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The expression of truncated form of CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) from genetically modified plant in *Escherichia coli*

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Background

During the study of horizontal gene transfer of the *aro*A CP4 gene encoding CP4 EPSPS from genetically modified feed through gastrointestinal tract to bacteria living in animal gut we have observed beside full length gene also functional truncated one present in bacteria [1]. The codon usage of both forms was originally optimized for plant [2]. Therefore we have used two different *E. coli* expression systems (with *tac* promoter and T7 promoter) to compare enzymatic properties and functional activities of both forms of CP4 EPSPS protein.

Results

For the protein expression we have prepared pKK1 and pKK2 plasmids – *tac* expression vectors derived from pKK233-2 plasmid, and pMD2 and pMD72, T7 expression vectors derived from pET28a plasmid. Gene *aro*A CP4 (full length as well as truncated one) from its ATG codon was amplified by PCR from GM plant and cloned into NcoI and HindIII sites of both expression vectors (see Figure 1). We have compared phenotypic properties of full length and truncated CP4 EPSPS forms by growth on solid M9 medium. The results from this experiment are summarized in Table 1. We have also investigated growth curves of *E. coli* Δ*aro*A strains containing either pKK1 or pKK2 plasmid on liquid M9 medium without aromatic compounds. Received results are shown on Figure 2. We have received suitable expression levels of both proteins with T7 expression system for further partial protein purification based on Ni^{2+} His tag procedure and comparative enzymatic assay.

Conclusion

We can conclude from our results that truncated form of CP4 EPSPS (shorter to 31 amino acid residues on N-terminal end) can confer full function of native protein and these findings also should be taken into account in risk assessment of possible HGT from GM food/feed. The enzymatic properties and comparison of both EPSPS forms will be subject of further studies.

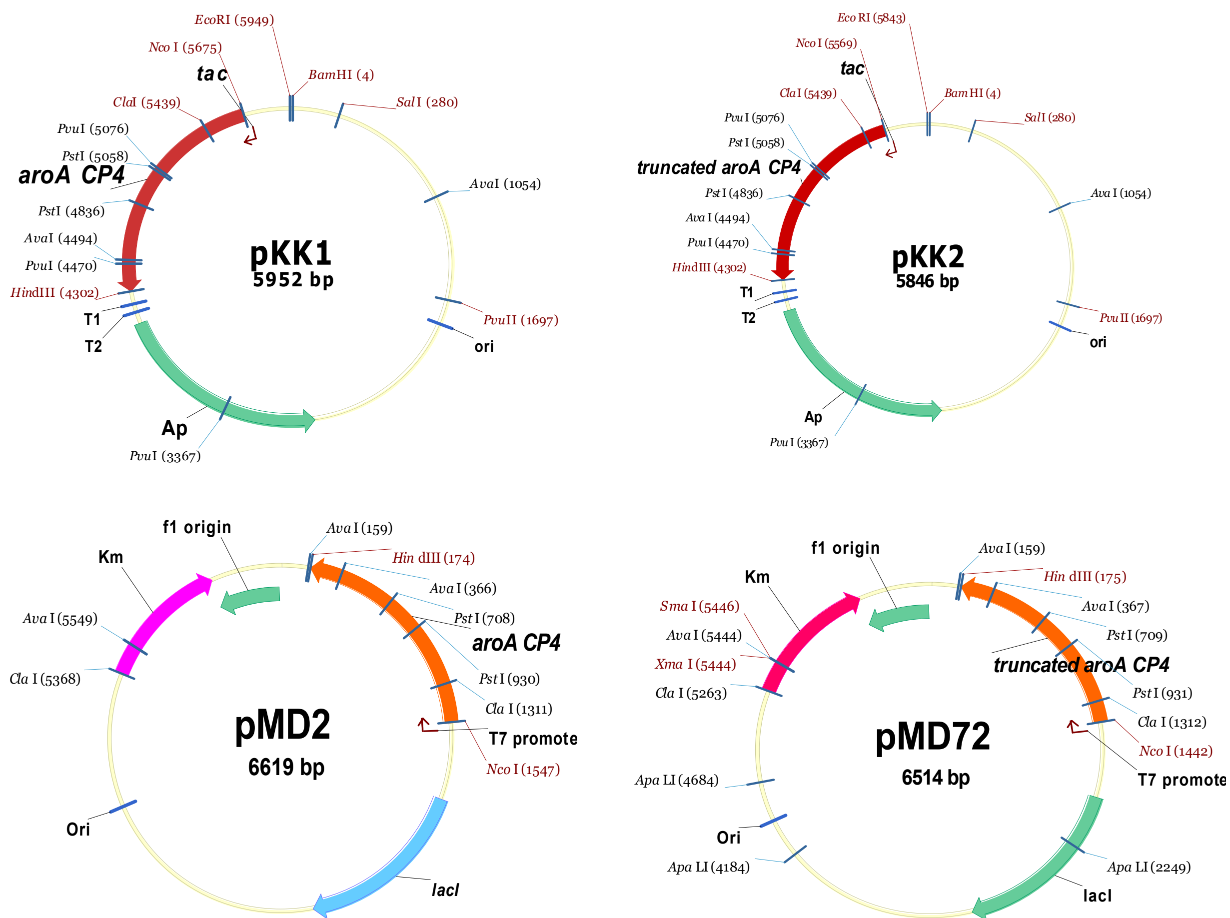

Figure 1
Physical maps of plasmids pKK1, pKK2, pMD2, pMD72.

Table 1: Complementation test of the CP4 *aro*A phenotypes.

Plasmids in *E. coli*strain	2% Glp	3% Glp	4% Glp	MM
pKK1 in SV1TcR	+++	++	+	+++
pKK2 in SV1TcR	+++	++	+	+++
pKK233-2 in SV1TcR	-	-	-	-
pKK233-2 in JM106	ND	ND	ND	+++

pKK1 – pKK233-2 with full length *cp4epsps*, pKK2 – pKK233-2 with truncated *aroA* CP4, Glp – glyphosate, + indicates the intensity of the growth, – indicates the absences of growth, MM – minimal M9 medium without aromatic amino acid supplements, ND – not done. SV1TcR strain carries Δ*aro*A mutation.

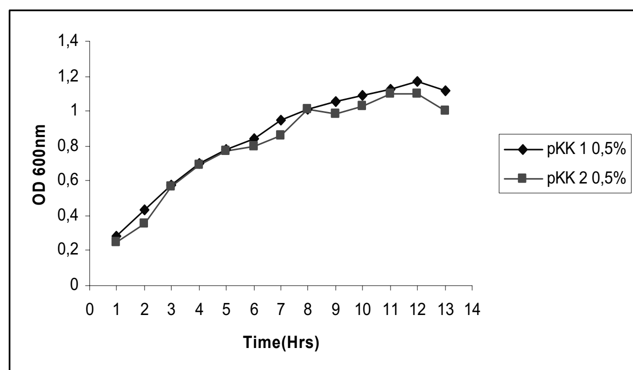

Figure 2
Growth curves of *E. coli* SV1TcR with plasmids pKK1 and/or pKK2 in M9 liquid medium. Both strains were grown in concentration of 0.5% glyphosate.

Acknowledgements
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