NATURAL BLUE DYE HETEROLOGOUS PRODUCTION IN BACTERIA

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ABSTRACT

The key to ensuring efficient production through bacterial cultivation is understanding all the factors involved in the bioprocess and how they affect cell metabolism. Indigoidine is a natural dye that has received a lot of attention recently due to its potential use in the textile and food industries. However, despite its great potential, the production of indigoidine by bacterial strains still has many variables to be improved in order to achieve a successful industrial process. Therefore, the aim of this study was to evaluate the production of indigoidine at two different temperatures by engineered *Bacillus subtilis*. The indigoidine synthetase gene was knocked in the bacterial genome. The resulting strain was cultivated in a complex medium for 45h at 30ºC and 37ºC. As a result, it was observed that the temperature of 30ºC was more favorable for the production of indigoidine, with a production rate 79% higher than in the culture at 37ºC. At 30ºC the growth rate was slower, but the cell population reached a higher density at maximum point. We conclude that the lower temperature favored heterologous production of the blue dye.

Keywords: Indigoidine. Heterologous production. Bioprocesses. Temperature.

1 INTRODUCTION

It's already known that change in cultivation temperature of microbial strains leads to a changes in the metabolism, thus influencing growth and metabolite production. Cellular structural modification, increased synthesis of genetic material, and variation in protein activity are important consequences of decreasing or increasing the cultivation temperature. Proteins constitute an average of 50% of the cell's dry weight, and temperature fluctuations can affect its structure, and consequently their activity[1,2].

Indigoidine is a natural blue dye with potential applications in the dye industry, such as for dyeing fabrics and as additive in drinks, foods, and cosmetics. Indigoidine is synthesized through the condensation of two L-glutamine molecules catalyzed by the ATP-dependent enzyme indigoidine synthetase (BpsA). BpsA is a highly stable enzyme with tolerance to a relatively wide range of pH and with the ability to function in different media compositions[3,4,5].

Much of the work carried out using bacterial strains to produce indigoidine was carried out at 30ºC, in most cases this temperature was selected taking into account the cell growth of the strain used and not necessarily the optimal temperature for enzyme activity and product stability. Therefore, the main objective of this work is to evaluate the production of indigoidine in an engineered *B. subtilis* strain and cell growth at 30ºC and 37ºC.

2 MATERIAL & METHODS

The DNA sequence corresponding to the *bpsA* gene from *Streptomyces lavendulae* responsible for the production of indigoidine was inserted into the *amyE* locus in the *B. subtilis* genome through recombination. The resulting strain was cultivated in 10 mL of complex culture medium (PW) in test tubes at 30ºC and 37ºC and shaking at 220 rpm for up to 43h. Culture growth was evaluated spectrophotometrically at 800 nm. At the end of cultivation, the culture was centrifuged at 15,000 xg for 20 min, and the supernatant was discarded. The pellet was then resuspended in DMSO and subjected to vigorous vortexing for 10 min, after which the suspension were centrifuged at 15,000 xg for 10 min and the supernatant reserved in a clean tube. This step was repeated six times. Finally, 200 uL of the reserved supernatant analysed at 600 nm for indigoidine quantification. The indigoidine titer was calculated using a calibration curve previously built using the pure substance.

3 RESULTS & DISCUSSION

B. subtilis is well bale to grow in bothe temperatures 30ºC and 37ºC reaching a very similar maximum culture density. However, the highest cell density was reached faster in the culture carried out at 37ºC, where an average of 2.6 absorbance units was reached after only 19 h. Although the cultivation carried out at 30ºC only reached its highest cell density at the end of the cultivation at 43h, it reached an average of 2.7 absorbance units, which does not show a significant difference in maximum growth between the two cultivations (Figure 1A).

Indigoidine was produced at both tested temperatures, however, production at 30ºC resulted in a six-fold improvement in indigoidine production compared to 37ºC (Figure 1B and C). When calculating the productivity of these cultures, meaning the production of indigoidine per total cell, this difference is maintained, and cultivation at 30ºC proves to be more advantageous for indigoidine production. This result may indicate that BpsA has its activity optimized at 30ºC compared to 37ºC for the synthesis of

indigoidine. However, this result alone is not enough to confirm this fact since the higher production of indigoidine at the lower temperature could be affected by other factors, such as compound stability and general metabolic state of the cells. Another possible explanation for this difference in production is the dissolved oxygen in the medium. Higher temperatures decrease the solubility and levels of oxygen in the culture medium and indigoidine biosynthesis requires oxygen. Another aspect to consider is the rate of oxygen consumption and the metabolic activity of the microorganism, which increase with temperature to an optimum level, which can further reduce dissolved oxygen levels[7,8,9].

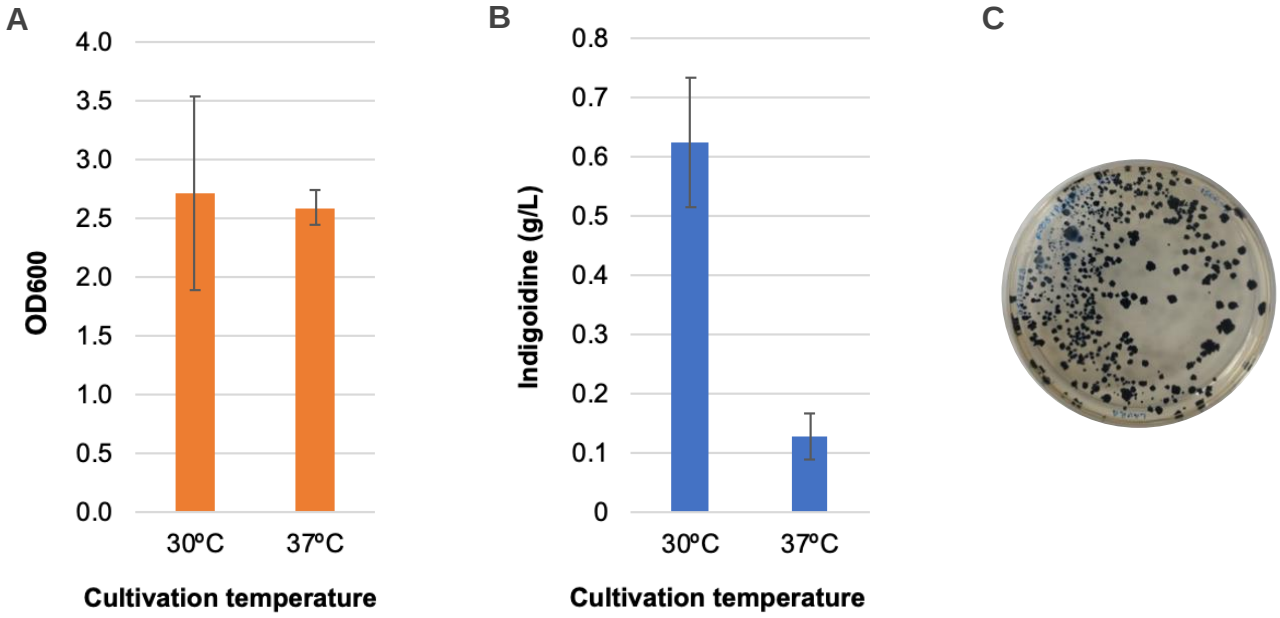

Figure 1. Heterologous production of natural blue dye in bacteria. (A) Bacterial population density after cultivation at 30ºC and 37ºC measured at 800 nm. (B) Indigoidine production after cultivation at 30ºC and 37ºC. (C) Blue bacterial colonies on an agar plate.

4 CONCLUSION

We conclude that *B. subtilis* is a promising chassis for the production of indigoidine heterologously. Indigoidine is not toxic to *B. subtilis* and the growth is not affected by production. Moreover, cultivation temperature is of great importance for the production of indigoidine in *B. subtilis*. Lowering the cultivation temperature from 37ºC to 30ºC significantly increased the production, showing that this parameter should be widely analyzed when considering the bioprocess for this blue dye. Finally, this work has successfully demonstrated that heterologous production of indigoidine in engineered *B. subtilis* is feasible.

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