



Expression screen of three *Vibrio salmonicida* proteins

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INTRODUCTION

Vibrio salmonicida is a psychrophilic gram-negative bacterium causing cold-water vibriosis in salmon, cod and rainbow trout. In order to study cold adaptation of different proteins linked to DNA regulation or modification on a structural level, we have selected three proteins to be expressed, purified and crystallized.

DNA adenine methylase (Dam) is involved in gene regulation and controls DNA replication and repair. Ferric uptake regulator (Fur) is involved in oxidative stress and controls the uptake of iron. RecJ is a ssDNA specific 5'–3' exonuclease involved in recombination, replication and various DNA repair systems.

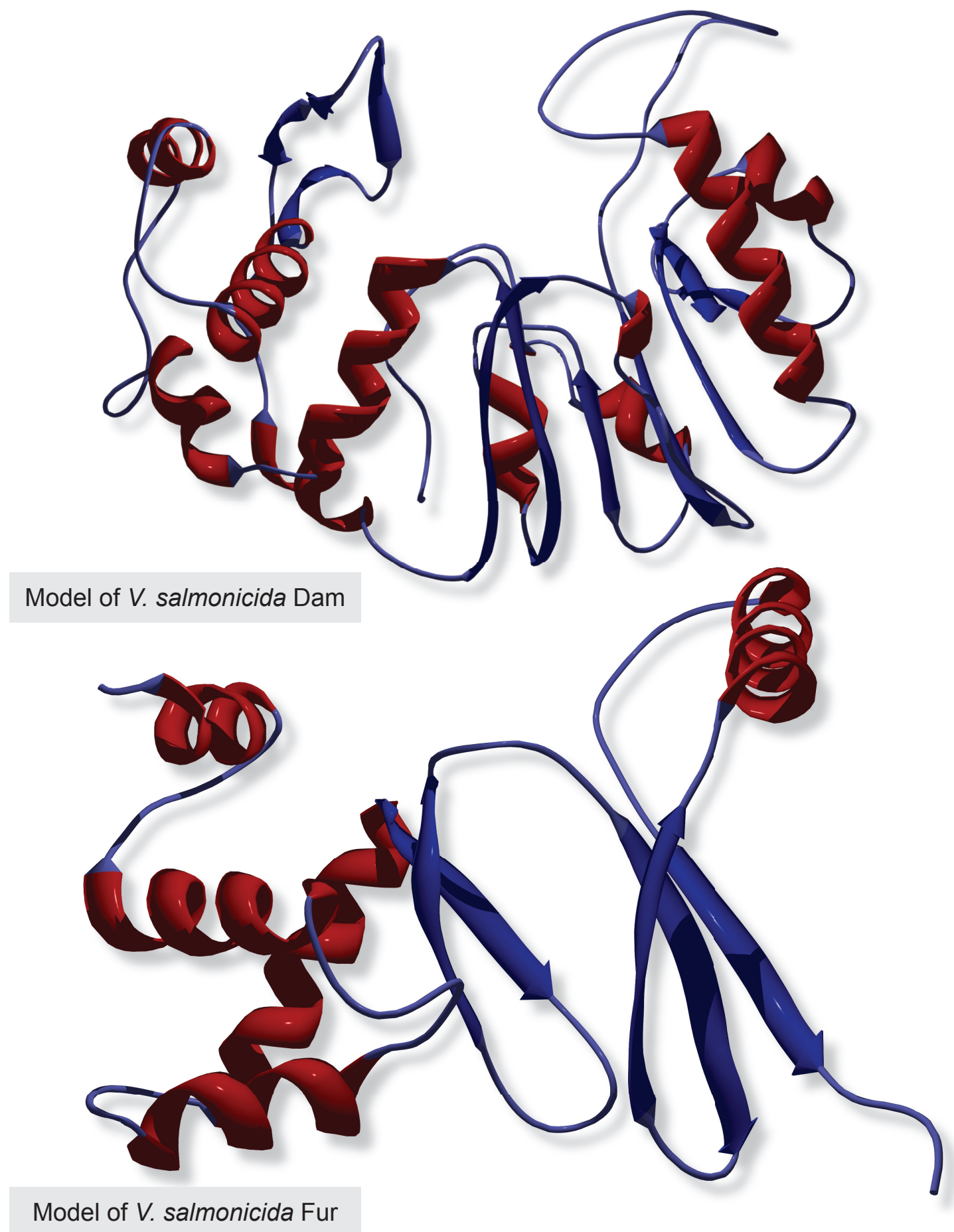


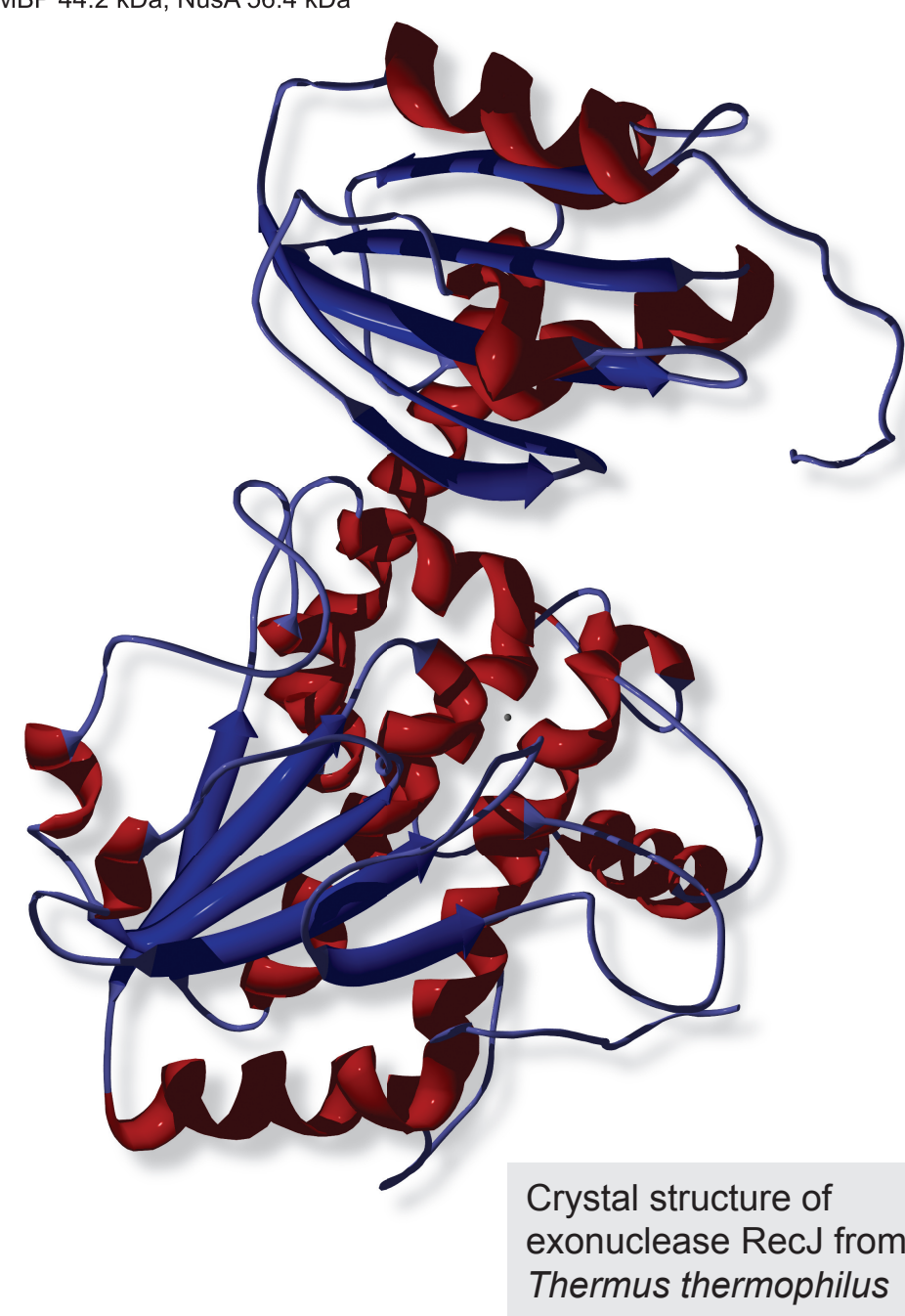
Table 2. Expression and solubility levels of Dam, Fur and RecJ when expressed as seven different fusions in *E. coli* BL21(DE3)AI or BL21(DE3) CodonPlus at 23°C or 37°C. ^{a, b, c}

Gene	Host strain	Temp	6*His		Gβ-1		Z		Thioredoxin		GST		MBP		NusA	
			Exp	Sol	Exp	Sol	Exp	Sol	Exp	Sol	Exp	Sol	Exp	Sol	Exp	Sol
Dam	BL21(DE3)AI	23°C	++	0	+	0	+	0	+	0	+	0	+/*	+	+	0
		37°C	++	0	+++	0	++	0	++	0	++	0	+/*	0	+++	0
	BL21(DE3)RIL CodonPlus	23°C	-	-	-	-	-	-	++	0	++	0	++/*	0	+++	0
		37°C	-	-	+	0	+	0	-	-	+	0	++/*	0	+++	0
Fur	BL21(DE3)AI	23°C	+++	0	+++	++	++	++	+++	++	+++	++	++	++	+++	++
		37°C	+++	0	+++	++	++	++	+++	+	+++	0	++	++	+++	+
	BL21(DE3)RIL CodonPlus	23°C	+	+	+++	++	+++	++	+++/*	0	++/*	0	+++	+++	+++	+++
		37°C	-	-	+++	++	+++	++	+	0	-	-	+++	++	+++	+++
RecJ	BL21(DE3)AI	23°C	+++	0	+++	0	+++	0	+++	0	+++	0	+++	+	+++	0
		37°C	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0
	BL21(DE3)RIL CodonPlus	23°C	+++	0	+++	0	+++	0	+++	0	+++	0	+++	++	+++	+
		37°C	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0

^a Expression levels given as: +++ = strongest band on SDS-PAGE gel, ++ = among the strongest bands, + = visible bands, * = incorrect size.
^b Solubility levels given as: ++ = minority in soluble fraction but among the strongest bands, + = visible band, 0 = nothing in soluble fraction, - = no expression.
^c Protein sizes: Dam 31.9 kDa, Fur 16.6 kDa, RecJ 63.7 kDa, Gb-1 8.6 kDa, Z 11.1 kDa, Thioredoxin 14.2 kDa, GST 26.2 kDa, MBP 44.2 kDa, NusA 56.4 kDa

Table 1. KTH Gateway adapted destination vectors

DEST-vectors	promotor	N-fusion	Cleavage site	Selection	Vector size (bp)	Added a.a. after cleavage	Fusion size (kDa)
pDEST TM 15	T7	GST		amp+chloram	7013	-	26.2
pDEST TM 16	T7	Thioredoxin	Enterokinase	amp+chloram	6675	ITSLYK KAG(S)	14.2
pDEST TM 17	T7	6xHis	-	amp+chloram	6354	-	2.4
pTH1	<i>tac</i>	MBP	Factor X _a	amp+chloram	8525	ISEPDQTS LYKKAG(S)	44.2
pTH3	T7- <i>lac</i>	Gβ-1	TEV protease	amp+chloram	7256	GDQTS LYKKAG(S)	8.6
pTH7	T7- <i>lac</i>	NusA	3C protease (PreScission)	carb+chloram	8637	GPLESTS LYKKAG(S)	56.4
pTH10	T7- <i>lac</i>	Z	3C protease (PreScission)	amp+chloram	7351	GPLESTS LYKKAG(S)	11.1



Crystal structure of exonuclease RecJ from *Thermus thermophilus*

METHODS

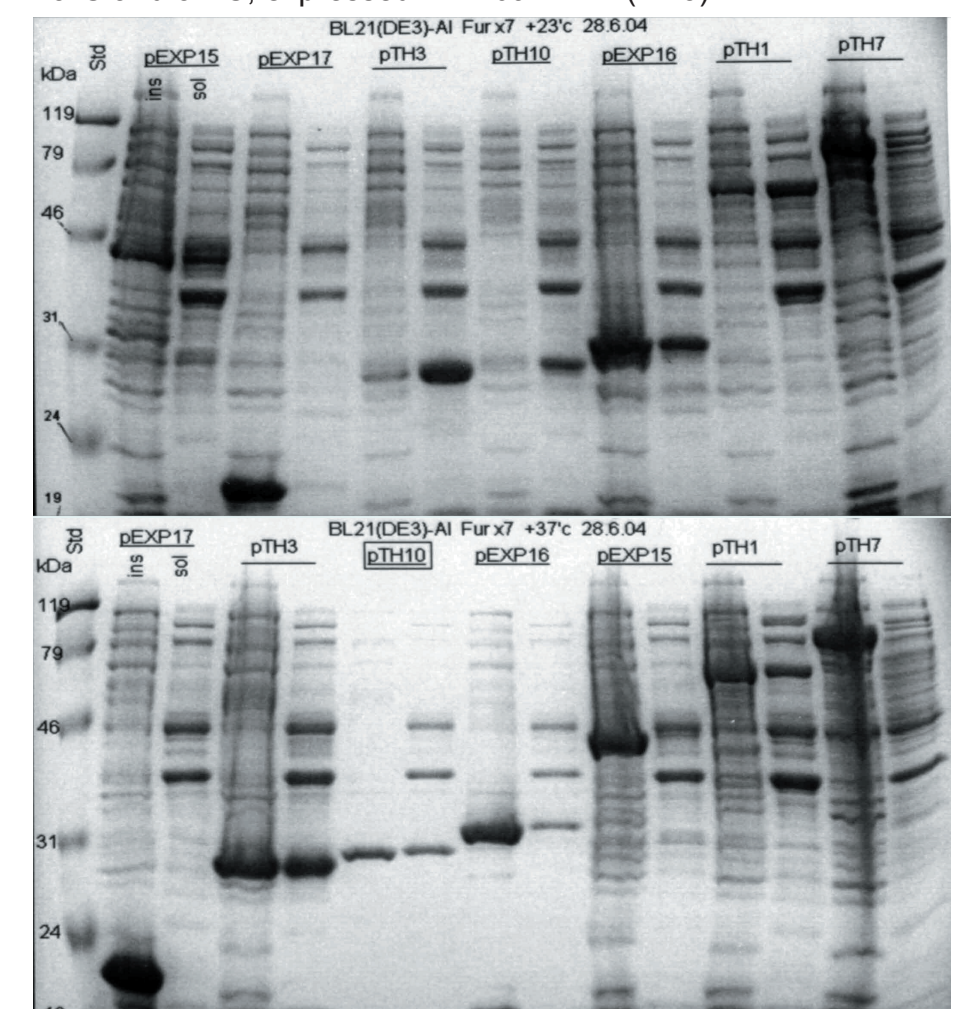
Expression of soluble native proteins was tried in several host strains and temperatures, with poor results. A screen system was set up to test the effect of seven different N-terminal fusion partners on the protein solubility (table 1). The effect of host strain and culture temperature was also tested. The GatewayTM system from Invitrogen was used for constructing expression clones. A screen for expression various destination vectors with or without an N-terminal affinity or solubility tag was made. The effect of host strain and culture temperature was also tested using two different *E. coli* expression strains and two different temperatures (table 2 and figure 1).

RESULTS & DISCUSSION

Best results were obtained with the smallest of the proteins, Fur, which was expressed in soluble form with the majority of fusion partners. For Dam and RecJ only one or two of the fusions produced soluble protein, and with Dam several fusions failed to express protein at all.

Fermentation has been carried out with pTH1-*dam*, pTH3-*fur* and pTH1-*recJ* in *E. coli* BL21(DE3)AI expression strain at 23°C. MBP-Dam and MBP-RecJ have been purified. MBP-Dam has been cleaved with FactorX_a and purified to homogeneity.

Figure 1. SDS-PAGE of Fur with different fusion partners at 23°C and 37°C, expressed in *E. coli* BL21(DE3)AI.



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- Yamagata A, Kakuta Y, Masui R and Fukuyama K. "The crystal structure of exonuclease RecJ bound to Mn²⁺ ion suggests how its characteristic motifs are involved in exonuclease activity." PNAS (2002), 99: 5908-5912.