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Short Communication

Transformation of protoplasts of *Cellulomonas flavigena*

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SUMMARY

Protoplasts of *Cellulomonas flavigena* (Cm^s) were transformed with plasmid pC194. Transformation frequency was 2.72×10^{-3} in MR-1 regeneration medium with 2 μg/ml chloramphenicol. Transformation conditions are described.

INTRODUCTION

Cellulolytic microorganisms are of potential industrial importance, in particular, *Cellulomonas flavigena* for single cell protein production [6]. Genetic modification of this microorganism is essential in order to obtain higher cellulase yields, which allows for better growth of this bacterium on cellulosic waste. Cellulase genes have been cloned and expressed in other microorganisms [8,15], and cloning in the same genus would ensure high levels of expression if plasmids are stably maintained since heterologous signals would be absent. *Cellulomonas flavigena* itself has not been transformed with foreign DNA.

MATERIALS AND METHODS

Microorganisms. Cellulomonas flavigena (Cm^s) was separated from mixed bacterial culture ATCC 3920. *Bacillus subtilis* SB34 containing (pC194) was a donation from Dr. Laurent Janniere, Biotechnology Institute, INRA, France.

Protoplast formation. Solutions, reagents and conditions were previously reported. [5,14].

Plasmid isolation. pC194 was isolated from *B. subtilis* as described by Kieselburg et al. [10]. The plasmid was characterized by electrophoresis in 0.8% horizontal agarose gel and visualized by short-wavelength UV light after being stained with ethidium bromide.

Protoplast transformation. 100 μl of pC194 DNA (574 μg/ml) in SMM 2× buffer were added to 0.5 ml of *C. flavigena* protoplast suspension, together with 1.5 ml of 40% PEG (M.W. 1000). Gentle mixing was performed for 2–3 min and 20 ml SMMP buffer were added. Protoplasts were collected by centrifugation at 1240 × *g* for 20 min. The cells were suspended in the same initial volume with SMMP buffer, followed by gently agitation for 90 min at 37 °C, to allow phenotypic expression. Decimal dilutions in SMMP were performed and were spread on regeneration medium MR-1, supplied with 0.35 M KCl as an osmotic stabilizer [14], with or without 2 μg/ml chloramphenicol. Transformants appeared after 4 or 5 days of incubation at 37 °C. Transformation efficiency was calculated as the ratio between the colony forming units (cfu) in the medium with antibiotic (transformants) and those that grew in MR-1 without antibiotic.

RESULTS

Plasmid pC194, which confers resistance to chloramphenicol, was extracted from *B. subtilis* SB234 and used to achieve PEG-induced genetic transformation of *C. flavigena* protoplasts.

Table 1 shows the results of transformation experiments performed where efficient transformation was achieved. Transformants appeared after 4–5 days of incubation on MR-1 regeneration plates with 0.35 M KCl

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TABLE 1

Transformation efficiency of *Cellulomonas flavigena* protoplasts by plasmid pC194

Experiment	Regenerants obtained (cfu)	Transformants obtained (cfu)	Transformants efficiency[a]
1	3.9×10^6	1.0×10^4	2.56×10^{-3}
2	4.4×10^6	1.2×10^4	2.72×10^{-3}

[a] Transformants per regenerants.

and 2 μg/ml chloramphenicol. Subsequently, a chloramphenicol MIC (minimal inhibitory concentration) test was applied to transformants. *C. flavigena* transformants grew in a medium containing 5 μg/ml, whereas the wild-type strains did not grow in concentrations of the antibiotic greater than 0.5 μg/ml (Fig. 1).

Attempts to extract pC194 were performed on two transformant strains, using the extraction techniques reported by Birnboim and Doly [3], Kieselburg et al. [10] and Eckhardt [7]. However, the plasmid could not be detected following agarose electrophoresis. The plasmid may exist either in an episomal form or may be integrated into the chromosome.

DISCUSSION

One of the necessary conditions to achieve the introduction of foreign DNA into a recipient cell is the complete elimination of the cell wall, as well as a high percentage of regeneration [1,2,12]. Plasmid pC194 from *B. subtilis* was selected for its characteristics of low molecular weight, readily identifiable phenotype and having multiple copies. In spite of the low percentage of protoplast regeneration, as low as 0.054% [14], the transformation efficiency obtained (2.72×10^{-3}) was comparable to the result obtained in other genera, such as *Corynebacterium glutamicum* (4.6×10^{-4}) [9], and 6.6×10^{-4} for *Clostridium acetobutylicum* [11]. The results shown in Table 1 indicate that the conditions are reproducible, since the order of magnitude of the transformants obtained is very similar.

The acquisition of chloramphenicol resistance was selected as a transformation indicator, as wild-type *C. flavigena* is sensitive to this antibiotic (0.5 μg/ml) and transformants resisted ten-fold this amount (5 μg/ml). This level of resistance is lower than the level tolerated by the donor strain, a phenomenon observed earlier by Chang and Cohen [5], McDonald and Burke [13] and Vehmaanpera [16] for *Bacillus*.

The inability to extract the plasmid from the transformant strains by any of the techniques employed suggests that the plasmid might have homologous sequences to the chromosome of *C. flavigena* and may have become integrated. Martin et al. [12] reported that pC194 behaved as an insertion element when it was introduced into *Bacillus thuringiensis* protoplasts, but it could be isolated as an extrachromosomal element after introduction into *B. subtilis* [5] and *B. megaterium* [4].

Despite the low regeneration frequency of *C. flavigena* protoplasts, it was possible to obtain Cm^r transformants. At present we are carrying out plasmid DNA detection by means of the hybridization technique.

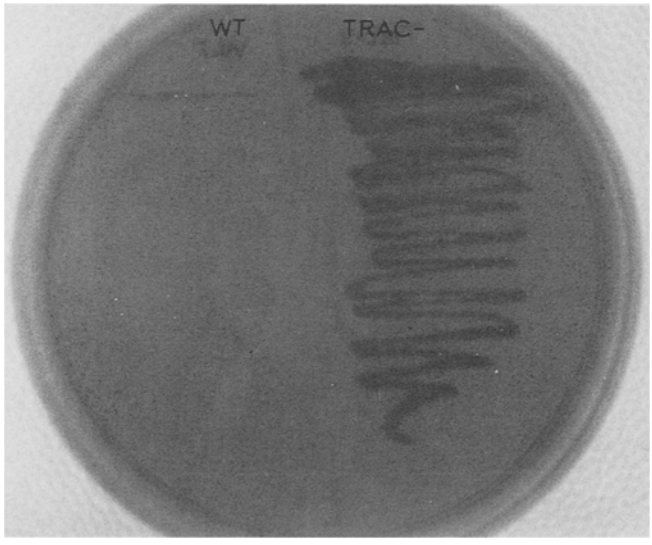

Fig. 1.

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