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Functional Complementation of *Escherichia coli* by the *rpoS* Gene of the Foodborne Pathogenic *Vibrio vulnificus*

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Abstract The *rpoS* gene product is a global transcriptional factor, which is involved in bacterial survival under various stress conditions. An *rpo*S-homologous gene was cloned from a septicemia-causing pathogenic *Vibrio vulnificus*. Introduction of this gene as a multicopy plasmid into various *E. coli* strains displayed functional complementation, for examples, increased survivability of an *rpoS*-defective *E. coli* cell and induction of known σ^S-dependent, stress-responding promoters of *E. coli* genes.

Key words: *rpoS*, functional complementation, foodborne pathogen

One of the sigma factors in some Gram-negative bacteria, σ^S (RpoS), is believed to be involved in survival by acting as a transcriptional regulator of multiple regulons that confer resistance against stresses normally experienced in the stationary phase [4, 12] or frequently encountered due to fluctuating environmental parameters [8, 19]. Thus, in many pathogenic bacteria belonging to the γ-subdivision of Proteobacteria, σ^S is required for eliciting phenotypes related to virulence, especially to the overcoming of stresses imposed by host systems [15, 24, 25, 27].

The causative agent of septicemia, *Vibrio vulnificus*, has been considered an important foodborne pathogen in humans due to its rapid pathogenic progresses and its high mortality rates, since its presence and identification was first documented in 1976 [5, 7, 23]. Questions have been raised concerning the presence of the *rpoS* homologous gene and the role of σ^S, if present, in the case of this pathogenic *V. vulnificus*.

In an effort to isolate global regulators involved in survival of *V. vulnificus*, the *rpoS* gene has been cloned [18]. The deduced amino acid sequence appeared to code for 343 amino acid residues. Compared to other known σ^S of *V. parahaemolyticus, V. cholerae,* and *E. coli* [2], there is a complete homology in 2.3–2.4 subregions of σ^S, and a significant conservation in subregion 2.1 (Fig. 1).

Effect of $rpoS_{Vv}$ on Expression of σ^S_{Ec}-Dependent Promoters

In *E. coli*, σ^S is known to regulate the expressions of several genes involved in cellular adaptation to diverse stresses. To examine if the σ^S homologue of *V. vulnificus* is also able to play an equivalent role in regulation of these genes, the plasmid pINE32 carrying the $rpoS_{Vv}$ gene was introduced to the *E. coli* strains containing one of the σ^S-dependent promoter::*lacZ* fusions (Table 1).

Exponential phase cultures of various *E. coli* strains grown in 0.05% glucose-based minimal M9 medium (42.2 mM Na_2HPO_4, 22.05 mM KH_2PO_4, 8.55 mM NaCl, 18.7 NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$) were lysed with sodium dodecyl sulfate and chloroform. Portions of the lysates were used for enzyme assay employing 10 mM *O*-nitrophenyl-β-D-galactoside as a substrate. β-Galactosidase activity was calculated using Miller's formula [16].

The β-galactosidase activities of four σ^S-dependent promoters were determined in the absence or in the presence of the $rpoS_{Vv}$. The reporter genes employed in this study were; *katE* [14], *bolA* [1], *pexB* [11], and *pexA* [22]. β-Galactosidase activities were found to be induced by the presence of pINE32 (Table 2). The degrees of induction by pINE32 were about two-fold, which is comparable to the induction achieved by *E. coli* σ^S [22].

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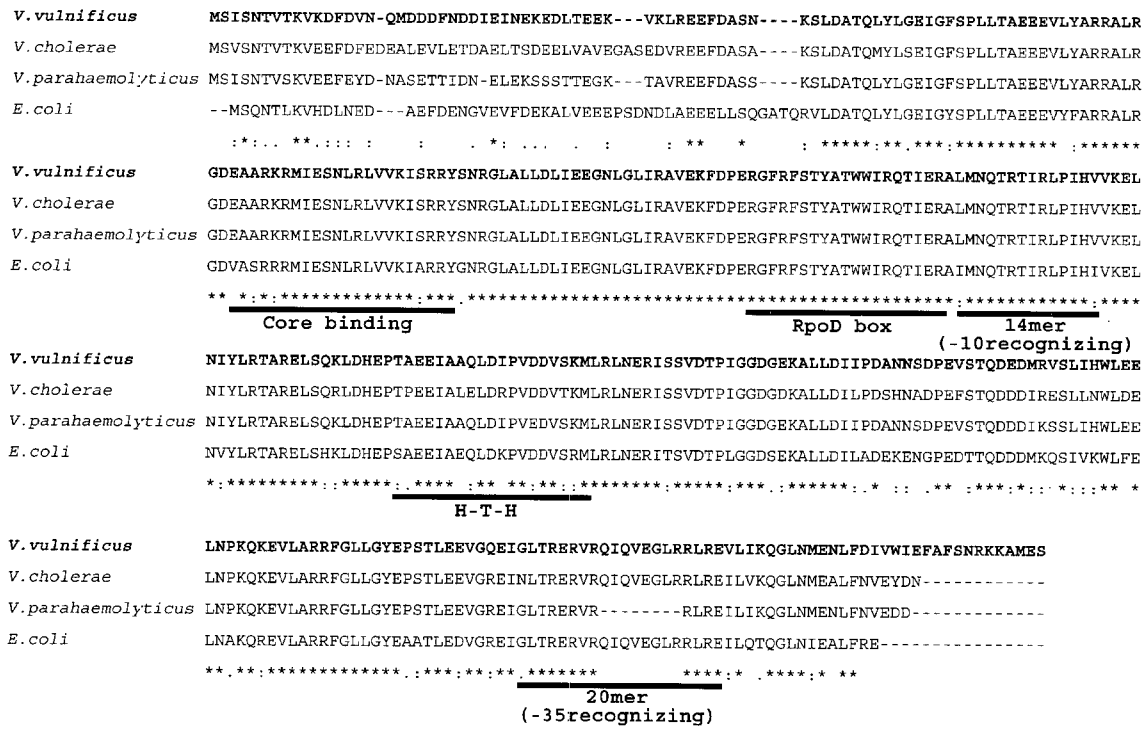

Fig. 1. Alignment of the deduced amino acid sequences of σ^S from *V. vulnificus* with related species, *V. cholerae*, *V. parahaemolyticus*, and *E. coli*.

The asterisk symbol indicates a fully conserved residue, the two-dotted symbol indicates a strongly similar residue, and the one-dotted symbol indicates a weakly similar residue.

Effect of $rpoS_{Vv}$ on Survial of *E. coli*

To further study the effect of $rpoS_{Vv}$ on *E. coli* cells, the survivability of four *E. coli* strains was also examined. The survival of *rpoS*-deficient *E. coli* AMS150 strain [13] was compared in parallel with those of the *rpoS*-proficient AMS6 strain [21], AMS150 containing the vector plasmid (pUC19), and AMS150 containing *V. vulnificus rpoS* (pINE32). Each strain was grown in culture to the exponential phase in LB medium supplemented with ampicillin if necessary, and then centrifuged, washed, and resuspended in the appropriate stress media, i.e., LB broth titrated to pH 2.4, LB containing a final NaCl concentration of 2.4 M, or LB including 15 mM of hydrogen peroxide. The initial cell density in the resuspension was approximately 10^6 cells/ml. During aerobic incubation at 37°C, samples were taken at the indicated time points and plated onto LB agar plates

Table 1. Strains and plasmids used in this study.

Strains or plasmids	Relevant characteristics	Source or reference
V. vulnificus ATCC29307	Clinical isolate; virulent	6
E. coli		
DH5α	Φ80d*lacZ*ΔM15 *recA*1 *endA*1 *gyrA*96 *relA*1 *thi*-1 *hsdR*17(r_K^-,m_K^-) *supE*44 *deoR* Δ(*lacZYA-argF*)U169	Laboratory collection
AMS6	K-12 (λ⁻ F⁻ Δ*lac*)	21
AMS150	AMS6 but *rpoS*::Tn*10*	13
AMS60	AMS6 but *pexA*::*lacZ*	11
AMS159	AMS6 but *katE*::*lacZ*	14
AMS170	AMS150 but *pexB*::*lacZ*	11
ZK918	W3110 Δ*lacU169 tna*-2 *rpoS*::*kan bolA*::*lacZ*	1
Plasmids		
pUC19	Cloning vector; *lacZ*α Ap^r	26
pINE32	pUC19 with 2.75 kb-*Sau*3AI fragment of *V. vulnificus* DNA containing the complete coding sequence of $rpoS_{Vv}$	18

Table 2. β-Galactosidase activity of *E. coli* cells containing various σ^S-dependent promoter::*lacZ* fusions in the presence and in the absence of $rpoS_{Vv}$ (pINE32).

E. coli strain	β-Galactosidase activity (MU)[a]		
	No plasmid	pINE32	Fold induction
AMS159 (*katE::lacZ* fusion)	20.2	39.5	1.96
ZK918 (*bolA::lacZ* fusion)	29.8	71.3	2.39
AMS170 (*pexB::lacZ* fusion)	1133	2792	2.46
AMS60 (*pexA::lacZ* fusion)	28.6	61.8	2.16

[a]The average values derived from two independent experiments were presented. The standard deviations were less than 5% of the corresponding average values.

supplemented with ampicillin, if necessary, to determine cell numbers.

Upon exposure to acidic condition, pH 2.4, both *rpoS*-deficient *E. coli* AMS150 and AMS150 with pUC19 demonstrated dramatically reduced survivability (Fig. 2A), whereas AMS150 containing pINE32 showed a capability to survive under acidic conditions, as did AMS6. The presence of pINE32 also endowed AMS150 cells with the ability to resist against hyperosmotic shock (2.4 M NaCl) or oxidative stress (15 mM H_2O_2), like the wild-type AMS6 (Figs. 2B and 2C). The strain AMS150 containing pINE32 showed slightly superior survivability to the strain AMS6. This increased resistance to stresses appeared to be derived from the difference in culture condition. *E. coli* cells with a plasmid were grown in medium containing an antibiotic. This could elicit a cross-protection phenomenon [12], and the exposure to an antibiotic increased the resistance against starvation and oxidative stress.

Effect of $rpoS_{Vv}$ on Catalase Activity of *E. coli*

Since an increased survival of AMS150 containing pINE32 was observed in the presence of H_2O_2, the activities of the two hydroperoxidases, HP I (KatG) and HP II (KatE), in these *E. coli* cells were examined. HP II is a σ^S-dependent catalase in *E. coli* [17, 20]. In *E. coli*, HP II is fully induced during the stationary phase, when the synthesis of σ^S is maximal.

Cellular extracts were made in 50 mM potassium phosphate buffer (pH 7.0) by sonication (Vibracell, Sonics & Materials, Inc.). The amount of protein in the cell lysate was measured by Bradford assay using bovine serum albumin as a standard. Upon separation on 8% nondenaturing polyacrylamide gel, the locations of HP were visualized by staining the gel with a solution of 1% $K_3Fe(CN)_6$ and 1% $FeCl_3$ [3]. Two *rpoS*-deficient strains, AMS150 and AMS150 containing pUC19, demonstrated only HP I activity. AMS150 containing pINE32 expressed HP II in addition to HP I (Fig. 3).

The *V. vulnificus rpoS* gene showed its involvement in regulating various stress-inducing genes in *E. coli* and increasing the survivability of *E. coli*. Our studies are in progress to characterize its roles in *V. vulnificus* under the

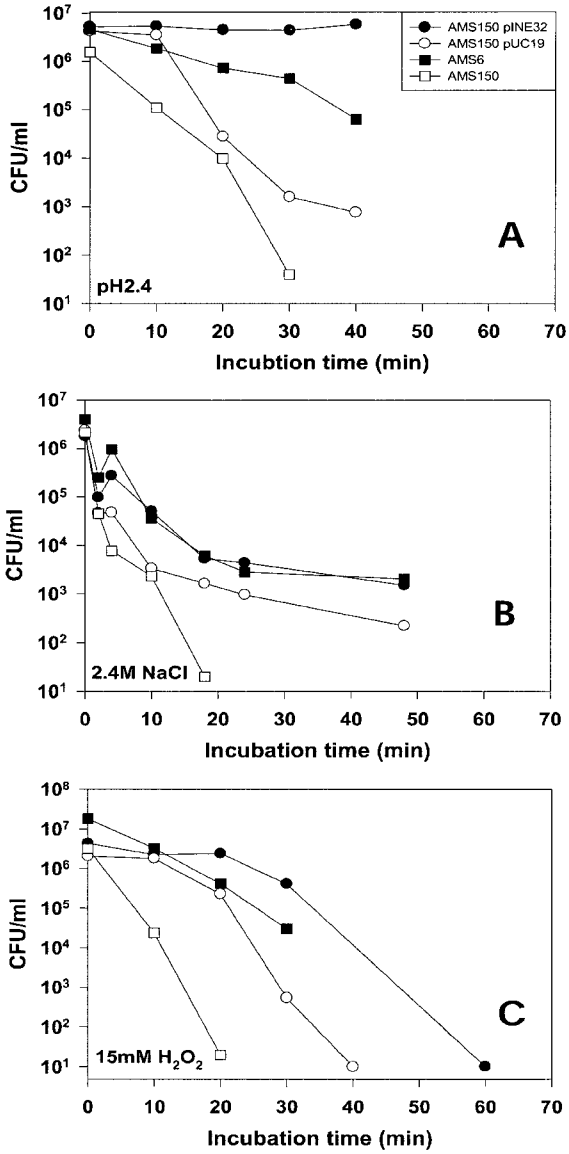

Fig. 2. Complementation of *rpoS*-deficient *E. coli* AMS150 by $rpoS_{Vv}$ (pINE32).
The survival of wild-type *E. coli* AMS6 and *rpoS*-deficient *E. coli* AMS150 cells containing either a vector plasmid (pUC19) or pINE32 were compared after treatment with an acid pH (pH 2.4; A), hyperosmotic shock (2.4 M NaCl; B), or oxidative stress (15 mM H_2O_2; C).

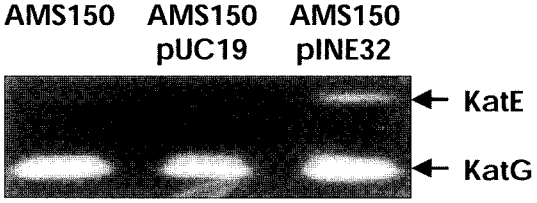

Fig. 3. A nondenaturing gel for staining of catalase activity.
Crude extracts (20 μg) prepared from the *E. coli* strains were loaded onto an 8% polyacrylamide gel and the HP activities observed. The typical KatG (HP I) and KatE (HP II) bands were visualized by staining the gel with 1% $K_3Fe(CN)_6$ and 1% $FeCl_3$.

conditions which this bacterial species frequently encounters within the host or in the estuarine environments [7, 9, 10], by investigating the signal transduction pathways involved by σ^S.

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