Curcumin production by engineered *Escherichia coli* biofilms

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Introduction

Curcuminoids are **polyphenolic compounds** isolated from the rhizome of *Curcuma longa,* and are a mixture of **curcumin**, desmethoxycurcumin and bisdemethoxycurcumin.

Despite being famous for their **therapeutic properties,** pure curcuminoids are **very expensive** and **rare** because the plant is seasonal and grows slowly.

The production of curcuminoids has been described using an **artificial pathway in *E. coli*** [1]. A 4-step synthetic pathway has already been tested in **planktonic *E. coli* cells,** being able to produce **563 mg/L of curcumin from ferulic acid** [2].

The main goal of this work was to attempt the production of curcumin, an added-value compound, using biofilms as production platforms.

Material and Methods

E. coli JM109(DE3) cells transformed with a plasmid carrying the three genes encoding for the artificial pathway of curcumin was used. Figure 1 illustrates the workflow of this study.

***E. coli* JM109(DE3)** cells transformed plasmid carrying the genes for the artificial pathway

Culture medium:
- Terrific broth (TB)

Surface:
- Polyvinyl chloride (PVC)

30 °C; 185 rpm; 9 days

Induction at day 3:
1 mM IPTG
8 mM ferulic acid

30°C vs 26°C

Planktonic and biofilm characterization:
- Culturable cells – colony-forming units
- Curcumin quantification – HPLC

Fig. 1 – Diagram of the materials and methodology used in these assays

Results

Regarding **planktonic fraction** (fig.2-A) at day 4, induced cultures at 30°C presented 14% more culturable cells than at 26°C. This difference became more evident on days 6 and 7, with 73% and 85% more culturable cells, respectively, at 30°C than at 26°C ($p < 0.05$).

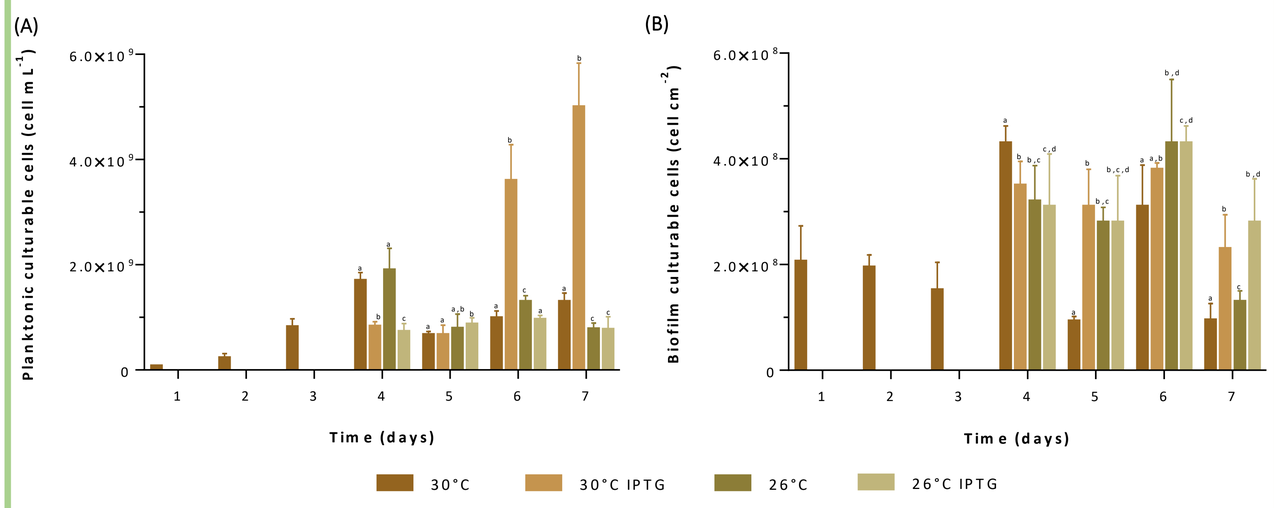

Fig. 2 – Planktonic (A) and biofilm culturable cells (B) during a 7-day experiment. The means and standard deviations (SDs) of three independent experiments are presented. Statistically significant differences on each time point were considered, and different lowercase letters indicate statistically significant differences between the conditions on each day ($p < 0.05$).

Regarding **biofilm fraction** (fig.2-B), biofilms grown at 30°C presented a similar pattern to that observed in the planktonic state. After day 4, viable cells at 30°C showed a decrease, maintaining levels below those observed for biofilms grown at 26°C, with and without induction. For biofilms incubated at 26°C, induction did not promote an increase in the number of biofilm culturable cells (except on day 7).

Curcumin production was about **14-fold higher in the biofilm state** than in the planktonic state at days 4 and 5 (fig. 3), with production values of on average of 0.14 and 1.77 fg.cell^{-1} on planktonic and biofilm states, respectively.

Regarding the biofilm state, 30 °C seemed to slightly benefit curcumin production.

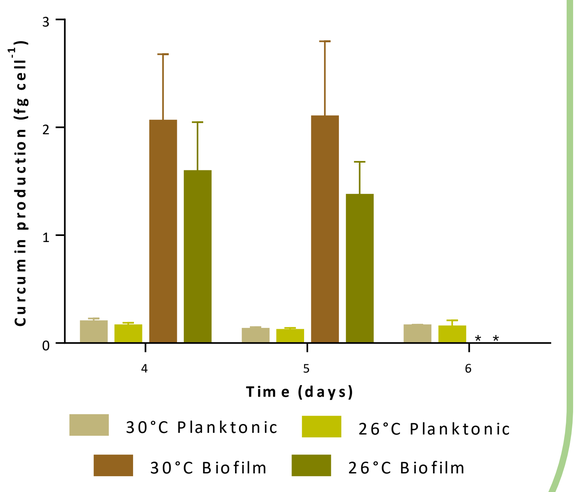

Fig. 3 – Curcumin production after induction at different temperatures. * no curcumin production was quantified.

Conclusion

In general**, induction negatively affected the growth of planktonic and biofilm cultures**, except at 30°C where the chemical induction promoted the planktonic growth.

Biofilm state tended to promote the production of curcumin compared to the planktonic state, and 30°C seemed to benefit the biofilm production.

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