The putative permease PhlE of \textit{Pseudomonas fluorescens} F113 has a role in 2,4-diacylphloroglucinol resistance and in general stress tolerance

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2,4-Diacetylphloroglucinol (PHL) is the primary determinant of the biological control activity of \textit{Pseudomonas fluorescens} F113. The operon \textit{phlACBD} encodes enzymes responsible for PHL biosynthesis from intermediate metabolites. The \textit{phlE} gene, which is located downstream of the \textit{phlACBD} operon, encodes a putative permease suggested to be a member of the major facilitator superfamily with 12 transmembrane segments. PhlE has been suggested to function in PHL export. Here the sequencing of the \textit{phlE} gene from \textit{P. fluorescens} F113 and the construction of a \textit{phlE} null mutant, F113-D3, is reported. It is shown that F113-D3 produced less PHL than F113. The ratio of cell-associated to free PHL was not significantly different between the strains, suggesting the existence of alternative transporters for PHL. The \textit{phlE} mutant was, however, significantly more sensitive to high concentrations of added PHL, implicating PhlE in PHL resistance. Furthermore, the \textit{phlE} mutant was more susceptible to osmotic, oxidative and heat-shock stresses. Osmotic stress induced rapid degradation of free PHL by the bacteria. Based on these results, we propose that the role of \textit{phlE} in general stress tolerance is to export toxic intermediates of PHL degradation from the cells.

INTRODUCTION


Micro-organisms have developed various ways to resist the toxic effects of the secondary metabolites they produce. These mechanisms include export of the metabolite out of the producing cell. The \textit{phlE} gene, located at the 3’ end of the \textit{phlACBD} operon in \textit{P. fluorescens} strains (Bangera & Thomashow, 1996, 1999; Delany, 1999) encodes a putative transmembrane permease with 12 predicted transmembrane segments (TMS) (Bangera & Thomashow, 1999). The hydrophobicity profile predicted by the Kyte–Doolittle model indicates that PhlE is structurally similar to NorA of \textit{Staphylococcus aureus}. NorA is a multidrug
transporter mediating resistance to a range of structurally
dissimilar drugs (Paulsen & Sukurray, 1993; Yoshida et al.,
1990). PhlE also has structural similarity with integral
membrane proteins associated with resistance which are
encoded within clusters responsible for polyketide biosyn-
thesis (Brault et al., 2000; Fernandez-Moreno et al.,
1991; Guillofle & Hutchinson, 1992; Marger & Saier, 1993;
Molnar et al., 2000). It has been reported that phlE
mutants produce less PHL than the parent strain
(Bangera & Thomashow, 1996, 1999). The phlE mutants
are also affected in production of a red pigment, usually
in the

Beside their role in drug resistance, many efflux pumps
play an important role in stress tolerance. Consequently,
in this report we have examined the role of PhlE not only
in PHL resistance but also in general stress tolerance in
P. fluorescens F113. We show that PhlE is important for
F113 survival at high concentrations of PHL and that it
plays an important role in protecting F113 from a range of
environmental stresses, where it may export toxic inter-
mediates of PHL degradation from the bacterial cell.

**METHODS**

**Bacterial strains, plasmids and culture conditions.** The bac-
terial strains and plasmids used in this study are listed in Table 1. P. fluorescens F113 and derivatives were routinely grown at 28 °C in
minimal medium (SA) (Scher & Baker, 1982) with sucrose (50 mM)
and asparagine (17.5 mM) as carbon and nitrogen sources, respectively.
SA medium was supplemented with 100 μM FeCl₃. *Escherichia coli*
strains were grown at 37 °C in Luria–Bertani broth (Sambrook et al.,
1989). Antibiotics were used at the following concentrations
(μg ml⁻¹): for *P. fluorescens*, tetracycline 75 and kanamycin 50; for
*E. coli*, tetracycline 25, kanamycin 30 and ampicillin 100.

**Recombinant DNA techniques.** Plasmid DNA isolations were
performed using the Qiagen plasmid mini kit according to manufac-
turer’s specifications. Genomic DNA isolation was performed using
standard methods (Sambrook et al., 1989). Plasmids were intro-
duced into *E. coli* and *P. fluorescens* by electroporation (Farinha &
Kropinski, 1990) or mobilized into *P. fluorescens* by tripalental mating
using the helper plasmid pRK2013 (Figurski & Helinski, 1979).

**Construction and complementation of phlE mutant.** A 0.7 kb
*BamHI–*SpeI fragment of pMP22 cloned into *BamHI–*SpeI
sites of the narrow-host-range vector pK18 (Pridmore, 1987) to pro-
duce pKE7. A 0.275 kb *BamHI* fragment within the *phlE* gene was
isolated from pKE7, blunt-ended and subcloned into the SmaI
restriction site of the suicide vector pK18 to produce pKD–3. This
construct was mobilized by tripalental mating into F113. Cells with
chromosomally integrated pKD–3 were selected for kanamycin resis-
tance. The position of the mutation was confirmed by Southern
blotting. The mutant was named F113–D3. To complement the
F113–D3 mutant, a PCR fragment containing the full sequence of the
*phlE* gene and 50 bp extra on both sides was obtained using
*phlE* (5’-CCGCTCGAGAGAGGGCTTCGAAAGCGCT-3’) and
*phlE2* (5’-CCCAAGCTTTGGCGAGTCCAGCAACAT-3’). The
PCR product was digested using *HindIII* and *XhoI* and cloned into
the broad-host-range cloning vector pBRR1MCS (Kovach et al.,
1994), restricted by the same enzymes to produce pCUD4. This
places the expression of *phlE* under control of the lacZ promoter.

**Sequencing and sequence analysis.** The *phlE* gene was
sequenced using the pMP2 plasmid containing the entire *phlE* gene
as template and the primers 5’-CCCCGGCCGGGACTCA-CC-
3’), SEQ2 (5’-CACGGCTACCTGACAGACATCC-3’) and
SEQ3 (5’-CCGGGCGCGGACTCA-CC-3’) as well as the M13 universal
primers. The sequence data were analysed using the MEGALIGN
DNASTAR software package (Madison, WI, USA), the Mfold
program (Mathews et al., 1999; Zuker et al., 1999) and the Sossui
system at www.expasy.ch.

**Table 1. Strains and plasmids used**

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Genotype*</th>
<th>Reference</th>
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<tbody>
<tr>
<td>P. fluorescens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F113</td>
<td>Wild-type; PHL⁺ HCN⁺ Prt⁺ Lac⁻</td>
<td>Shanahan et al. (1992)</td>
</tr>
<tr>
<td>F113–D3</td>
<td>PHL⁺ HCN⁺ Prt⁺ Lac⁻ PhlE⁻</td>
<td>This study</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DH5α</td>
<td>Δ80lacZΔm15 (ΔlacZYA-argF) U169 hisD17 recA1 endA1 thi-1</td>
<td>Sambrook et al. (1989)</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRRK2013</td>
<td>Helper plasmid; Tra⁺ Mob⁺ ColE1 KmR</td>
<td>Figurski &amp; Helinski (1979)</td>
</tr>
<tr>
<td>pK18</td>
<td>Cloning vector; ColE1 KmR</td>
<td>Pridmore (1987)</td>
</tr>
<tr>
<td>pBRR1MCS</td>
<td>Broad host range cloning vector; CmR LacZa</td>
<td>Kovach et al. (1994)</td>
</tr>
<tr>
<td>pCUD4</td>
<td><em>phlE</em> gene in pBRR1MCS</td>
<td>This study</td>
</tr>
<tr>
<td>pMP2</td>
<td>2 kb <em>Spel</em> fragment of pME8.3 carrying the entire <em>phlE</em> gene in pMP220; TcR</td>
<td>This study</td>
</tr>
<tr>
<td>pKE7</td>
<td>0.7 kb *BamHI–*SpeI fragment of pMP22 in pK18; KmR</td>
<td>This study</td>
</tr>
<tr>
<td>pKD–3</td>
<td>0.275 kb internal <em>BamHI</em> fragment of <em>phlE</em> in pK18; KmR</td>
<td>This study</td>
</tr>
<tr>
<td>pMP8.3</td>
<td>8.3 kb <em>EcoRI</em> fragment carrying the entire PHL biosynthetic locus with truncated <em>phlE</em> gene in pMP220; TcR</td>
<td>Delany (1999)</td>
</tr>
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</table>

*PHL, 2,4-Diacetylphloroglucinol; HCN, hydrogen cyanide; Prt, protease; Cm, chloramphenicol; Km, kanamycin; Tc, tetracycline.
Stress response assays. To measure the ability of the wild-type and the mutant variants to survive heat shock, cells were grown overnight in LB at 28 °C with shaking (150 r.p.m.). The cells were then washed twice with LB and resuspended to a density of approximately 5000 c.f.u. ml⁻¹. One milliliter of the diluted cell suspension was transferred to prewarmed Eppendorf tubes at 50 °C. Viable counts were determined by plating 100 µl from each tube per time point on LB plates. To measure the sensitivity to osmotic shock, cells were grown and washed as described above and resuspended to a density of approximately 50 000 c.f.u. ml⁻¹. One milliliter of this cell suspension was inoculated into 10 ml LB or SA containing NaCl or sucrose. After incubation at 28 °C with shaking (200 r.p.m.), aliquots of 100 µl were taken at 0, 2, 4 and 5 h and plated on LB plates to determine the c.f.u. Sucrose concentrations of 1 and 2 M, and NaCl concentrations of 0-5 and 0-75 M were tested. The concentrations of sucrose used in this analysis are 20 and 40 times higher than the concentrations found under normal growth conditions. Sensitivity to hydrogen peroxide (H₂O₂) was measured on LB plates. Cells from 17 h growth in LB medium were plated on LB plates to determine the c.f.u. The plates were incubated for 36 h at 28 °C. The zone of inhibition was measured. To measure the cell sensitivity to hydrogen peroxide, cells were grown and washed as described above and resuspended to a density of approximately 50 000 c.f.u. ml⁻¹. One milliliter of the diluted cell suspension was transferred to prewarmed Eppendorf tubes at 50 °C. Viable counts were determined by plating 100 µl from each tube per time point on LB plates. To measure the sensitivity to osmotic shock, cells were grown and washed as described above and resuspended to a density of approximately 50 000 c.f.u. ml⁻¹. One milliliter of this cell suspension was inoculated into 10 ml LB or SA containing NaCl or sucrose. After incubation at 28 °C with shaking (200 r.p.m.), aliquots of 100 µl were taken at 0, 2, 4 and 5 h and plated on LB plates to determine the c.f.u. Sucrose concentrations of 1 and 2 M, and NaCl concentrations of 0-5 and 0-75 M were tested. The concentrations of sucrose used in this analysis are 20 and 40 times higher than the concentrations found under normal growth conditions. Sensitivity to hydrogen peroxide (H₂O₂) was measured on LB plates. Cells from 17 h growth in LB medium were plated on LB plates at a density of 10⁶ c.f.u. per plate and sterile Whatman 3MM filter disks impregnated with 20 µl 30 % (v/v) H₂O₂ were placed on the plate. The plates were incubated for 36 h at 28 °C and the zone of inhibition was measured. To measure the cell sensitivity to hydrogen peroxide, cells were grown overnight on SA at 28 °C with shaking (150 r.p.m.), washed twice and diluted in Ringer’s solution (Oxoid). A set of solutions with different concentrations of HPLC-purified PHL was prepared in ethanol (70 %) and added to SA medium before solidification to give final concentrations of 0, 50, 100, 150, 200, 300, 400 and 500 µg ml⁻¹. Cells of F113 and F113-D3 were plated and incubated for 36 h at 28 °C.

Measurement of PHL levels. P. fluorescens F113 and variants were assayed for the level of PHL production by HPLC using the method described by Shanahan et al. (1992). The free PHL was extracted from the culture supernatant. To extract cell-associated PHL, cells were broken by ultrasonic treatment. The broken cells were centrifuged at 10 000 g for 5 min and PHL was extracted from the supernatant.

RESULTS AND DISCUSSION

Sequence analysis of the phlE gene from P. fluorescens F113

The phlE gene is located at the 3′ end of the phlACBD operon. The 5′ end of phlE of P. fluorescens F113 was recently sequenced along with the entire phlACBD operon (GenBank accession no. AF497760). In the present study, we determined the full sequence of the gene (AJ542662). To examine whether phlE constitutes an independent transcriptional unit, the DNA sequence of the phlE-phlD intergenic region was analysed for the presence of any secondary structure using the MFOLD program (Mathews et al., 1999; Zuker et al., 1999). A 22 bp palindrome, consisting of two GC-rich (73 %) inverted repeats of 11 bp separated by 4 bp, was identified downstream of the phlD translation stop codon. The sequence upstream of the palindrome is rich in C (41 %) and poor in G (12 %). These features are characteristic of the ρ-dependent terminators. Additional evidence that the stem–loop formed by the two inverted repeats could function as a terminator comes from its calculated free energy, ΔG = −14.7 kcal (61.5 kJ), indicating that it could form a stable hairpin structure and function as a ρ-dependent terminator of phlD transcription. Furthermore, the sequence (5′-CA-GGGCTTTGGAAGCGCT-3′) located between positions −37 and −23, relative to the ATG translational start of phlE, shows similarity with the consensus sequence (5′-MRRNYTGGACG-N₄-TTGCGWNNW-3′) recognized by σ₅₄. The most important feature of this consensus is the perfectly conserved GC and GG elements (shown in bold type) positioned 12 and 24 nt downstream from the transcriptional start of genes under the control of σ₅₄ (Barios et al., 1999). This structure is not conserved in P. fluorescens QZ-87 (Bangera & Thomashow, 1999). This suggests that the phlE gene of P. fluorescens F113 may be transcriptionally independent of phlD and could be transcribed from a ρ₅₄-dependent promoter.

The phlE gene consists of an ORF of 1269 bp, corresponding to a predicted protein of 423 aa with a predicted molecular mass of 45.2 kDa. PhlE proteins from F113 and QZ-87 are highly related and share 88.2 % identity and 97.4 % similarity throughout their entire amino acid sequence. It is noteworthy that the newly identified phlE of P. fluorescens HP-72 (AB125214) encodes a predicted protein showing two deletions of 3 aa each located between amino acids 270 and 300. Hydropathy analysis predicts that PhlE is organized in two sets of six hydrophobic α-helices, of 23 aa each, separated by a central hydrophilic loop. The central region, which is predicted to have a periplasmic location, corresponds to the most divergent amino acid sequence between the PhlE proteins of different P. fluorescens strains. Interestingly, PhlE of HP-72 has only eight hydrophobic α-helices of 23 aa each and a long predicted periplasmic C-terminal region. It is not known if these structural differences reflect functional differences with other PhlE proteins. Homology searches show that PhlE has similarity with Staphylococcus aureus NorA (Paulsen & Sukkary, 1993; Yoshida et al., 1990), E. coli Bcr (Bentley et al., 1993) and EmrD (Naroditskaya et al., 1993), and Bacillus subtilis Bmr (Neyfakh et al., 1991) and Blt (Ahmed et al., 1995). These proteins are members of the major facilitator superfamily having 12 TMS. We analysed the primary amino acid sequence of 10 randomly chosen members of the 12-TMS family. Interestingly, the analysed proteins share strong similarity in molecular mass (43±2±2-23 kDa) and in hydrophobic residue content (198±12 aa). The molecular mass and the hydrophobic residue content of PhlE from both F113 and QZ-87 are within these ranges. The 12-TMS family members have suggested functional motifs containing conserved amino acid residues. Motif D2 (GxxxxPXxP) and motif G (GxxGxPL) are specific to the 12-TMS family. Motif B (IxxxRxxqGxxa) and motif C (gxxxGPxxGxxI) are common to both 12 and 14 TMS (Putman et al., 2000). Motif D2, found at the N-terminal region of 12-TMS family members, was found in the same location in PhlE. PhlE also has a G motif in α-helix 11; this motif is present in the same region of 12-TMS family members (Putman et al., 2001). Motifs B and C are also present at conserved locations. Overall these results suggest
that PhlE is a transmembrane protein likely to be a member of the major facilitator superfamily having 12 TMS.

**Effect of mutation of phlE on PHL synthesis and PHL resistance**

Plasmid pKD-3, which contains an internal 0.275 kb BanII fragment of the phlE gene and a kanamycin resistance cassette, was integrated into the F113 chromosome via a single homologous recombination within phlE. Southern blotting analysis confirmed that F113-D3 had a disrupted phlE allele (data not shown). Colonies formed by F113-D3 are lighter in colour than the ones formed by F113 due to the absence of the red pigment, as reported by Bangera & Thomashow (1996, 1999). To investigate whether PhlE is implicated in PHL transport, we performed a time-course experiment monitoring the production of both free and cell-associated PHL in *P. fluorescens* F113 and in the phlE mutant derivative. Fig. 1 shows that mutation of phlE does not affect F113-D3 growth. F113-D3 produces less total PHL than the wild-type; levels of both free and cell-associated PHL are lower than in the wild-type strain. When we compared the ratio of cell-associated to free PHL, there were no significant differences between the wild-type and the mutant at any of the time points (Fig. 1). Over-expression of phlE in the F113-D3 mutant by introduction of pCUD4 (Table 1), which has approximately 15 copies per cell, restored PHL production to levels that were up to threefold higher than the wild-type. This complementation excludes the possibility that the phenotypic alteration of PHL production seen in the phlE mutant is due to polar effects of the mutation in phlE on downstream genes. Overall these data suggest that under normal growth conditions PhlE is not the only factor determining the distribution of PHL between the cells and the medium. In a study of indole synthesis in *E. coli*, Kawamura-Sato et al. (1999) reported that mutation of the acrEF genes, encoding a multidrug efflux pump, causes a reduction in indole production. This defect can be corrected if the mutant strain is cultured in a high concentration of tryptophan, suggesting the existence of an alternative indole efflux pump. The findings are thus superficially similar to our own on PHL production in *P. fluorescens* F113. They suggest that bacteria producing toxic compounds have multiple systems for their transport from the cell and that they engage a mechanism(s) for reducing the level of metabolite production when the transport is impeded. The mechanism by which mutation of phlE leads to a reduction of PHL synthesis is not known. The impact of PhlE on PHL production is not at the level of transcription of the phlACBD operon. A phlACBD::lacZ transcriptional fusion revealed no differences in transcriptional activity between wild-type and phlE mutant backgrounds (data not shown).

*P. fluorescens* F113 produces and exports PHL to a maximum concentration of 50–70 µg per OD<sub>600</sub> unit ml<sup>−1</sup>. F113 is resistant to this level of PHL, but is sensitive to higher levels. To study the influence of PhlE on PHL resistance, *P. fluorescens* F113 and its phlE mutant derivative were cultured on SA agar supplemented with increasing concentrations of PHL. Fig. 2 shows that when cells were grown at a PHL concentration of 300 µg ml<sup>−1</sup> or higher, the survival of both strains was markedly decreased. The number of surviving cells of the phlE mutant strain was 32- and 62-fold less than the wild-type at PHL concentrations of 300 and 400 µg ml<sup>−1</sup>, respectively. These findings directly implicate PhlE in PHL resistance in *P. fluorescens* F113.

**Role of PhlE in general stress tolerance**

Both eukaryotic and prokaryotic cells increase expression of some multidrug transporter genes in response to environmental shock caused by heat (Miyazaki *et al*., 1992), osmotic (Ma *et al*., 1995) or oxidative (Chou *et al*., 1993; George &...
Levy, 1983) stresses. However, the role that transporters have in stress tolerance has not been extensively examined.

To determine whether PhlE contributes to the tolerance of *P. fluorescens* F113 against different stresses, F113 and F113-D3 were compared for their sensitivity to increased osmolarity, heat shock and oxidative stress provided by the addition of exogenous H$_2$O$_2$.

To measure the effect of heat shock, cells were grown at 28°C to stationary phase and were exposed to 50°C. The *phlE* mutant was more sensitive to this heat shock than the wild-type F113 (Fig. 3); after 4 min at 50°C, the number of viable cells was 20-fold higher in the wild-type compared to the *phlE* mutant. There was, however, no difference in behaviour between the wild-type and complemented mutant F113-D3/pCUD4.

To test whether PhlE has a role in protecting F113 against oxidative stress caused by H$_2$O$_2$, cells were grown to stationary phase in LB, seeded on LB plates at 10$^6$ cells per plate and a disk of Whatman 3MM filter paper impregnated with 20 μl 30% (v/v) H$_2$O$_2$ was deposited on the plate. The diameter of the zone of inhibition, which indicates the relative sensitivity to H$_2$O$_2$, was 5.4 ± 0.2 cm for F113, 6.8 ± 0.1 cm for F113-D3 (*phlE*) and 4.3 ± 0.1 cm for F113-D3/pCUD4 (n = 3). H$_2$O$_2$ was thus more effective against F113-D3 than the wild-type, whereas overexpression of *phlE*, in F113-D3/pCUD4, increased the level of tolerance.

To analyse the effect of osmotic shock, F113, F113-D3 (*phlE*) and F113-D3/pCUD4 strains were grown in SA or LB medium and then subjected to osmotic shock by the addition of NaCl (to 0.75 M) or sucrose (to 2 M) to the medium. The effect of 0.75 M NaCl on the viability of these strains is shown in Fig. 4. The *phlE* mutant F113-D3 was considerably more sensitive to this osmotic stress than the wild-type strain F113, whereas strain F113-D3/pCUD4 has an increased tolerance compared to wild-type. Similar results were seen when 2 M sucrose was used to induce stress (data not shown). When bacteria were grown in LB medium rather than SA, the impact of these osmotic stresses was less pronounced, but the same differential behaviour of the strains was observed (data not shown). Overall the differential responses of the wild-type and the *phlE* mutant to heat, oxidative and osmotic stress indicate a role for PhlE in general stress tolerance in *P. fluorescens*.

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**Fig. 2.** PHL resistance test. F113 (circles) and F113-D3 (triangles) were grown to stationary phase and then plated on SA plates containing different concentrations of PHL. The number of surviving cells is expressed as a percentage of the number of c.f.u. on control plates not supplemented with PHL. Values given are the means ± SD of triplicate independent measurements.

**Fig. 3.** Effect of mutation of *phlE* on tolerance to heat shock. Stationary-phase cultures of F113 (squares), F113-D3 (*phlE*) (triangles) and F113-D3/pCUD4 (circles) grown in LB were washed, diluted in LB and transferred to pre-warmed tubes at 50°C. Cells were heat-shocked at 50°C for indicated time and the number of surviving cells counted on LB plates. Values presented are the means ± SD of triplicate independent measurements.

**Fig. 4.** Effect of mutation of *phlE* on tolerance to osmotic stress. To assay survival of exposure to high concentrations of NaCl, F113 (circles), F113-D3 (squares) and F113-D3/pCUD4 (triangles) cells were grown to stationary phase in SA, washed, diluted and transferred to tubes containing SA supplemented with 0.75 M NaCl. Values given are the means of three independent measurements, which differed from the mean by less than 10%.
F113. The restoration of stress tolerance of F113-D3 to wild-type or higher levels by the introduction of phlE also indicates that the increased sensitivity of the mutant to stress is solely due the phlE mutation.

Possible relationship between response to stress and PHL metabolism

The observed role of phlE in both tolerance to exogenous PHL and in general stress tolerance prompted us to ask whether there is any relationship between these two phenomena. A decreased capacity to export PHL from the cell could account for the increased sensitivity of F113-D3 to exogenous PHL. An attractive hypothesis is that the effect of stress is to increase PHL production and that the increased sensitivity of the phlE mutant to stress is due to the enhanced accumulation of PHL within the mutant cells. We tested this hypothesis by analysis of the effects of osmotic stress (0-75 M NaCl) on PHL levels in the wild-type. Application of osmotic stress led to a rapid disappearance of PHL from the medium together with a concomitant appearance of MAPG (Fig. 5). As well as acting as the precursor of PHL synthesis, MAPG is also known to be a degradation product of PHL (Schnider-Keel et al., 2000; A. Abbas, unpublished results). Control experiments established that loss of PHL was not due to precipitation from the medium and required the presence of the bacteria. These findings, which indicate a hitherto unsuspected relationship between stress and PHL degradation, lead us to propose that one role of phlE in general stress tolerance is to export toxic intermediates of PHL degradation from the cells. PHL production and stress tolerance are functions that must contribute to the biocatalytic ability of P. fluorescens F113, so that further work on the mechanism of PHL and MAPG transport and the role of PhlE in regulation of PHL synthesis and stress tolerance is clearly warranted.

ACKNOWLEDGEMENTS

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REFERENCES


Fig. 5. Effect of osmotic shock on the kinetics of PHL and MAPG production. F113 cells were grown for 17 h on SA, diluted and transferred to tubes containing SA supplemented with 0-75 M NaCl. The control tubes were not supplemented with NaCl. Samples from control (solid lines) and shocked (dashed lines) cultures were taken at the indicated times and the free PHL (filled symbols) and MAPG (open symbols) content quantified by HPLC. The concentration of PHL is expressed in nmol PHL per OD600 unit. Values given are the mean of three independent measurements, which differed from the mean by less than 10%.


