SUPPLEMENTARY MATERIAL

Journal: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY

Article Title: "Direct involvement of the CreA transcription factor in penicillin biosynthesis and expression of the *pcbAB* gene in *Penicillium chrysogenum*"

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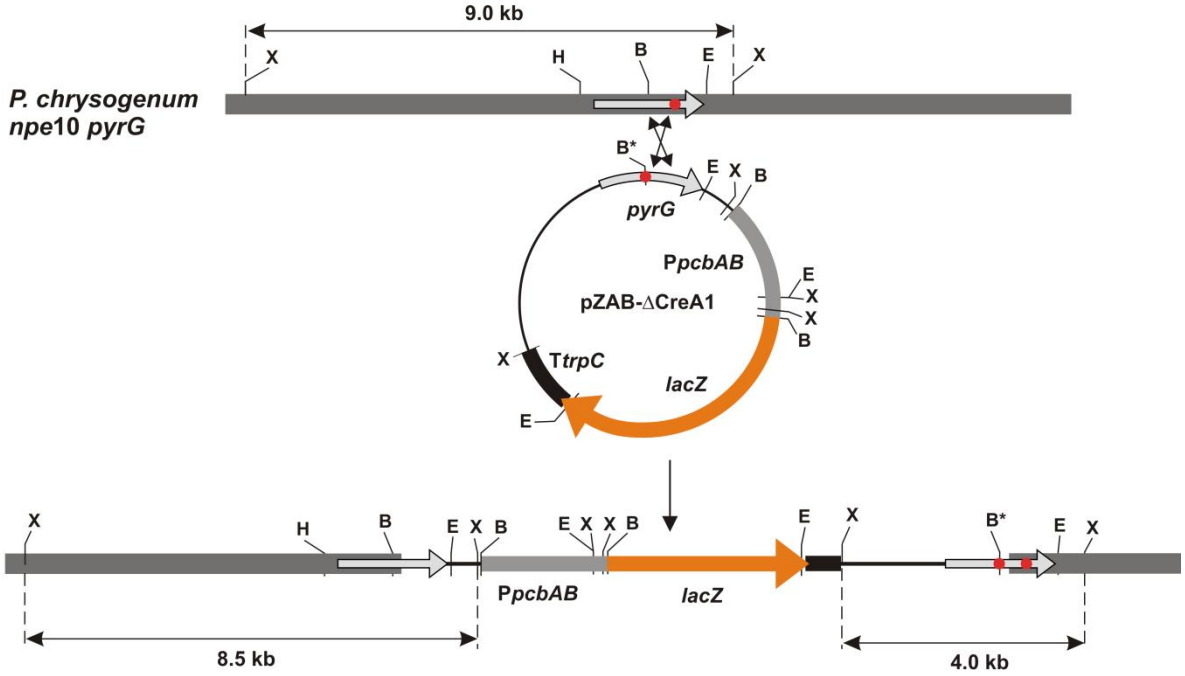
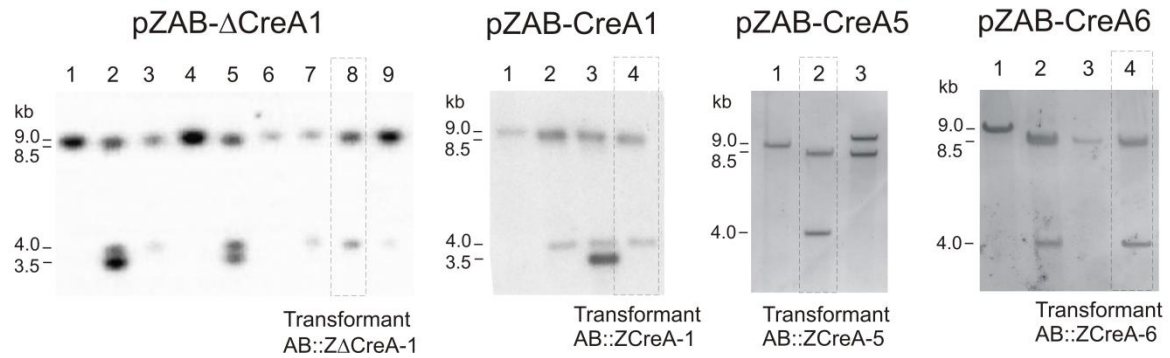
a


9.0 kb
P. chrysogenum
npe10 pyrG
X
H
B
E
X
B*
pyrG
PpcbAB
pZAB-ΔCreA1
TtrpC
lacZ
8.5 kb
4.0 kb

b


pZAB-ΔCreA1
pZAB-CreA1
pZAB-CreA5
pZAB-CreA6
kb
9.0
8.5
4.0
3.5
Transformant AB::ZΔCreA-1
Transformant AB::ZCreA-1
Transformant AB::ZCreA-5
Transformant AB::ZCreA-6

Online Resource 1 Isolation of single copy transformants with the pZ2b-derived plasmids containing the *pcbAB* promoter fused to the *lacZ* gene and mutations of CreA binding sites, following the strategy described by Gutiérrez et al. (1999). **a.** Scheme of the integration event of the pZAB-ΔCreA1 plasmid (as model for all other plasmids in Table 2) at the *pyrG* locus of strain *P. chrysogenum npe*10 *pyrG*. Loss-of-function mutations in the *pyrG* gene present in the genome of the recipient strain and in the pZ2b-derived plasmids are indicated by a red dot. A single crossing-over in the DNA region between both mutations (indicated with a crossed double arrow) enables targeted integration at the genomic *pyrG* locus with reconstitution of a functional *pyrG* gene, which allows selection of transformants in minimal medium. Restriction enzymes are: *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H) and *Xho*I (X). The *Bam*HI site in the *pyrG* gene of plasmid pZ2b was mutated to get a non-functional *pyrG* gene, and is indicated with an asterisk (B*). **b.** Selection by Southern blot of single copy transformants with the P*pcbAB*::*lacZ* fusion integrated at the *pyrG* locus. Total DNA from transformants was digested with *Xho*I. A 360 bp *Sal*I-*Eco*RI fragment from the 3'-end of the *pyrG* gene was used as probe in all hybridization experiments. When a single integration event at the *pyrG* locus occurred two bands appear in the blots, with sizes of 8.5 and 4.0 kb, as happens in lane 8 of the left panel for instance. Additional bands of 3.5 kb or other sizes reveal the presence of additional copies integrated elsewhere in the genome. The plasmid used for transformation is indicated at the top of each panel, and the selected transformant is highlighted with a rectangle. Lane 1 in all panels contains DNA from the recipient strain *npe*10 *pyrG*, which produces a hybridization signal of 9.0 kb.

Lactose

Strain	Penicillin (μg/mg dry weight) ± standard deviation				
	24 h	48 h	72 h	96 h	120 h
Wis 54-1255	4.067 ± 0.365	7.860 ± 0.821	16.613 ± 0.886	23.638 ± 1.213	22.944 ± 1.115
Wis/pJL43-RNAi	3.945 ± 0.383	7.932 ± 0.819	17.168 ± 1.016	23.555 ± 1.253	22.542 ± 1.188
Wis/CreAi-1	4.890 ± 0.339	7.786 ± 0.712	16.944 ± 0.976	23.863 ± 1.168	23.268 ± 1.226
Wis/CreAi-2	4.476 ± 0.424	9.056 ± 0.931	18.756 ± 1.167	28.864 ± 1.418	27.833 ± 1.345
Wis/CreAi-7	4.578 ± 0.395	8.896 ± 0.896	19.111 ± 0.968	28.222 ± 1.395	27.562 ± 1.291
Wis/CreAi-8	4.321 ± 0.410	8.240 ± 0.753	18.444 ± 0.922	27.764 ± 1.267	26.733 ± 1.323
Wis/CreAi-13	4.279 ± 0.353	8.249 ± 0.777	18.185 ± 1.064	27.543 ± 1.312	26.023 ± 1.377
Wis/CreAi-15	4.179 ± 0.383	8.052 ± 0.902	18.267 ± 1.207	27.117 ± 1.279	26.555 ± 1.224
Wis/CreAi-18	4.378 ± 0.404	8.417 ± 0.832	18.543 ± 1.045	27.668 ± 1.207	26.115 ± 1.188
Wis/CreAi-19	3.257 ± 0.377	7.644 ± 0.818	18.111 ± 0.952	26.378 ± 1.188	25.927 ± 1.253

Glucose

Strain	Penicillin (μg/mg dry weight) ± standard deviation				
	24 h	48 h	72 h	96 h	120 h
Wis 54-1255	3.356 ± 0.417	6.172 ± 0.644	11.682 ± 0.968	15.224 ± 1.075	15.765 ± 1.182
Wis/pJL43-RNAi	3.215 ± 0.425	6.231 ± 0.634	11.553 ± 0.867	14.952 ± 1.263	15.276 ± 1.281
Wis/CreAi-1	3.178 ± 0.397	6.675 ± 0.588	10.973 ± 0.886	15.065 ± 0.988	14.867 ± 1.203
Wis/CreAi-2	3.749 ± 0.347	8.632 ± 0.762	18.126 ± 1.183	25.367 ±1.643	25.765 ± 1.474
Wis/CreAi-7	3.852 ± 0.389	8.938 ± 0.855	18.231 ± 1.329	24.962 ± 1.557	25.043 ± 1.615
Wis/CreAi-8	3.772 ± 0.410	8.032 ± 0.715	17.753 ± 0.933	24.156 ± 1.372	24.115 ± 1.532
Wis/CreAi-13	3.592 ± 0.340	8.228 ± 0.644	17.270 ± 1.217	23.774 ± 1.515	23.259 ± 1.479
Wis/CreAi-15	3.826 ± 0.374	8.428 ± 0.613	16.855 ± 0.988	23.955 ± 1.345	23.568 ± 1.774
Wis/CreAi-18	3.613 ± 0.312	8.283 ± 0.817	18.116 ± 1.263	24.451 ± 1.523	24.026 ± 1.645
Wis/CreAi-19	3.449 ± 0.357	7.886 ± 0.738	17.439 ± 1.182	23.584 ± 1.430	22.788 ± 1.365

Lactose

Strain	pH				
	24 h	48 h	72 h	96 h	120 h
Wis 54-1255	6.35	6.50	6.55	7.10	7.53
Wis/pJL43-RNAi	6.29	6.48	6.53	7.03	7.46
Wis/CreAi-1	6.32	6.56	6.62	7.12	7.42
Wis/CreAi-2	6.47	6.54	6.63	7.31	7.66
Wis/CreAi-7	6.50	6.43	6.60	7.25	7.61
Wis/CreAi-8	6.38	6.52	6.59	7.10	7.50
Wis/CreAi-13	6.35	6.54	6.63	7.09	7.55
Wis/CreAi-15	6.41	6.48	6.56	7.16	7.47
Wis/CreAi-18	6.28	6.42	6.52	7.11	7.39
Wis/CreAi-19	6.44	6.57	6.59	6.91	7.41

Glucose

Strain	pH				
	24 h	48 h	72 h	96 h	120 h
Wis 54-1255	6.18	5.50	5.38	5.29	5.17
Wis/pJL43-RNAi	6.15	5.47	5.36	5.32	5.15
Wis/CreAi-1	6.12	5.53	5.31	5.25	5.20
Wis/CreAi-2	6.20	5.55	5.42	5.38	5.25
Wis/CreAi-7	6.18	5.60	5.48	5.40	5.29
Wis/CreAi-8	6.10	5.57	5.45	5.36	5.23
Wis/CreAi-13	6.07	5.41	5.56	5.20	5.14
Wis/CreAi-15	6.14	5.52	5.35	5.29	5.22
Wis/CreAi-18	6.08	5.49	5.38	5.30	5.18
Wis/CreAi-19	6.11	5.58	5.46	5.39	5.27

Online Resource 2 Specific penicillin production of *creA*-attenuated transformants and the control strains Wisconsin 54-1255 and Wis/pJL43-RNAi, at five different time points during the culture. Cultures were performed in flasks as described in Materials and Methods. Data represent the mean and standard deviation of three independent experiments, with two flasks per condition, and two bioassays for each sample. The two lowermost panels show the pH of the medium; the initial pH was 6.1 for both the glucose- and the lactose-containing medium.