DNA adenine methylase and RecJ exonuclease from Vibrio salmonicida

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INTRODUCTION

Vibrio salmonicida is a psychrophilic gram-negative bacterium causing cold-water vibriosis in salmon, cod and rainbow trout. To date, little is known about the molecular mechanism of host invasion, colonization and virulence. Previous studies have shown that DNA adenine methylase (Dam) negative strains of *Salmonella typhimurium* are essentially avirulent. Our goal is to find out if also the *V. salmonicida* Dam is involved in virulence.

Dam methylation regulates genes and controls DNA replication and repair. RecJ is a ssDNA specific 5'-3' exonuclease involved in recombination, replication and DNA repair. Based on structural and functional studies the characteristics of possible cold adaptation of these two DNA modifying enzymes will be described.

METHODS

The Gateway™ system from Invitrogen was used for constructing expression clones. A screen for expression with 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 1).

Expressed soluble fusion proteins with MBP-tag were purified using affinity chromatography and gel filtration. Activity of MBP-Dam was evaluated by a DNA methylation assay (figure 1). A new non-radioactive method based on fluorescence was developed for MBP-RecJ exonuclease (figure 2).

Table 1. Expression and solubility levels of Dam and RecJ when expressed as seven different gene fusions in *E. coli* BL21(DE3)AI or BL21(DE3)RIL CodonPlus at 23°C or 37°C.[a,b,c]

			6*His		Gb–1		Z		Thioredoxin		GST		MBP		NusA	
Gene	Host strain	Temp.	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	23°C	–	–	–	–	–	–	++	0	++	0	++/*	0	+++	0
	CodonPlus	37°C	–	–	+	0	+	0	–	–	+	0	++/*	0	+++	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	23°C	+++	0	+++	0	+++	0	+++	0	+++	0	+++	++	+++	+
	CodonPlus	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 band, – = no visible band, * = 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, 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.

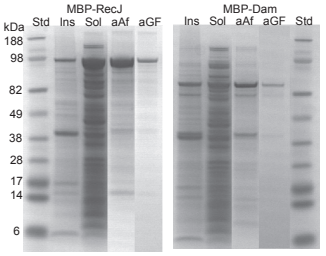


Figure 3. Purity of MBP-RecJ and MBP-Dam preparations. Ins = insoluble extract, sol = soluble extract, aAf = after affinity purification, aGF = after gel filtration.

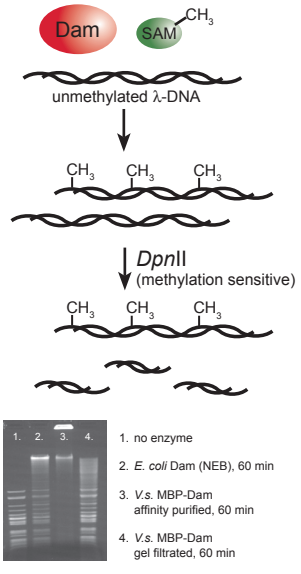


Figure 1. DNA adenine methylase assay and MBP-Dam activity. SAM = S-adenosyl-methionine.

RESULTS

Both Dam and RecJ were insoluble or unstable in their native form and with most tags. Soluble protein was obtained in both cases with an MBP-fusion. Slightly lower culture temperature (+23°C) seems to favour the production of soluble protein. With the current purification procedure the purity of the fusion protein is approximately 80% (figure 3). Both MBP-Dam and MBP-RecJ are active at 37°C.

FUTURE

The purification procedure will be improved in order to obtain pure enough material for structure determination by X-ray crystallography. Further enzyme characterization and development of exonuclease assay is underway.

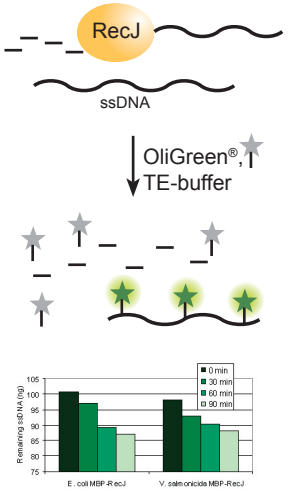


Figure 2. A schematic representation of the newly developed exonuclease assay based on fluorescence of ssDNA binding OliGreen® dye (Molecular Probes).