Inducing the expression of violacein using the T7 promoter in *Escherichia coli* BL21 (DE3)

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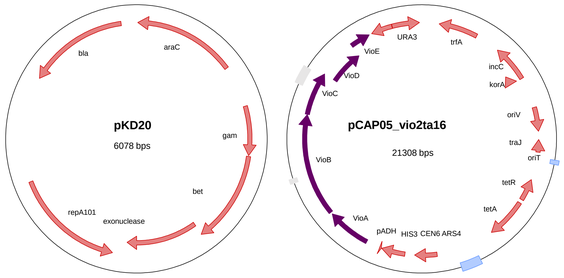
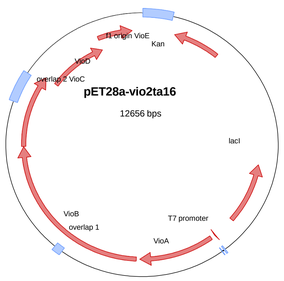
Abstract

Violacein is a purple pigment with a number of pharmacologically significant properties found in a number of bacteria species including *Chromobacterium violaceum* and *Pseudoalteromonas*. The Goal of this study was to induce expression of the violacein gene cluster (VioA-VioE) utilizing a non-native promoter, T7. In order to do this we used Λ red to insert the violacein gene cluster from *Pseudoalteromonas luteoviolacea* 2ta16 into pET28a and then introduced it to *E. coli* BL21 (DE3). Once the violacein gene cluster was introduced to *E. coli* with plasmid pCAP05 expression without the T7 promoter. Higher concentrations of violacein were observed in plated colonies at lower temperatures (30C). No expression was observed in liquid cultures without the T7 promoter and the inducer, IPTG. After Λ red however, the plates appeared different, some cells with the pET28a plasmid expressed much more violacein, while others expressed none. In liquid cultures two trials were run, the first inconclusive with spectrophotometry results that merited further investigation, and the second a success with limited, but clear, violacein expression.

Methods



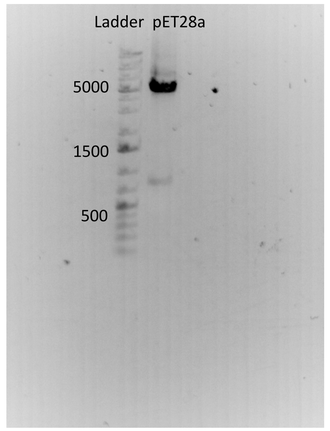
Each plasmid used in this study contributed to the construction of pET28a-vio2ta16 by Λ red. pKD20 contains a promoter activated by arabinose , the gene cluster coding for enzymes that perform Λ red, and an ampicillin resistance gene. The resistance gene ensures pKD20 can be selected for as new plasmids are introduced. The next plasmid pCAP05-vio2ta16 contains the target violacein gene cluster and a tetracycline resistance gene. After these two plasmids were introduced with electroporation, the final plasmid containing the T7 promoter and a kanamycin resistance gene, pET28a was inserted. The final genetic iteration of BW25113 was selected for by the three selection antibiotics mentioned previously and was grown in a liquid culture containing arabinose. The arabinose triggered Λ red creating pET28a-vio2ta16.



Once the BW25113 cells performed Λ red the DNA was extracted and purified , pET28a-vio2ta16 was inserted to BL21 (DE3) through electroporation, a process involving disrupting the cell membranes of target cells with a shock and allowing the target plasmid to diffuse into said cells. After cooling down, the BW cells were given a recovery period in LB broth without selection antibiotics. These cells were plated on the second plate to the right labeled BL21 with pET28a-vio2ta16.

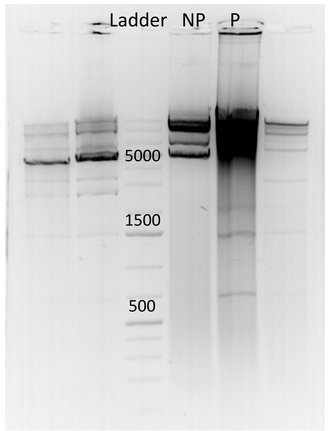
The colonies of BL21 (DE3)/pET28a-vio2ta16 and BL21/pET28a show up as purple and not purple respectively. To test the two colony types gel electrophoresis was run (as depicted in the second gel image). Both of the resulting gels were inconclusive. Although it is not certain that the BL21 cells that are purple contains pET28a-vio2ta16 and those that aren't contain pET28a, plating inoculated colonies of each type maintained a uniform coloration, the purple colony forming similarly colored colonies when transferred and the non-purple colonies still not expressing violacein (the NP colonies after inoculation and transfer are in the third plate image to the right. Liquid cultures were also grown with IPTG . Sets of liquid cultures, BL21that had successfully performed lambda red, BL21 that hadn't conducted lambda red, and a culture containing BL21 with pCAP05 were incubated at three different temperatures, 18C, 30C, and 37C. This is because greater violacein expression was observed in colder colonies when the native promoter was still present implying that the regulatory DNA present in the gene cluster is temperature sensitive. The liquid cultures were spun down after 24hrs of growth and the plates incubated in each temperature were observed.

pET28a Prior to Insertion into BW25113



Prior to the insertion of pET28a into BW cells, we ran a PCR of pET28a in order to amplify it. We used gel electrophoresis to ensure the PCR had run properly. The plasmid (to the right of the ladder) was amplified properly as the third and forth rung represent the number of base pairs pET281 should have. The lighter bar further down is other DNA or proteins in the sample. This additional DNA presence may be a leftover from the digestion of the methylated DNA template.

DNA From BL21 Cells After Transformation

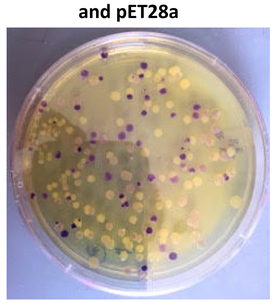


Columns four and five contained the DNA extracted from the non-purple and purple colonies respectively after transformation. The thick smudge representing the purple colonies was an unexpected result. The DNA was altered or destroyed at some point in the purifying process. Therefore we can't know whether or not the pET28a-vio2ta16 was added to BL21 until another extraction and electrophoresis can be performed on purple BL21 colonies.

Results

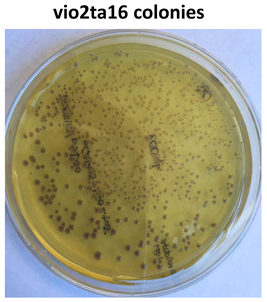
BW25113 with pCAP05-vio2ta16

BW25113 with pET28a-vio2ta16 and pET28a

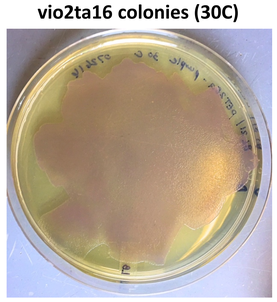


BL21 non purple pET28a colonies

BL21 purple pET28a-vio2ta16 colonies



BL21 purple pET28a-vio2ta16 colonies (30C)



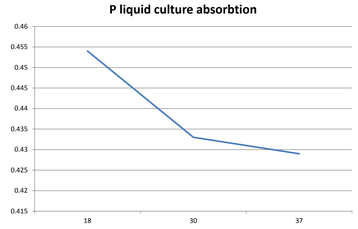
BL21 purple pET28a-vio2ta16 colonies (37C)

Analysis of Liquid Culture BL21/pET28a-vio2ta16

Absorption of Liquid Cultures Incubated at Different Temperatures (575nm)

	18	30	37
P	.454	.433	.429
NP	.267	.697	.246
pCAP	.055	.387	.103

Using a spectrophotometer, the absorptions of the nine liquid cultures were analyzed. The NP and pCAP cultures didn't grow uniformly. The P colonies however, gave interesting results. Assuming no violacein production in the liquid culture (as Zhang X and Enomoto K (2011) found) absorption and temperature should be negatively correlated as in colder conditions the metabolic rate of *E.coli* should slow and the culture should grow slower. However, with P colonies the absorption goes up as the temperature decreases. This could be due to violacein production. When the colonies were spun down and pelleted no difference in pigmentation was observed.



In the second set of liquid cultures pigmentation was observes in pelleted P and pCAP cultures incubated at 30C. The pCAP colonies expressed without IPTG as before in the initial plates of BW25113/pCAP05-vio2ta16 + pKD20 The colonies grown at 18C didn't grow in time and those grown at 37C didn't express any pigment.

The plates grown from a P colony all expressed violacein, further confirming successful lambda red. The colony grown at 30C did express violacein without IPTG. Purple BL21 expressed violacein on a plate but in an odd pattern. The violacein pigment was only expressed on the edge o the colony, not the center, the opposite of BW25113 with pCAP05-vio2ta16.

Discussion and Conclusions

The synthesis of violacein is complex. Inability to induce with the T7 promoter implies other driving factors for expression. The expression occurred with the native promoter at 30°C in initial BW11325 cells indicating that temperature is an important factor in production of violacein. This was observed again in both liquid cultures and plated colonies. One reason for this is likely regulatory DNA within the violacein gene cluster. Previous studies were unable to induce expression in liquid cultures. When found in *Pseudoalteromonas luteoviolacea* quorum sensing is involved. Violacein's wide range of potential pharmacological uses merits further study as it has antibacterial, antiviral, antitrypanosomal, and antitumoral properties. Potential use in industry, makes understanding violacein expression a worthy avenue for future study. With a few more weeks here at UCSD I would be interested in determining the optimal temperature and conditions for violacein production.

References

Zhang X, Enomoto K (2011) Characterization of a gene cluster in its putative promoter region for violacein biosynthesis in *Pseudomonas* sp. 520P1. Appl Microbial Biotechnol (2011) 90:1963-1971

Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted Streptomyces gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. (2003) 18; 100(4): 1541-6

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