pH 8 (Fig. 2d). This finding is consistent with an *E. coli* study where a 30 % increase in *ompC* expression was observed at lower pH (Thomas & Booth, 1992), and this was confirmed in the present study using pOYL338W. The opposite trend occurred for *S. marcescens* *ompF* (pOY9ompF), where at pH 8, the expression from the *ompF* promoter was approximately twice as much as that at pH 6. *S. marcescens* *micF* expression was similar to *ompC* expression, such that as pH increased, the production of *micF* decreased (Fig. 3d).

DISCUSSION

In most Gram-negative bacteria, the OmpR-EnvZ regulatory system controls osmoregulation of the porin genes at the transcriptional level (Nikaido, 2003; Cai & Inouye, 2002; Matsubara *et al.*, 2000; Pratt *et al.*, 1996). Our previous investigation revealed that the upstream nucleotide sequence of the cloned *S. marcescens* *ompF* gene closely resembles that of *E. coli* *ompF* (Hutsul, 1996). However, the OBS and IHF recognition sequences, which are important for repression of *ompF* transcription by the OmpR-EnvZ regulatory system, are absent in *S. marcescens* (Fig. 1). This suggests that osmoregulation via this system, as it is described in *E. coli*, probably cannot take place in *S. marcescens*.

In addition, we found no change in the transcription of the *S. marcescens* *micF* gene in the presence of 5, 8 or 10 % sucrose. This finding is in sharp contrast to an *E. coli* study

Table 2. Induction of *S. marcescens* pOY9ompF and pOY9ompC expression in the presence of single environmental stimuli, and combinations of two and three stimuli

Values are means ($\pm$ SD), $n=3$ for each condition. β -Galactosidase activity was calculated according to Miller (1972).

Condition	β -Galactosidase activity (Miller units)	
	<i>ompF</i>	<i>ompC</i>
10 % sucrose	2765 $\pm$ 102	1765 $\pm$ 88
28 °C	5107 $\pm$ 54	345 $\pm$ 23
42 °C	2311 $\pm$ 51	1432 $\pm$ 36
pH 6	2976 $\pm$ 44	1244 $\pm$ 31
pH 8	5013 $\pm$ 68	351 $\pm$ 13
1 mM salicylate	3765 $\pm$ 110	1099 $\pm$ 92
8 mM salicylate	2454 $\pm$ 54	1674 $\pm$ 26
28 °C + 10 % sucrose	4987 $\pm$ 95	389 $\pm$ 12
pH 8 + 10 % sucrose	4888 $\pm$ 106	405 $\pm$ 19
pH 6 + 10 % sucrose	1355 $\pm$ 29	2234 $\pm$ 28
28 °C + 8 mM salicylate	4851 $\pm$ 67	476 $\pm$ 28
42 °C + 1 mM salicylate	2296 $\pm$ 39	1489 $\pm$ 78
8 mM salicylate + pH 8	4765 $\pm$ 103	406 $\pm$ 13
10 % sucrose + 8 mM salicylate + 28 °C	4561 $\pm$ 105	410 $\pm$ 31
10 % sucrose + pH 6 + 28 °C	4107 $\pm$ 75	476 $\pm$ 16
28 °C + 8 mM salicylate + pH 6	4233 $\pm$ 114	497 $\pm$ 28

that reported a fivefold increase in the level of *micF* RNA under high osmolarity (Delilhas, 1995). Research on the involvement of *micF* in the osmoregulation of porin genes has yielded contradicting results. One study reported that *micF* plays a major role in the negative osmoregulation of porin genes in cells grown in media of low-to-intermediate osmolarity (Ramani *et al.*, 1994); however, those experiments were performed at a temperature that stimulates *micF* transcription by tenfold, and, as such, the effects observed may not be directly related to osmoregulation. Another *E. coli* study concluded that the *micF* gene does not play a critical role in osmoregulation, after the authors witnessed a normal level of *ompF* regulation in a *micF* mutant strain (Matsuyama & Mizushima, 1985).

We propose that another regulatory system must be involved in the osmoregulation of *S. marcescens* *ompF* and *ompC* porin genes. As demonstrated in this study, *S. marcescens* regulates the synthesis of the OmpF and OmpC proteins with very strict control under stressful circumstances that greatly mimic their natural environment. Two hypotheses may be able to explain how this regulation is achieved. The first is that a change in the nucleotide sequence in the Pribnow box region occurs, which results in complete osmoregulation (Aiba *et al.*, 1987). *E. coli* *ompF* promoters with four different bases at the first position of the Pribnow box

Fig. 3. Induction of *S. marcescens* *micF*:*lacZ* transcription by (a) osmotic pressure, (b) salicylate, (c) temperature and (d) pH. All β -galactosidase assays were performed in triplicate. The controls in (a) and (b) did not contain sucrose and salicylate, respectively.

were compared for their activity, and it was found that an A to T substitution of the first base resulted in a sharp reduction in *ompF* expression (Ozawa *et al.*, 1987). The second hypothesis is that higher osmotic pressure increases the degree of negative DNA supercoiling, which could then act to promote *ompC* gene expression, since changes in DNA supercoiling are synergistic with the changes in OmpR-P in eliciting *ompC* expression (Graeme-Cook *et al.*, 1989; Hulton *et al.*, 1990). However, the exact mechanism by which osmotic stress can influence the degree of negative supercoiling has not been determined. It is likely that changes in the cytoplasmic concentrations of sucrose molecules are the intermediary effectors (Thomas & Booth, 1992).

Salicylate, being a weak acid, triggers an increased transcription of *E. coli* *ompC* and *micF*, while it leads to a concomitant decrease in OmpF production (Pratt *et al.*, 1996). Addition of higher concentrations of salicylate would lead to an increase in the threshold levels of 4-5S *micF* RNA available for binding to *ompF* RNA (Andersen & Delilhas,

1990), thereby causing a reduction in the expression of *ompF*. Similarly, in this study, we found that all of the concentrations of salicylate reduced *S. marcescens ompF* expression, and increased the expression of *S. marcescens ompC* (Fig. 2b). An increase in *S. marcescens micF* expression was also observed in cells grown with 1, 5 and 8 mM salicylate. Therefore, we find that, unlike osmolarity, salicylate strongly blocks the transcription of *ompF* by increasing the levels of *micF*. It is not surprising that *ompC* and *micF* are similarly regulated by salicylate in *S. marcescens*, since common OmpR-binding sequences are present in both transcriptional control regions (Hutsul, 1996).

Elevated temperature is another stimulus that increased the levels of *S. marcescens micF* and *ompC* mRNA, and decreased the levels of *ompF*. Unlike the findings for changes in osmolarity, we propose that most of the effect on *ompF* repression with temperature increase is due to *micF*. Specifically, MicF would be involved in the post-transcriptional negative regulation of *ompF*, similar to that in *E. coli*. In *E. coli*, the *micF* RNA forms a stable duplex with the RBS of *ompF* mRNA, thereby inhibiting *ompF* translation, and destabilizing the mRNA. OmpF porin production presumably decreases with temperature increase, and, considering that OmpF has wider pores, this would protect *S. marcescens* from bile salts and other toxic compounds in the intestinal tract.

S. marcescens ompC and *micF* transcription are both induced by growth of cells in acidic pH conditions. Transcription of *micF* increased by 63 % at pH 6, as compared with pH 8 (Fig. 3d). In *E. coli*, this environmental factor represses OmpF production through the EnvZ–OmpR system (Nikaido, 2003). In *S. marcescens*, the OBS recognition sequences are absent, and thus we propose that the effect of OmpF repression in acidic environments is due to *micF*. Having an increased synthesis of the narrower-channelled OmpC porins in an acidic environment may contribute to the retention of buffer molecules, such as γ -aminobutyrate, in the periplasm (Nikaido, 2003).

Our initial assumption was that there would be a synergy between different conditions; however, this was not necessarily the case. When cells were grown in the presence of a combination of conditions, such as 28 °C + 10 % sucrose, 28 °C + 8 mM salicylate, 28 °C + 10 % sucrose + 8 mM salicylate, 28 °C + pH 6 + 10 % sucrose, and 28 °C + 8 mM salicylate + pH 6, the effect of growth temperature overpowered the effects of high osmolarity, presence of salicylate and acid pH (Table 2). In these conditions, because the lower temperature of 28 °C is a favourable condition for the expression of *ompF* porin, the effect of more detrimental conditions, such as the maximum concentrations of salicylate and sucrose, and an acidic pH, seemed to have little influence. A favourable temperature of 28 °C, and presence of stressors, such as salicylate and sucrose, were compensated for by the reduction in the normal pore size of OmpF. In an *E. coli* study by Zhang & Ferenci (1999), when lactose was used as the sole carbon source in a minimal medium,

strains emerged with mutations that altered the residues in the constriction region, or that resulted in a short deletion in the channel-constricting loop L3 of OmpF. We suggest that mutations may have occurred in the PEFGG motif within loop L3, changing it to PEFDG, which would reduce the calculated 1.1 nm OmpF pore size. As such, these strains would produce OmpF porins with a much narrower channel, and demonstrate reduced sucrose or salicylate uptake. *S. marcescens ompC* has been sequenced (Hutsul, 1996), and it was found that the PEFGG motif conserved within enterobacterial porins is not conserved in the *S. marcescens* OmpC porin, where it is replaced with the sequence PEFDG. Through liposome-swelling assays (Nikaido *et al.*, 1991), we have determined that the aspartic acid residue (D) at position 112 reduces diffusion of sucrose molecules through loop L3 (Hutsul, 1996). We are currently undertaking liposome-swelling assays using site-specifically altered OmpC to determine if the D residue at position 112 of OmpC also reduces diffusion of salicylate molecules through loop L3. We suggest the possibility exists that such strains would have a reduced OmpC pore channel (<1.0 nm), such that the positive effects of sucrose and salicylate are negligible, and there is an overall decrease in the expression of *ompC* at 28 °C. At 42 °C, mutations might have occurred in the PEFDG motif within OmpC loop L3, changing it to the PEFGG consensus sequence. Hence, these strains would produce OmpC porins with a wider channel, and would demonstrate increased sucrose and salicylate uptake at 42 °C, and an overall increase in the expression of *ompC* at 42 °C. In assays where a combination of parameters was examined, with the exception of temperature, basic pH was the predominating factor for the expression of *ompF*, even in the presence of more detrimental conditions, such as 10 % sucrose and 8 mM salicylate. Again, we propose that possibly there has been a reduction in the pore size of OmpF in order to 'keep out' sucrose and salicylate, in the same fashion as explained by Zhang & Ferenci (1999).

In summary, we report that *S. marcescens* outer membrane porins exhibit regulation in response to osmotic pressure, salicylate, temperature and pH variability. The *micF* system is one of the main regulatory mechanisms responsible for controlling the expression of OmpF and OmpC porins under all the environmental conditions tested here, with the exception of osmotic shock. In addition, from our multi-condition analyses, we conclude that temperature is the predominant effector, followed by pH. We propose that this finding is due to a reduction and an increase in the pore sizes of OmpF and OmpC porins, respectively.

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