Using fusions with luxAB from Vibrio harveyi MAV to quantify induction and catabolite repression of the xyl operon in Staphylococcus carnosus TM300

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Abstract: The luxA, B genes from the Gram-negative marine bacterium Vibrio harveyi MAV were used in Staphylococcus carnosus TM300 as a reporter system for regulated expression of xylose utilization. The luciferase genes were fused to the xyl operon from Staphylococcus xylosus C2a. Expression of bioluminescence was induced through addition of xylose and repressed in the presence of glucose. A method to quantitate bioluminescence directly from the culture is described.

Key words: Staphylococcus xylosus; Bioluminescence; Gene regulation; Reporter genes; Staphylococcus carnosus

Introduction

Staphylococcus carnosus TM300 belongs to the coagulase negative staphylococci and has been used as a starter culture for dry sausage fermentation [6,10]. It has gained importance in molecular biology and biotechnology as it can be efficiently employed as a cloning host and is able to stably maintain and express foreign genes. Unfor-

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tunately, not much is known about its molecular biology and only few reporter systems for gene expression, like an extracellular lipase [13], are available for this organism. The use of the luciferase genes from Vibrio harveyi MAV has been described in Gram-negative [1] and Gram-positive [2,11] bacteria and seemed also suitable for S. carnosus. To evaluate its use in S. carnosus, transcriptional as well as translational fusions of luxA and luxB coding for the luciferase subunits were constructed to the xyl operon from S. xylo-
sus. The genetic organization of xyl genes is displayed in Fig. 1a [12,13]. Regulated light emission [14] from S. carnosus containing these fusions was quantified using a luminometer.
Materials and Methods

Bacterial strains and plasmids

*Staphylococcus carnosus* TM300 [3] was used as a cloning host throughout this study. The *lux* genes were from *Vibrio harveyi* MAV and were used either as separate genes *luxA* and *luxB* derived from plasmid pLX203ab [9] or as a *luxAB* fusion derived from plasmid pLX703fab9 [3].

Growth media

Luciferase assays were performed in Mopso reduced medium [13] with 0.5% glycerol as a non-regulative carbon source and addition of 1% xylose to induce the *xyl* operon and additional 0.5% glucose for determining catabolite repression. Staphylococci were grown with shaking at 37°C.

Determination of luciferase activity

Colonies were subjected to n-decanal vapor by streaking the substance on the lid of the Petri dish and light emission was detected by exposure to a Kodak X-Omat film. For quantification with the luminometer Lumat LB 9501, Berthold (see Results), the substrate was injected as a sonified emulsion of 90 parts H₂O, nine parts ethanol and one part n-decanal.

Results

Construction of a transcriptional *xyl-luxA,B* fusion

Plasmid pLX203ab (see Fig. 1b) was restricted with *Pvu*II and *Bam*HI and the resulting 2.2 kbp *luxA,B Bam*HI fragment was ligated with *Bam*HI/*Bgl*II restricted pIC20H [7]. Candidates with the *Bam*HI site adjacent to the 5' end of the *luxA* gene were screened for luciferase activity expressed from the *lac* promoter on pIC20H. The resulting construction named pWH505 was restricted with *Hind*III and the 2.25 kbp *lux* fragment ligated with *Hind*III linearized pC194 [5] yielding pWH506. The *xyl* regulatory region with *xylR* was isolated from pXRL10 [13] by restriction with *Bam*HI and cloned into *Bam*HI linearized pWH506. Candidates which showed xylose inducible light emission contained *luxA,B* under control of the *xyl* operon and were named pWH507 (see Fig. 1b).

![Fig. 1.](attachment:figure1.png)

(a) Organization of the *xyl* operon from *S. xylosus*. *xylR* is the gene for the Xyl repressor, *xylA* for the xylose isomerase, *xylB* for the xylulokinase. *P, O* designate the *xyl* promoter and operator, respectively. (b) Transcriptional (pWH507) and translational (pWH389) fusions between the luciferase genes from *V. harveyi* and the *xyl* operon from *S. xylosus*. ORF A-D designate the open reading frames of plasmid pC194, MCS the multiple cloning site. *luxA* and *luxB* abbreviate the genes for the luciferase subunits, SD the ribosome binding site. *xylR* is the Xyl repressor gene, *xylA* the gene for the xylose isomerase. −10 and −35 elements of *xyl* promoters are indicated by boxes. The relevant restriction sites are: H: *Hind*III, B: *Bam*HI.
**Construction of a translational xylA-luxAB fusion**

It has been described that use of a luxAB gene fusion results in stronger light emission than use of the separate genes [3]. The luxAB fusion from plasmid pLX703fab9 was ligated with a *Pst*I cleaved derivative of pC194 containing the multiple cloning site of pIC20H. The xyl region with 167 bp of the xylA reading frame was isolated from *Pst*I digested pXyl12 [12,13]. The resulting construction was termed pWH389 (see Fig. 1b) and showed xylose inducible light emission. A *Bst*XI deletion within the xylR gene resulted in a constitutive phenotype. This derivative was named pWH389ΔXR.

**Quantitative analysis of xyl mediated luciferase activity**

Light emission was measured as relative light units, RLU, calculated as number of impulses divided by ten. Light emission reached a peak at 1.5 s after substrate injection and decreased over a period of several minutes. We have obtained reproducible results with a measuring time of 15 s starting at the time of decanal injection. The bacteria were grown in Mopso reduced medium at 37°C to an optical density (578 nm) of 0.4-0.5. After addition of tetracycline to 50 μg/ml, the cultures were incubated at 23°C without shaking for 60 min prior to the measurement. This treatment was necessary, because light emission increased steadily over a period of several hours at 23°C, while cell density only changed marginally in the absence of tetracycline. This effect was eliminated by addition of the antibiotic. The samples were vortexed briefly before assaying and 100 μl of the culture was used for determining light emission by injecting 100 μl of the substrate. The optical density of the culture was determined after this step. For quantification, the relative light activity (RLA) was calculated as follows:

\[
RLA = \frac{RLU_\text{s} - RLU_\text{R}}{OD_{578}} \times 10
\]

RLU_s = light emission of the culture determined for 15 s
RLU_R = light emission of a lux^- control strain

Each determination was done using three independent colonies and all measurements were repeated twice. Reproducibility of the method was demonstrated by analyzing the same culture three times yielding deviations of less than 4%. Light emission was linear with growth over an optical density (578 nm) between 0.2 to 0.8 as shown in Fig. 2. This corresponds to the log phase. At the beginning of the stationary phase above 0.5 OD luxAB expression per cell is reduced (see Fig. 2).

Inducibility and glucose repression of the xyl operon was determined using pWH507, pWH389 and pWH389ΔXR (see Fig. 1B). The transcriptional fusion on pWH507 is less active than the translational fusion on pWH389. Repression in the presence of glucose is about 100-fold with both plasmids, whereas luminescence encoded by pWH389ΔXR is only about 50-fold glucose repressed. xylR dependent xylose induction is about

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**Table 1**

<table>
<thead>
<tr>
<th>S. carnosus</th>
<th>No supplement</th>
<th>1% xylose</th>
<th>1% xylose and 0.5% glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>transformed with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC194-MCSb b</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>pWH507</td>
<td>3 × 10² ± 3.0 × 10³</td>
<td>1 × 10⁶ ± 0.1 × 10⁶</td>
<td>1 × 10⁴ ± 4 × 10³</td>
</tr>
<tr>
<td>pWH389</td>
<td>4 × 10² ± 0.6 × 10²</td>
<td>6 × 10⁶ ± 0.5 × 10⁶</td>
<td>4 × 10⁴ ± 4 × 10³</td>
</tr>
<tr>
<td>pWH389ΔXR</td>
<td>1 × 10⁷ ± 1.0 × 10⁶</td>
<td>1 × 10⁷ ± 1.0 × 10⁶</td>
<td>2 × 10⁵ ± 2 × 10⁴</td>
</tr>
</tbody>
</table>

a mean ± SD of three independent clones of two determinations; b pC194-MCSb contains the polylinker of pIC20H in pC194 and was used as a control to determine background light emission.
Fig. 2. Light emission from the plasmid pWH389 in *S. carnosus* TM300 as RLU (× 1000) during growth in Mopso reduced medium with 0.5% glycerol as non-regulative carbon source and 1% xylose. It may be taken from this curve that light emission per cell is constant during logarithmic growth which occurred up to 0.8 OD (not shown).

10^2-fold with pWH507 and about 10^4-fold with pWH389 (see Table 1).

**Discussion**

We demonstrate that the luciferase genes *luxA* and *luxB* from the Gram-negative organism *Vibrio harveyi* MAV can be used in *Staphylococcus carnosus* TM300 as a reporter system. Previous quantifications of light emission relied mostly on the use of scintillation counters, which offer high sensitivity, but do not allow immediate detection of light emission after substrate injection. We report a protocol for quantitative determination of light emission in *S. carnosus* using a luminometer with automatic substrate injection. This approach increases the sensitivity of the method since the highest light intensity occurs right after addition of decanal, which apparently diffuses through the cell membrane. Disruption of the cells is not necessary for this determination thus facilitating the use with staphylococci, as they are resistant to mechanical disruption by sonication and French press and homogenization using glass beads cannot be reproducibly performed. Quantification of light emission was possible because expression of the luciferase genes did not alter the growth behavior of the host organism. A negative effect on growth was described for *Bacillus* ssp. [5] and also appeared to be the case with high *lux* activity in *E. coli* [8].

Previous experiments with *xyl-lipA* fusions showed that repression of the xylose utilizing genes is dependent on a functional *xylR* [13]. Glucose repression could not be determined using the lipase system [13], because it was glucose responsive itself. By use of this system, both levels of regulation [13] of the *xyl* operon from *S. xylosus* could be quantitated in vivo. In particular, the *lux* system proved useful for quantitative determination of glucose repression in this organism. Since it is slightly more efficient (2-fold) in *xylR*+ strains a functional Xyl repressor may contribute to glucose repression. This could either be due to glucose mediated exclusion of the inducer xylose or competition between glucose and xylose for Xyl repressor binding. However, the main contribution (50-fold) to glucose repression is not *xylR* dependent.

**References**

8 Müller, G. unpublished results.