Effects of glutamine synthetase on indigoidine production in *Escherichia coli*

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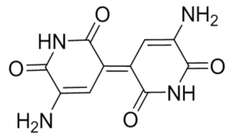
Introduction

Sc-indC is an indigoidine synthetase gene that has been previously cloned from *Streptomyces chromofuscus* into *Escherichia coli* BAP1. The gene was shown to produce the blue pigment indigoidine in *E. coli*. Glutamine is a necessary precursor to indigoidine. The gene *glnA* codes for the enzyme glutamine synthetase in *E. coli*. In this work, we examined whether introduction of *glnA* would increase the supply of glutamine and thus improve indigoidine production.

Indigoidine 

Indigoidine is not only a natural blue pigment, but also a powerful radical scavenger and antioxidant agent. This compound enables phytopathogens to tolerate oxidative stress, organic peroxides and superoxides during the plant defense response. Recently, indigoidine has also been found to possess antimicrobial activity.

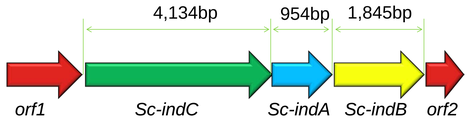
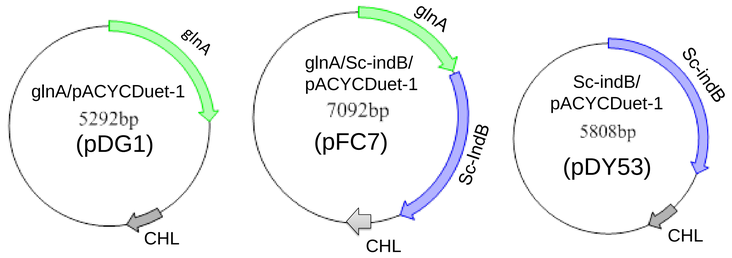


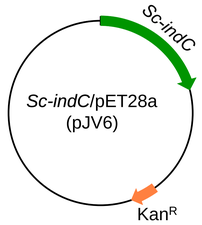
Fig. 1 The indigoidine biosynthetic gene cluster in *S. chromofuscus* ATCC 49982.

Methods

- Cultured *E. coli* BL21 in LB medium, and extracted the genomic DNA using phenol/chloroform extraction.
- PCR to amplify the target gene *glnA*.
- *Sc-indC* was ligated into pET28a to yield pJV6.
- *glnA* was ligated into pACYCDuet-1, yielding pDG1.
- *Sc-IndB* was ligated into pACYCDuet-1, yielding pDY53.
- *glnA* and *Sc-IndB* were ligated into pACYCDuet-1, yielding pFC7.
- The plasmids were expressed or co-expressed in *E. coli* BAP1 for product analysis.

Construction of Plasmids





List of transformants

- *E. coli* BAP1/pJV6
- *E. coli* BAP1+pDY53/pJV6
- *E. coli* BAP1/pDG1+pJV6
- *E. coli* BAP1+pFC7/pJV6

Time Course Evaluation

- Colonies were picked, and cultured overnight in 5 mL of LB with 5 μL of necessary antibiotics, in a 37°C shaker.
- 200 mL of LB was inoculated with 1 mL of overnight culture, and continued incubation in shaker.
- When OD of the 200 mL cultures reached ~0.6, 40 μ L of 1M IPTG was added to induce indigoidine production.
- Cultures were then incubated in a 200 rpm shaker at 18°C (indigoidine production has been shown previously to be most productive at lower temperatures).
- 1 mL of samples were removed at regular time intervals, centrifuged, substrate was tested for OD. DMSO was added to cells to extract indigoidine, and OD was then tested.

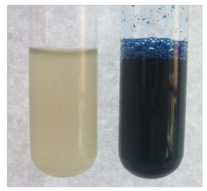
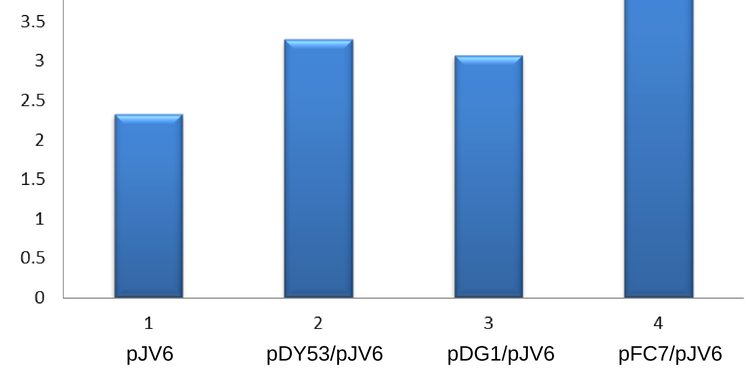


Fig. 2 Production of indigoidine in *E. coli*. (1) control; (2) Production of indigoidine by *E. coli* BAP1/pJV6.

Results

- From OD results and the established standard curve, the final titers of indigoidine in different *E. coli* strains were determined.
- Final titers were measured after ~20 hours

Time Course	pJV6 *indC*	pDY53/pJV6 *indC+indB*	pDG1/pJV6 *indC+glnA*	pFC7/pJV6 *indC+glnA +indB*
1	2.19 g/L	3.50 g/L	3.20 g/L	4.22 g/L
2	2.46 g/L	3.05 g/L	2.94 g/L	4.27 g/L
Average	2.33 g/L	3.28 g/L	3.07 g/L	4.25 g/L



Conclusion and Future Research

- *glnA* has been shown to increase indigoidine production in our experiments.
- Additional repeated expermiments will be done to further confirm our results.
- This method of co-expressing *glnA* and *Sc-indC* will be tested against the method of feeding of glutamine to cultures.
  - Glutamine is expensive, therefore we hope to determine the cost effectiveness of co-expression over the feeding method.