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Evidence for the presence of β-lactamase in *Streptomyces glaucescens* and its inhibition by sodium clavulanate

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***Streptomyces glaucescens* is shown to possess β-lactamase activity which is inhibitable by clavulanate. This is important in regard to its use as a cloning host for enzymes of β-lactam biosynthesis.**

Keywords: β-lactamase; *Streptomyces glaucescens*; clavulanate; cloning

We previously reported [6] the cloning of the gene encoding cephalosporin 7α-hydroxylase, *cmcI*, from *Streptomyces clavuligerus* NRRL 3585 in the multicopy vector pIJ385 [1] its expression in *Streptomyces lividans* 66 (strain No. 1326 [1]), and the deleterious effect of β-lactamase-like activity in cell-free extracts (CFE) of the recipient on the detection of expression. β-Lactamase activity in *Streptomyces glaucescens* has never been reported, although 75% of *Streptomyces* strains produce β-lactamase [3]. We thus attempted to obtain improved expression of the *cmcI* gene using *S. glaucescens* ETH22794 [2] as the host. In this report, we describe the results and the evidence for the presence of a β-lactamase activity in *S. glaucescens*.

S. glaucescens was transformed with pIJ385 and pIJ385R1.5 + (pIJ385 carrying the cloned *cmcI* gene) as described earlier for *S. lividans* [6]. The transformants were grown in media containing thiostrepton (50 μg ml^{-1} in solid medium, 10 μg ml^{-1} in liquid medium), which is the resistance determinant carried by pIJ385 and its derivatives. For inoculation of liquid cultures, a 7-day old surface culture (1 cm^2) on CG agar [2] was transferred into 20 ml of TSB broth [4] in a 250-ml unbaffled flask. After incubation for 52 h at 30°C and 250 rpm on a rotary shaker, 1 ml of the culture was transferred into 50 ml of TSB broth in a 500-ml baffled flask. The inoculated flask was shaken for 48 h at 30°C and 250 rpm before harvest of the mycelium. Cell-free extracts (CFEs) of the mycelium were prepared and used to assay the hydroxylation of cephalosporin C (CPC) as described previously [5].

When a CFE from *S. glaucescens* carrying pIJ385 was used at a protein concentration of 11 mg ml^{-1} in the reaction, the substrate, CPC, added at an initial concentration of 250 μg ml^{-1} almost entirely disappeared by 2 h (Figure 1a and b). However, in the presence of sodium clavulanate (SC), this CPC-degrading activity was reduced (Figure 1c). Forty-seven percent of the CPC remained in the presence of 0.5 mM SC, 57% with 2 mM SC, 68% with 4 mM, and 71% with 8 mM concentrations of the inhibitor. This β-lactamase-like activity was more sensitive to the inhibition by SC than the similar activity observed in the CFE of *S. lividans* [6]. The degrading activity was also observed in CFEs of untransformed *S. glaucescens* and in its transformants carrying pIJ385R1.5 + (data not shown).

Preliminary experiments showed that SC at concentrations ranging from 1 to 24 mM did not affect the hydroxylation of cephalosporin C by *S. clavuligerus* CFEs (data not shown). When a CFE (14 mg protein ml^{-1}) from *S. glaucescens* carrying pIJ385R1.5 + was used for the hydroxylation reaction in the presence of 11 mM SC, HPLC analysis showed that hydroxycephalosporin C was formed in a 15-min incubation (Figure 2b) whereas no product was detected when a CFE from *S. glaucescens* carrying the vector alone was used (Figure 2a). In co-chromatograms, the hydroxycephalosporin C formed co-migrated with that formed by an *S. clavuligerus* CFE (Figure 2b–d).

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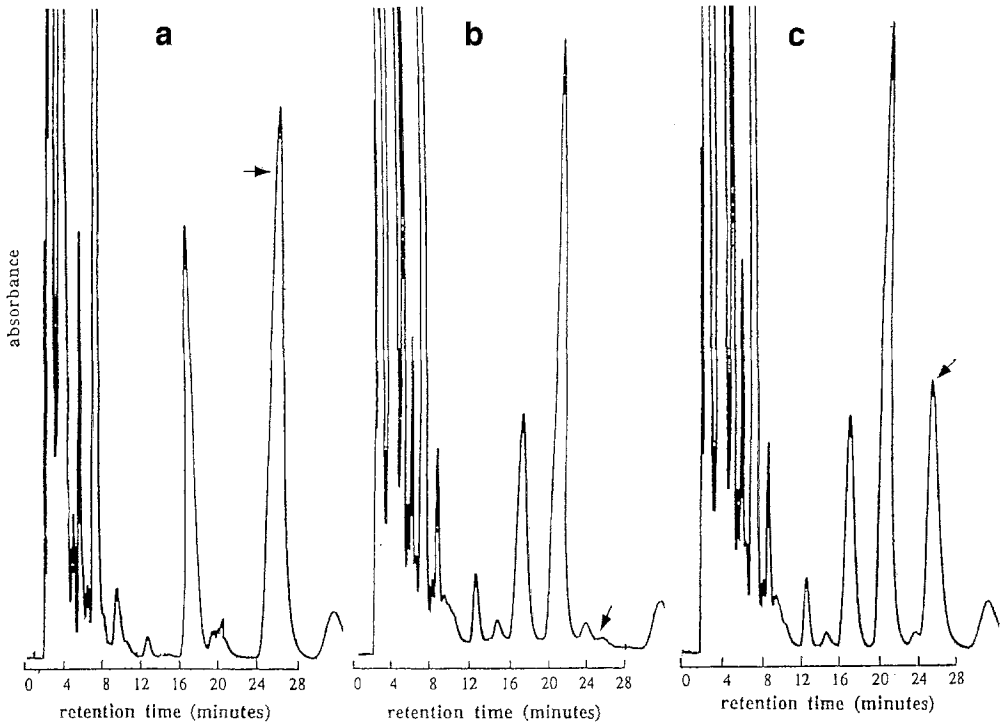

Figure 1 Degradation of cephalosporin C (CPC) by a CFE of *S. glaucescens* carrying pIJ385 in the presence and absence of sodium clavulanate (SC). Arrows indicate the position of CPC in the HPLC chromatogram. (a) Reaction time zero in the absence of SC. (b) 2 h reaction time in the absence of SC. (c), 2 h reaction time in the presence of 0.5 mM SC.

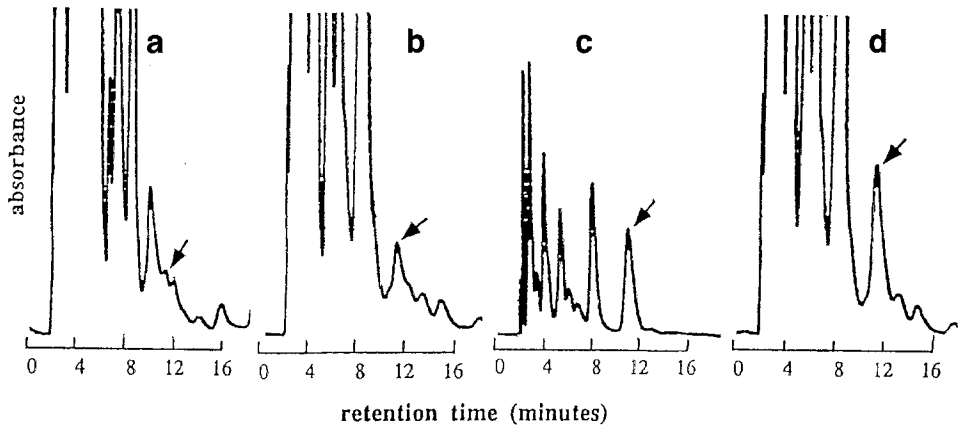

Figure 2 HPLC analysis of 7 α-hydroxycephalosporin C (HCPC) formed in 15-min cell-free enzymatic reactions. Arrows indicate the position of the HCPC peak. (a) CFE from *S. glaucescens* carrying pIJ385; 25 μl of the reaction supernatant phase were injected. (b) CFE from *S. glaucescens* containing pIJ385R1.5 +; 25 μl of the reaction supernatant phase were injected. (c) CFE from *S. clavuligerus*; 2.5 μl of the reaction supernatant phase were injected. (d) Co-chromatogram of (b) and (c); 22.5 μl of the supernatant phase from reaction (b) plus 2.5 μl of the supernatant phase from reaction (c) were injected.

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