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Full Length Research Paper

The role of RpoS, H-NS and AcP on the pH-dependent OmpC and OmpF porin expressions of *Escherichia coli* at different pH

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It is generally accepted that OmpC and OmpF porin proteins have important roles to play in the survival of *Escherichia coli* under different environmental conditions. However, the influence of different environmental conditions on porin expression is not as well known. This work was carried out to find out the effect of *envZ*, *ompR*, *rpoS*, *pta* and *hns* mutations on the pH-dependent *ompC* and *ompF* expression in *E. coli* in minimal medium at different pH. The expression of *ompF* was higher in cells grown in an alkaline pH, and the expression of *ompC* increased at the acidic pH value. There was low-level expression of *ompC* and *ompF* in *envZ* mutants. The expression of *ompC* was increased in the presence of NaCl at three tested pH values while that was reduced in the case of OmpF. The level of expression of *ompF* in the strain with the *rpoS* mutation was greatly increased at all pH values and in the presence and absence of NaCl. The expression of *ompC* in the *pta* mutant was greatly increased compared to the wild type *E. coli* at all pH values in the presence and absence of NaCl. There was no expression of *ompC* and *ompF* in the *ompR* mutants. Mutations in *hns* and *pta* had variable effects on the expression of *ompC* and *ompF* in the presence and absence of NaCl. Overall, this work shows that although OmpC and OmpF porin protein synthesis was affected according to pH, it was not a direct role of RpoS, HNS, EnvZ and Acp at pH-depending porin expression.

Key words: *Escherichia coli,* Porin, EnvZ, OmpR, RpoS, AcP, H-NS.

INTRODUCTION

Porin proteins, 35 to 36 kDa low molecular weight, water-filled channels, control the permeability of <600 kDa hydrophilic polar solutes across the outer membrane of Gram-negative bacteria (Achouak et al., 2001). In the aquatic environment, bacteria are subjected to environmental conditions, such as changes in pH, osmolarity, temperature, and starvation which lead to stress of the organisms (Grant and Long, 1981). Under these conditions, bacteria have adapted a range of different strategies for survival. Alterations in membrane permeability are generally caused by changes in the composition of the outer membrane porin proteins (Overbeek and Lugtenberg, 1980). The synthesis of porins is regulated under environmental conditions (Pratt et al., 1996). Under normal conditions, the total amount of OmpF and OmpC porin proteins remains unchanged but their synthesis is altered by a variety of factors, such as osmolarity and starvation (Özkanca and Flint, 2002). Also Darcan et al. (2003) and Muela et al. (2008) showed the rearrangement of outer membrane proteins in *Escherichia coli* entering the Viable But Non Culturable (VBNC) state.

The mechanisms of OmpC and OmpF porin regulation are particularly complex. Many factors are involved in the transcriptional and translational control of porin synthesis.

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Table 1. *Escherichia coli* strains used in this study.

Strain	Relevant Genotype	Source
MH225	MC4100 U(*ompC*-lacZ$^+$)10-25 (Wild type)	(Liu and Ferenci, 2001)
MH513	MC4100 *araD*+U(*ompF*-lacZ$^+$)16-13 (Wild type)	(Liu and Ferenci, 2001)
BW3343	MH513 *envZ60*::Tn*10*	(Liu and Ferenci, 2001)
BW3345	MH225 *envZ60*::Tn*10*	(Liu and Ferenci, 2001)
BW3303	MH513 *ompR*::Tn*10*	(Liu and Ferenci, 2001)
BW3304	MH225 *ompR*::Tn*10*	(Liu and Ferenci, 2001)
BW3301	MH513 *rpoS*::Tn*10*	(Liu and Ferenci, 2001)
BW3302	MH225 *rpoS*::Tn*10*	(Liu and Ferenci, 2001)
BW3305	MH513 *hns*: :*neo*	(Liu and Ferenci, 2001)
BW3306	MH225 *hns*: :*neo*	(Liu and Ferenci, 2001)
BW3601	MH513 *pta*: :*kan*	(Liu and Ferenci, 2001)
BW3602	MH225 *pta*: :*kan*	(Liu and Ferenci, 2001)

The best understood system of porin regulation control involves a two component phosphorelay system through the EnvZ/OmpR proteins (Hoch 2000; Yoshida et al., 2006). A high level of OmpR-P stimulates the expression of *ompC* and represses the expression of *ompF*, whereas a low level of OmpR-P induces *ompF* transcription. The amount of OmpR-P in a cell is reduced at low osmolarity, and increased at high osmolarity under the regulation of the EnvZ protein. However, porin regulation is not totally dependent on EnvZ (Forst et al., 1988), several alternative histidine kinase donors (Matsubara and Mizuno 1999) and some small molecules including acetyl phosphate (AcP), phosphoramidate and carbamyl phosphate can phosphorylate OmpR (Heyde et al., 2000; McCleary et al., 1993). As well as EnvZ and OmpR, many global regulators, such as the alternative sigma factor (RpoS) (Pratt et al., 1996), integration host factor (IHF) (Goosen and Van de Putte, 1995), histone-like DNA binding proteins (H-NS) (Suzuki et al., 1996) and AcP (Heyde et al., 2000) can regulate porin expression at the level of transcription.

It has been reported in the literature that the amounts of OmpC and OmpF in the outer membrane is affected by the pH of the environment (Heyde and Portalier, 1987). It has been suggested that pH-dependent porin regulation is under the control of EnvZ (Heyde and Portalier, 1987). But Sato et al. (2000) reported that the expression of OmpC at acidic pH is independent of EnvZ, but the mechanism of control in this case has so far not been elucidated. In addition, AcP has a role in the regulation of OmpC and OmpF in *envZ* mutant strain of *E. coli* (Heyde et al., 2000). These researchers also reported that AcP had an indirect and direct function to phosphorylate OmpR and also reported that the expression of porins in *E. coli* was dependent on the available carbon source (Heyde et al., 2000).

There are a lot of molecules working with EnvZ/OmpR two-component regulatory system so as to response environmental stimuli and control OmpC and OmpF porin expression. Recently there have been new factors investigated (Chen et al., 2004; Keller et al., 2006). Therefore it has not been known which factor affect OmpC and OmpF synthesis under different stress conditions. Although alteration in the expressions of OmpC and OmpF at different pH has been reported by several researchers, especially on acidic pH, the regulation and main factors involved have not been determined (Heyde et al., 1988; Thomas and Booth, 1992; Sato et al., 2000).

In this study, we tried to investigate the roles of HN-S, AcP and RpoS molecules on OmpC and OmpF porin expression depending on pH changes. *ompF* and *ompC* transcription was followed under acidic (pH 5.3), neutral (pH 7.1) and alkaline (pH 8.3) conditions in the presence and absence of NaCl as an osmoregulator. The effects of mutations in *envZ, ompR, hns, rpoS* and *pta* on the expression of porin proteins were also investigated. In all cases expression of the relevant porin was followed using a *lacZ* reporter system.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bacterial strains used in this study are listed in Table 1. Mutant starins were tested according to antibiotic resistance properties. A minimal medium was used for growth of these bacterial strains (Sato et al., 2000). This medium contained K_2HPO_4 (5 mM), NH_4Cl (20 mM),) $MgCl_2$ (1 mM), $CaCl_2$ (0.1 mM), $FeSO_4.7H_2O$ (0.1 mM), glucose (1% w/v) and thiamine (20 µg/ml) (Sigma). 50 mM N-Tris (hydroxymethyl)-methylglycine (Tricine-Serva) was added for media of pH 8.5, 50 mM 3-(N-morpholino) propane sulphonic acid (MOPS, Sigma) for media of pH 7.2 and 50 mM 2-(N-Morpholino) ethanesulfonic acid monohydrate (MES, Serva) was added for media of pH 5.5. For osmolarity experiments, NaCl was added to a final concentration of 0.2 mM. Thiamine was filter sterilised and added separately to the medium. Glucose and stock cultures of $MgCl_2$ and $FeSO_4.7H_2O$ were heat sterilised separately and added to the medium after cooling. The pH of the medium was adjusted by the addition of KOH.

Bacteria were grown in 5 ml nutrient broth (Merck) at 37°C for 24 h. Then 100 µl culture was inoculated into 50 ml nutrient broth and

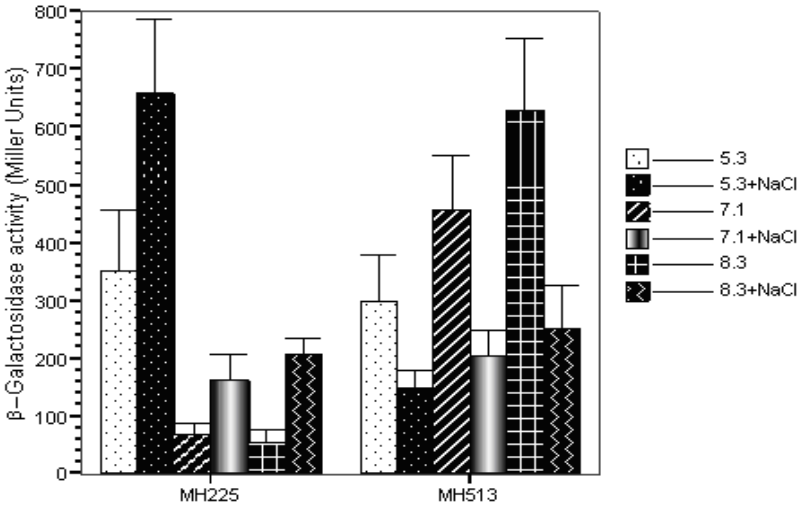

Figure 1. The expressions of ompC and ompF at different pH values. MH225 (*ompC*-lacZ*)* and MH513 (*ompF-lacZ*) were grown in minimal medium at different pH values in the presence and absence of NaCl. The data are mean values ± standard deviations from four independent experiments.

left in a shaker for 18 h at 37°C. The culture (10 ml) as harvested by centrifugation at 3440 x g for 10 min then cells washed twice with distilled water (10 ml) and pellet resuspended in 10 ml water in the end. Subsequently 0.1 ml of culture was transferred to the minimal medium (100 ml) which adjusted to different pH values and were grown at 37°C and 160 rpm. Growth was monitored by measuring the absorbance change at 600 nm. Because the pH of the growth medium decreased rapidly when glucose was used as an energy source, cells were harvested for estimation of enzyme activity when the absorbance reached between 0.3 and 0.4.

β-Galactosidase activity assays

β-Galactosidase activity was measured by the method of Miller (1992) using one drop of toluene and 100 µl of cells diluted with 900 µl Z buffer. The data are presented in the form of units of β-galactosidase per OD_{420} unit as defined by Miller (1992) and expressed as Miller Units (Miller, 1992). Each bar represents the mean ± standard deviation of four independent experiments. In order to measure the expression more quantitatively, a fusion gene with lacZ was used. Samples were taken from the bacterial cultures when the absorbance at OD_{600} had reached 0.3 to 0.4 during the exponential growth phase.

RESULTS

The expression of *ompC* and *ompF* at different pH

The results suggest that the expressions of the porins, OmpC and OmpF, were changed by exposure to different growth pH. It is clear that the expression of *ompC* increased at acidic pH (353 ± 52 Miller units) compared to neutral (67 ± 11 units) and alkaline pH (54 ± 11 units) as shown for *E. coli* strain MH225 (Figure 1). The expression of *ompF* was lower at acidic pH (300 ± 40 units), than at neutral (458 ± 46 units) or alkaline pH (629 ± 60 units) as shown for strain MH513 (Figure 1). As shown in Figure 1, the addition of NaCl to the growth medium led to a significant increase in the expression of *ompC* at all pH values (206 ± 15 units at alkaline pH, 163 ± 22 units at neutral pH and 658 ± 64 units at acidic pH) but the expression of *ompF* was at least halved at all pH values (250 ± 38 units at alkaline pH, 206 ± 22 units at neutral pH and 149 ± 15 units at acidic pH). There was still a higher expression of *ompF* at alkaline pH compared to acidic pH in the presence or absence of NaCl in the growth medium (Figure 1).

The effects of *ompR* and *envZ* on the expression of *ompC* and *ompF*

E. coli strains carrying mutations in the *envZ* and *ompR* genes were used to determine the effects of absence of these genes on the expressions of OmpC and OmpF at different pH. There was no LacZ activity in *E. coli* BW3303 (*ompF*-lacZ *ompR*::Tn*10*) and *E. coli* BW3304 (*ompC-lacZ ompR*::Tn*10*) at any pH tested (Figure 2). In an *envZ*⁻, *E. coli* strain (BW3343, *ompF-lacZ envZ*::Tn*10*), the expression of *ompF* at each pH was reduced from 300 ± 40 units to 149 ± 10 units at the acidic pH and from 629 ± 60 units to 75 ± 14 units at the alkaline pH. The pattern of expression was changed compared to the wild-type (*E. coli* MH513) with the highest activity being at the acidic pH than at the alkaline pH in the wild-type (Figure 2). These results suggest that OmpF expression showed more dependency on EnvZ at alkaline pH. OmpF also be expressed by an *envZ* independent system. The expression of OmpC was reduced at the acidic pH from 353 ± 52 units for the wild-type *E. coli* MH225 to 189 ± 32 units in the mutant *E. coli*

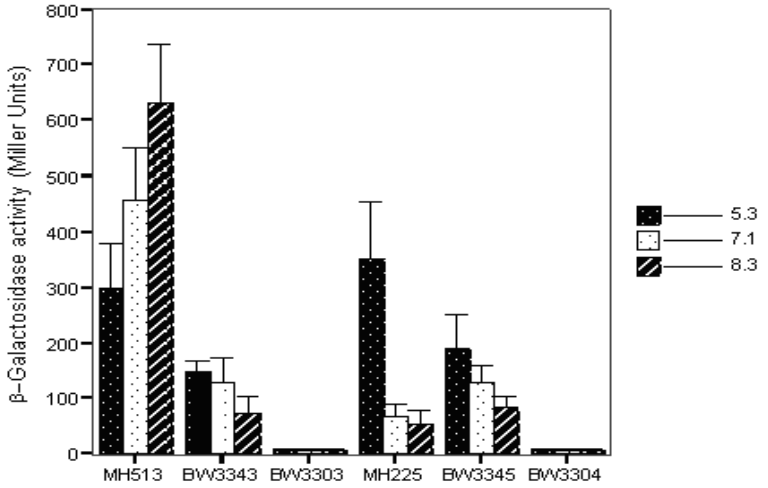

Figure 2. The expressions of OmpC and OmpF porin in *E. coli* MH513 (*ompF*-lacZ parent strain), BW3343 (*ompF-lacZ envZ60::Tn10*), BW3303 (*ompF-lacZ ompR::Tn10*), MH225 (*ompC*-lacZ parent strain), BW3345 (*ompC-lacZ envZ60::Tn10*) and BW3304 (*ompC-lacZ ompR::Tn10*) at different pH values.

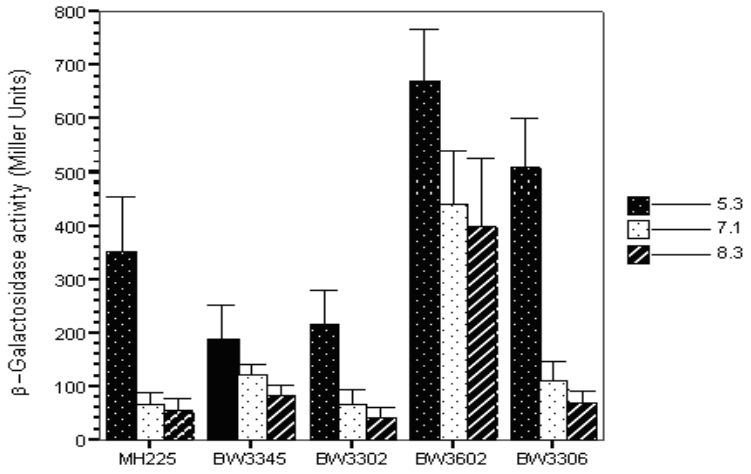

Figure 3. The expression of OmpC in *E. coli* MH225 (*ompC*-lacZ parent strain), BW3345 (*envZ60::Tn10*), BW3302 (*rpoS::Tn10*), BW3602 (*pta::kan*) and BW3306 (*hns::neo*) at different pH values in the absence of NaCl.

strain (BW3345, *ompC-lacZ envZ*::Tn*10*) whereas it increased slightly at both the neutral and alkaline pH from 67 ± 11 units to 121 ± 10 units and 54 ± 11 units to 84 ± 10 units, respectively (Figure 2). The expression of *ompC* in the *envZ E. coli* mutants (BW3345) was reduced in medium with added NaCl at all pH values (Figure 3, 4, 5 and 6). But the expression of OmpF in *envZ* mutants (BW3343) neutral and alkaline pH was increased slightly with the addition of NaCl.

The roles of RpoS, H-NS, AcP in the expression of *ompC and ompF*

As shown in Figure 3, the *rpoS* mutant strain (BW3302, *ompC-lacZ rpoS*::Tn*10*) had lower expression of *ompC* at the acidic pH with 216 ± 33 units than the wild-type strain (MH225) with 353 ± 52 units. However, the level of *ompC* expression at the other pH values was almost the same as that of the wild type strain with around 67 ± 13 units at neutral pH and 42 ± 9 units at alkaline pH. When NaCl was added to the minimal medium at all pH values, the expressions of *ompC* at *rpoS* mutant strain were seen to increase and as same as wild type (MH225) all pH values (Figure 4). As shown in Figure 5, the *rpoS* mutant strain (BW3301, *ompF-lacZ rpoS*::Tn*10*) had a slightly higher expression of *ompF* at all pH values with 446 ± 23 units at the acidic pH, 742 ± 124 units at the neutral pH, and 846 ± 57 units at the alkaline pH compared to 300 ± 40,

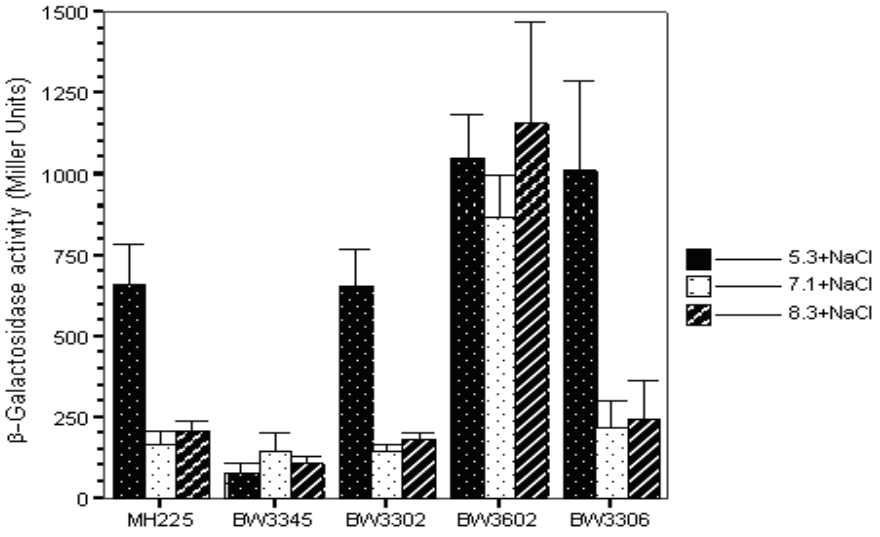

Figure 4. The expression of OmpC in *E. coli* MH225 (*ompC*-lacZ parent strain), BW3345 (*envZ60::Tn10*), BW3302 (*rpoS::Tn10*), BW3602 (*pta::kan*) and BW3306 (*hns::neo*) at different pH values in the presence of NaCl.

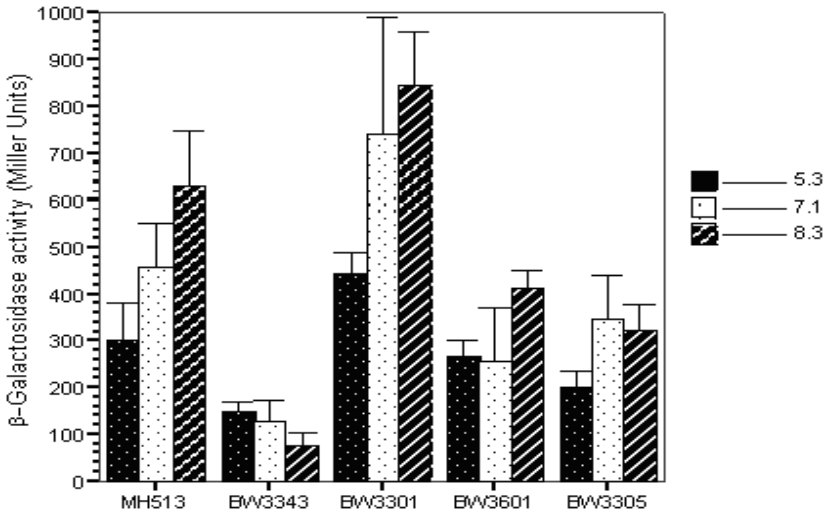

Figure 5. The expression of OmpF in *E. coli* MH513 (*ompF*-lacZ parent strain), BW3343 (*envZ60::Tn10*), BW3301 (*rpoS::Tn10*), BW3601 (*pta::kan*) and BW3305 (*hns::neo*) at different pH values in the absence of NaCl.

458 ± 46 and 629 ± 60 units respectively for the wild-type strain (MH513). When NaCl was added at all pH values, the expression of *ompF* was seen to decrease and as same as wild type (MH513) at all pH values (Figure 6). But there was still higher expression of *ompF* at *rpoS* mutation than wild type at all pH values.

In comparison with the wild-type strain (MH225), the expression of *ompC* was higher at all pH values in the *pta* mutant (BW3602, *ompC-lacZ pta*::*kan*) which lacks AcP (Figure 3). The expression of *ompC* was almost 7.5 times higher at the alkaline pH at 401 ± 62 units in BW3602 compared to the MH225 wild type strain with 54 ± 11 units. At the neutral pH, the difference was 67 ± 11 units in the wild-type and 442 ± 48 in the *pta* mutant, at the acid pH, while OmpC expression is 353±50 in wild type and 672± 85 in *pta* mutation (Figure 3). When NaCl was added, it seemed that OmpC was higher synthesis according to wild type at all the pH values (Figure 4). In comparison with the wild-type strain (MH513), the expression of *ompF* was slightly reduced at all pH values in the *pta* mutant (BW3601, *ompF-lacZ pta*::*kan*) (Figure 5). The expression of *ompF* was 268 ± 17 units compar-

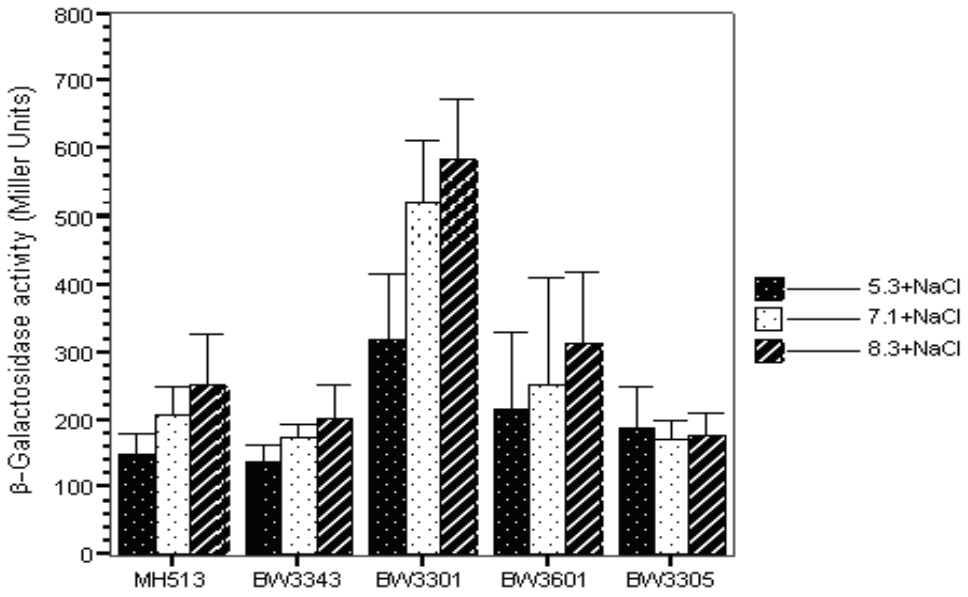

Figure 6. The expression of OmpF in *E. coli* MH513 (*ompF-lacZ* parent strain), BW3343 (*envZ60::Tn10*), BW3301 (*rpoS::Tn10*), BW3601 (*pta::kan*) and BW3305 *(hns::neo)* at different pH values in the presence of NaCl.

ed to 300 ± 40 units at the acidic pH, 258 ± 57 units compared to 458 ± 46 units at the neutral pH and 414 ± 19 units compared to 629± 60 units at the alkaline pH for the mutant and wild-type respectively (Figure 5). When NaCl was added, there was OmpF expression as same as wild type (Figure 6).

The OmpC expression of *E. coli* strain with an *hns* mutation at the neutral pH 110±18, at alkaline pH 69±11 and acidic pH 511±44 were measured (BW3306, *ompC-lacZ hns*::*neo)*. The *E. coli* strain with an *hns* mutation (BW3306, *ompC-lacZ hns*::*neo)* had consistently (but not significantly) slightly higher expression of *ompC* at alkaline pH (Figure 3). However there was a significant increase in *hns* mutation at acidic pH and neutral pH. The *E. coli* strain with an *hns* mutation (BW3305, *ompF-lacZ hns*::*neo)* also had slightly lower expression of *ompF* with 200 ± 17 units compared to 300 ± 40 units at the acidic pH, 347 ± 47 units compared to 458 ± 46 units at the neutral pH and 324 ± 28 units compared to 629 ± 60 units at the alkaline pH in the mutant and wild-type strains, respectively (Figure 5).

The effect of *hns* mutation on the expression *ompC* and *ompF* was carried out in minimal medium with and without NaCl at different pH. Therefore, there was a marked increase in the expression of *ompC* with loss of *hns* activity. Our results demonstrated that the expression of *ompC* was stimulated in both the *hns* mutant (BW3306) and wild- type *E. coli* in the presence of NaCl, especially at the acidic pH (Figure 4). The expression of *ompC* in the *hns* mutant was marginally increased compared to the wild-type levels in the absence of NaCl.

DISCUSSION

We have previously shown that porin mutations of *E. coli* have serious effects on its growth and survival (Darcan et al., 2003). The expression of *ompC* and *ompF* can be regulated by osmolarity. In the experiments reported here, the relative concentrations of each porin in the outer membrane changed when NaCl was added to the medium. Other studies have shown that the *ompC-ompF* double mutant of *E. coli* could survive for longer in seawater at a slightly acidic pH than at other neutral and alkaline pH (Darcan, 2005). At the same time, Kaeriyama et al. (2006) demonstrated that OmpC and OmpF are required for hyperosmotic adaptation at pH values above 8.0, but not below 8.0. Darcan et al. (2003) suggested that there was a meaningful relation between *envZ* mutation and viable but nonculturable bacterial state (VBNC). In these experiments, the expression of the outer membrane porins, OmpC and OmpF in cells of *E. coli* was followed through the activity of the reporter gene, β-galactosidase, under a range of different environmental conditions. The *ompC-lacZ* and *ompF-lacZ* constructs were contained in the same parental strains and each strain had a separate single mutation affecting one of the genes believed to be involved in the regulation of OmpC and OmpF under changing environmental conditions.

The expressions of *ompC* and *ompF* at different pH

The expressions of OmpC and OmpF porin proteins were followed at three pH values by measuring the β-

galactosidase activity produced in organisms where *lacZ* was linked to either *ompC* or *ompF*. Our results suggest that the expression of the porins, OmpC and OmpF, was changed by exposure to different growth pH. It is clear that the expression of *ompC* increased at acidic pH compared to neutral and alkaline pH as shown for *E. coli* strain MH225 (Figure 1). The expression of *ompF* was lower at acidic pH, than at neutral or alkaline pH as shown for strain MH513 (Figure 1). This is in agreement with the works at literature (Thomas et al., 1992, Sato et al., 2000). Moreover, they showed that when the osmolarity was increased, the expression of OmpC also increased independently from the effect of pH. This suggests that pH and osmolarity could separately affect the expression of *ompC*. However, the expression of *ompC* reported to be repressed with the increasing osmolarity at pH 8.0 (Sato et al., 2000). Wang et al. (2007) showed that OmpC was necessarily required for hyperosmotic adaptation of *E. coli* in the alkaline medium. Thomas and Booth (1992) suggested that OmpC expression was controlled by osmolarity but independently of pH. When minimal medium was supplemented with 0.2 M NaCl, the expression of the OmpC porin was increased but that of the OmpF porin was reduced.

According to these results, expression of OmpC and OmpF porin proteins were altered according to pH changes. However the mechanism of main factor for this alteration has not been completely known yet. In this work, therefore pH dependent regulation of OmpC and OmpF expression was tried to clarify by using *envZ, ompR, hns, rpoS, pta* mutant cells in order to find out main factor.

The effects of OmpR and EnvZ on the expressions of *ompC* and *ompF*

E. coli strains carrying mutations in the *envZ* and *ompR* genes were used to determine the effects of absence of these genes on the expressions of OmpC and OmpF at different pH. There was no LacZ activity in *E. coli ompR* mutants. These results suggest that OmpF expression showed more dependency on EnvZ at alkaline pH. OmpF also be expressed by an *envZ*-independent system. The expression of *OmpC* in the *envZ E. coli* mutants (BW3345) was reduced in medium with added NaCl at all pH values (Figures 3, 4, 5 and 6). But the expression of OmpF in *envZ* mutants (BW3343) at neutral and alkaline pH was increased slightly with the addition of NaCl.

Previous studies indicated that the expression of *ompC* and *ompF* in an *ompR* mutant of *E. coli* under all the stress conditions was entirely depending on *ompR* regulator (Forst et al., 1990; Sato et al., 2000; Liu and Ferenci, 2001). The expression of these porins in *envZ* mutants of *E. coli* (BW3343 and BW3345) was reduced compared to the wild-type but not completely stopped. Sato et al. (2000) indicated that OmpC and OmpF porins of *E. coli* were expressed at an acidic pH in the absence of the EnvZ sensor protein, but the mechanism of such regulation has still not been known.

In contrast to porin expression at an alkaline pH was dependent on the presence of the EnvZ sensor. Under conditions where NaCl was lacking, the results were consistent with previous data, but when NaCl was added to the minimal medium at an acidic pH, the expression of *ompC* was seen to increase. There was no porin expression in the *ompR* mutants (BW3303 and BW3304) (Figure 2). These results confirm that OmpR is a central regulator in the expression of these two porins. Any of the effects seen here due to other genes may be an indication of interaction between these gene products and that of *ompR* as the true effect.

The role of RpoS in the expressions of *ompC* and *ompF*

According to findings, there was high level of *ompF* expression in *rpoS* mutant of *E. coli* (BW3301) compared to wild type at the three different pH values tested. This expressions is not dependent pH. It has previously been shown that *rpoS* has a repressive effect on *ompF* expression in stationary phase cells (Pratt et al., 1996). It has also been reported that the amount of RpoS protein was increased at low pH, by high osmotic stress, and at high cell densities (Hengge-Aronis, 2002). A mutation of the *rpoS* gene enhanced the expression of *ompF* under glucose and nitrogen limitation (Liu and Ferenci, 2001). The above results indicated that the low expression of *ompF* in wild-type *E. coli* could have occurred at the acidic pH rather than the other tested pH values due to repression of RpoS. So OmpF expression was repressed by RpoS. It is said that high OmpF expression in *rpoS* mutant *E. coli* appeared to lose effect of repression of RpoS.

According to findings, the expression of OmpC level was low in *rpoS* mutant at the *E. coli* acidic pH. The expression of *ompC* was almost the same as that of the wild-type *E. coli* except for a small reduction at the acidic pH. Earlier studies have demonstrated that *ompC* promoter is not affected by RpoS as much as the *ompF* promoter (Liu and Ferenci, 2001). A mutation in *rpoS* did not influence the expression of *ompC* under nitrogen and carbon starvation conditions (Liu and Ferenci, 2001). It is also possible that the RpoS product is not stable or functional when the external pH is acidic. Apart from, the role of RpoS could be very indirect and explained through competition for the core RNA polymerase between different sigma factors (Farewell et al., 1998). So, RpoS had no direct role on pH-dependent OmpC and OmpF expression

The role of AcP on the expressions of *ompC* and *ompF*

The production of AcP from glucose is under the control

of two enzymes. Synthesis of AcP from acetyl-CoA and Pi is catalysed by phosphotransacetylase, the product of the *pta* gene. Synthesis of acetyl phosphate from ATP and acetate is catalysed by acetate kinase, the product of the *ackA* gene (McCleary et al., 1993). It is known that, unless there is an addition of acetate to the growth medium of a *pta* mutant of *E.coli,* AcP expression does not occur. It is known that there is a role of AcP in the phosphorylation of OmpR (McCleary et al., 1993). Therefore, we expected a reduction in the expression of *ompC* and an increase in the expression of *ompF* in the *pta* mutants (BW3601 and BW3602) used in these studies. However, the expression of *ompC* was detected at significantly higher levels compared to those in the wild-type *E. coli* under all the tested conditions. The expression of *ompF* was almost the same as that of the wild type *E. coli* at an acidic pH in the absence of NaCl. There was also a reduction in expression at the other tested pH conditions. When NaCl was added to the growth medium, the reduction in the expression of *ompF* was greater than in the absence of NaCl, but in general, less reduction in expression was detected compared to that seen in the wild-type *E. coli* at the three pH values. The above results suggest that AcP has a possible role in the reduction of the expression of *ompF* under conditions of different osmolarity. Moreover, in a medium without added NaCl, the main reason of the reduced expression of *ompF* at pH 7.1 and pH 8.3 could be related to a lack of AcP that caused the reduction in the amount of OmpR-P in the cell. However, an increase in the expression of *ompC* should rule out this possibility because it requires a higher level of OmpR-P. It was reported by Bouche et al. (1998) that AcP resulted in a negative effect on the expression of *rpoS*. This result indicates that a decline in the concentration of AcP in a cell led to an increase in the expression of *rpoS*, which, consequently, has a repressive effect on the expression of *ompF*. When the osmolarity of the medium was increased, the expression of *ompF* was found to be higher in the *pta* mutant than in the wild-type *E. coli*. Furthermore, there was a slight reduction in the level of expression of *ompF* with the addition of NaCl. This indicates that AcP has a role to play in the decline in the expression of *ompF* as dependent osmolarity. The presence of Acp plays a role at reduce of OmpF expression with increased osmolarity.

It is not expected that the higher expression of *ompC* in the *pta* mutant is because of a lack AcP. Heyde et al. (2000) findings implied that in the absence of EnvZ, AcP could contribute to OmpR phosphorylation during growth at pH 6.0. In the presence of EnvZ, and at an alkaline pH, porin expression was almost the same as in the wild-type, but expression was reduced by 50% at an acidic pH. However, the results shown here indicated that the expression of *ompC* was much higher in the *pta* mutant (BW3602) than in the wild-type at all the pH values tested and there are not a role of AcP on pH-dependent porin expression in presence EnvZ. The most likely explanation would be that for the expression of *ompC*, there should be a mechanism for OmpR phosphorylation which can be induced by a lack of AcP or by other unknown factors. In addition, Liu and Ferenci (2001) suggested that the lack of AcP induced EnvZ activity, consequently there was an increase in OmpR-P and as a result that of OmpC as well. Apart from this, we should take into consideration another factor that was not explored which could be effective on the expression of *ompC*. The expression of *ompC* increased significantly through an unknown mechanism in the absence of AcP. But this OmpC porin protein synthesis increase is totally independent from pH. In contrast, the expression of OmpF was inhibited under high osmolarity. Chen et al. (2004) suggested that MicC RNAs act in conjunction with the EnvZ-OmpR two component system to control the OmpF/OmpC porin protein ratio in response to a variety of environmental stimuli but the function of this RNA is not yet completely clear. Our results suggest that there could also be some other proteins which function is to control the expression of *ompC*. It has been reported previously that the formation of super-helix DNA (Graeme-Cook et al., 1989), Lrp (Leucine-Responsive Protein) (Ferrario et al., 1995), IHF (Integration Host Factor) (Goosen et al., 1995), HU (Histone-Like Protein) (Painbeni et al., 1997), StpA (Deighan et al., 2000), cAMP (Liu and Ferenci, 2001), *micF* RNA (Delihas and Forst, 2001) and MicC (Chen et al., 2004) was related to the expression of *ompC*. However their individual, mixed or combined roles have not been clarified yet.

The role of H-NS on the expressions *ompC* and *ompF*

In a previous study, a *hns* mutation had a significant positive influence on the expression of *ompC* (Suzuki et al., 1996). The reason for this increase in the expression of *ompC* was due to the negative influence of H-NS on *ompC* transcription which could be via binding to the intergenic region between *ompC* and *micF* resulting in expression of both genes (Suzuki et al., 1996). The same researchers also showed that a *hns* mutant had a higher expression of *ompC* than wild-type cells growing in a low osmolarity medium, but 0.15 M sucrose addition resulted in a reduction of the expression of *ompC*. However, according to our findings, there was no H-NS role on OmpC porin expression depended on pH stress.

There was a reduction in the expression of *ompF* in the *hns* mutant (BW3305) compared to wild-type *E. coli* in the absence of NaCl but this reduction in expression was reduced in the presence of NaCl at the three pH values tested. In contrast to the situation with *ompC*, no *in vitro* evidence exists to imply that H-NS has a binding site in the *ompF* promoter region that positively regulates *ompF* transcription. These results suggested the possibility that this decline in the expression of *ompF* could have been due to the repression of RpoS by H-NS having an indirect impact on *ompF* transcription as suggested by Lange and Hengge-Aronis (1994). It is known that H-NS has an inhi-

bitory role on *rpoS* and can affect the stability of RpoS (Lange and Hengge-Aronis, 1994). When this inhibition effect is removed through a *hns* mutation, the expression of *rpoS* increases, and therefore, the expression of *ompF* would be reduced. In another study with a *hns* mutant of *E. coli*, a 10-fold increase in the expression of *rpoS* during the exponential growth phase in a low osmolarity medium was seen (Bart et al., 1995). A reduction in OmpF expression in *hns* mutant *E. coli* could indirectly controlled by RpoS. Another report shows that the addition of 15% sucrose to minimal medium, reduced the expression of *ompF* in mutant *E. coli* by 40% compared to wild-type strains due to increase in *micF* RNA (Suzuki et al., 1996). This could be another reason for the reduction in expression of *ompF* seen in this study. As a result, H-NS had a little role on OmpF expression at neutral and alkaline pH that was detected.

These results demonstrated that AcP has a significant role to play in the expression of the *ompC* gene. There was significantly higher expression of *ompC* in the mutant strain compared to the wild-type strain. On the other hand, RpoS and HNS had little impact on the expression of *ompC*, especially at neutral and alkaline pH. While OmpC expression was increased with the presence of RpoS, It was repressed due to the presence of H-NS and AcP. In the presence of NaCl, similar results were obtained with increased expression of *ompC* at the acidic pH in the *pta and hns* mutants (Figure 4). There was also increased expression of *ompC* at neutral and alkaline pH in the *pta* mutant. The expression of *ompC* in all the other mutants showed no significant difference from the wild-type strain at any pH (Figure 4).

In the presence of NaCl were obtained with decreased expression of *ompF* at all pH values with the *rpoS* mutant compared to the absence of NaCl. These results showed similarity to *hns* and *pta* mutants (Figure 6). OmpF expression was reduced due to NaCl addition. In case of *hns* mutation, there was no change in OmpF expression at acidic pH in contrast to other pH values because there was reduction seen. The addition NaCl did not lead to changes in OmpF expression in *pta* mutant at neutral pH. In the absence of NaCl, the expression of OmpF in *pta* and *hns* mutants was lower than wild type cells at three tested pH values. But the addition of NaCl to the medium increased the OmpF expression to the same level. The expression of *ompF* in all the other mutants showed no significant difference from the wild-type strain at any pH (Figure 6).

Conclusion

Overall these studies have revealed that there is a complex situation involving a number of genes in controlling the expression of outer membrane porin proteins in *E. coli*. These genes are regulated by a complex of external factors, but there are not direct roles of AcP, H-NS and RpoS on pH-dependent OmpC and OmpF porin expressions.

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