Engineering and Investigating the Effects of Renewable Chemical Production in Bacteria

by

Omar Abed

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Graduate Supervisory Committee:

David R. Nielsen, Chair
Arul Varman
Xuan Wang

ARIZONA STATE UNIVERSITY

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ABSTRACT

Metabolic engineering of bacteria has become a viable technique as a sustainable and efficient method for the production of biochemicals. Two main goals were explored: investigating styrene tolerance genes in *E. coli* and engineering cyanobacteria for the high yield production of L-serine. In the first study, genes that were shown to be highly differentially expressed in *E. coli* upon styrene exposure were further investigated by testing the effects of their deletion and overexpression on styrene tolerance and growth. It was found that *plsX*, a gene responsible for the phospholipid formation in membranes, had the most promising results when overexpressed at 10 µM IPTG, with a relative OD_{600} of 706 ± 117% at 175 mg/L styrene when compared to the control plasmid at the same concentration. This gene is likely to be effective target when engineering styrene- and other aromatic-producing strains, increasing titers by reducing their cytotoxicity.

In the second study, the goal is to engineer the cyanobacterium *Synechococcus* sp. PCC 7002 for the overproduction of L-serine. As a robust, photosynthetic bacteria, it has potential for being used in such-rich states to capture CO_2 and produce industrially relevant products. In order to increase L-serine titers, a key degradation gene, *ilvA*, must be removed. While *ilvA* is responsible for degrading L-serine into pyruvate, it is also responsible for initiating the only known pathway for the production of isoleucine. Herein, we constructed a plasmid containing the native *A0730* gene in order to investigate its potential to restore isoleucine production. If functional, a *Synechococcus* sp. PCC 7002 Δ*ilvA* strain can then be engineered with minimal effects on growth and an expected increase in L-serine accumulation.

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CHAPTER 1

INVESTIGATING GENE EXPRESSION IN *E. COLI* FOR STYRENE TOLERANCE

1.1 Introduction

Advancements in biotechnology and sequencing have given us the tools and technologies required to increase our understanding of the genomics, proteomics, metabolomics, and transcriptomics of organisms, especially the model organism *E. coli*. These resources have allowed us to develop countless new synthetic pathways along with improving the flux towards desired products through the metabolic engineering of such pathways. One large thrust of this field is to use bacteria for the sustainable production of biofuels and biochemicals through the use of renewable sugar feedstocks. One such product is styrene, an important feedstock for the production of many synthetic plastics and rubbers which we used in daily life, and is currently being made in industry starting from the petroleum derivatives benzene and ethylene.[1] With the concerns of oil shortage and climate change are on the rise, it is important to find alternative means for the production of chemicals that society depends on.

Bacterial strains capable of styrene productions have been made in the past decade, but titers are often limited due to the toxicity caused by the compound. In order to better understand which genes can be targeted to prevent the inhibition of cell growth, RNA analysis was performed to find *E. coli*'s transcriptional response to styrene exposure.[2] Key genes that were seen to be highly differentially expressed and/or are regarded as important for tolerance were subsequently studied.

1.2 Materials and Methods

The genes of interest that were subsequently analyzed are *bhsA*, *cspA*, *cspG*, *fadM*, *ibpA*, *ibpB*, *lpp*, *marA*, *ompF*, *ompR*, *plsX*, *pspA*, *rpoH*, *skp*, *tolA*, *tolQ*, *tolR*, and *yhdV*. In order to develop a holistic understanding of these genes, both deletion and IPTG-inducible overexpression strains were prepared using the *E. coli* Keio knockout collection and ASKA library, respectively. The plasmids were transformed into chemically competent BW25113 *E. coli*. The only exception was for *rpoH*, where a deletion strain was not made as it is considered an essential gene.

The media used was composed of, in g/L, $Na_2HPO_4 \cdot 7H_2O$ (12.67), KH_2PO_4 (3.00), NaCl (0.50), NH_4Cl (1.0), and glucose (30). Before using the media, 5.0 mL of 50 g/L $MgSO_4$ and 230 µL of 50 g/L $CaCl_2$ were added per liter of media. For ASKA strains, chloramphenicol was added for a final concentration of 15 µg/mL. Seeds for the experiments were grown for 12-16 hours at 32 °C at 200 rpm. For the experiment, 20 mL of the media was transferred to a 40 mL glass vial and 400 µL of the seed was added. In order to achieve a final concentration of 100 mg/L styrene, 2.2 µL of styrene was added to the vial, then sealed shut with a screw cap to prevent styrene from escaping. The glass vial was then placed in a shaking incubator at 200 rpm and 37 °C. For all deletion and overexpression strains, triplicates were run with and without styrene, and the OD_{600} was taken after 6 hours. All overexpression strains were run at 10 µM and 100 µM IPTG in order to test for any varying effects.

In order to study the natural growth of the strains without styrene over a longer period of time, they were run in 96 well plates with the same media. A 2% inoculation volume was used, and samples were run for 24 hours with the OD_{600} taken every 15 minutes. Deletion strains were run in triplicates and overexpression strains at both 10 µM and 100 µM IPTG concentrations were run in quadruplicates. The average for each strain and condition was then plotted over the 24-hour time period, and the final timepoints were graphed with standard deviation error bars.

1.3 Results

While the initial study[2] compared the effects of the overexpression and deletion genes to controls without styrene as well as compared the genes to themselves with and without styrene, it is also important to study the relative growth of these strains to controls both with styrene added, as seen in Figures 1.3.1-3. These show how effective the overexpression or deletion of the genes are at improving styrene tolerance of the *E. coli* cells. Graphs depicting the first two comparisons can be found in Appendix A.

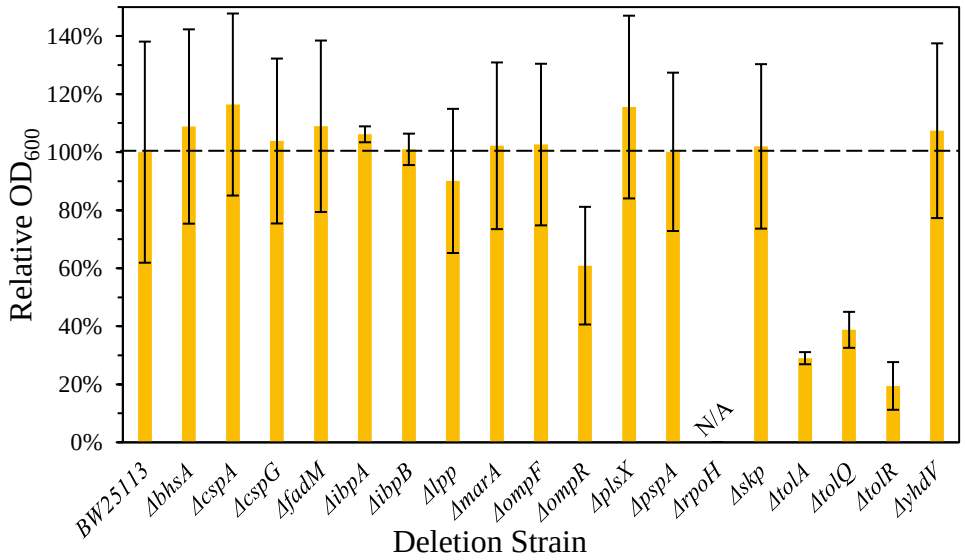

Figure 1.3.1: Relative OD_{600} of each deletion gene to the control BW25113 under the presence of 100 mg/L styrene.

It can be seen in Figure 1.3.1 that most deletion genes are within the relative standard deviations of the control BW25113. However, a few exceptions exist. One of these, *ΔompR*, is responsible for osmoregulation[3] and has a relative error of 60.9 ± 20.3%. Additionally, *ΔtolA*, *ΔtolQ*, and *ΔtolR* had significantly lower relative OD_{600} at 29.0 ± 2.1%, 38.8% ± 6.2%. and 19.4 ± 8.2%, respectively. These three genes, all part of the Tol-Pal system, play a central role in maintaining the integrity of the outer membrane.[3]

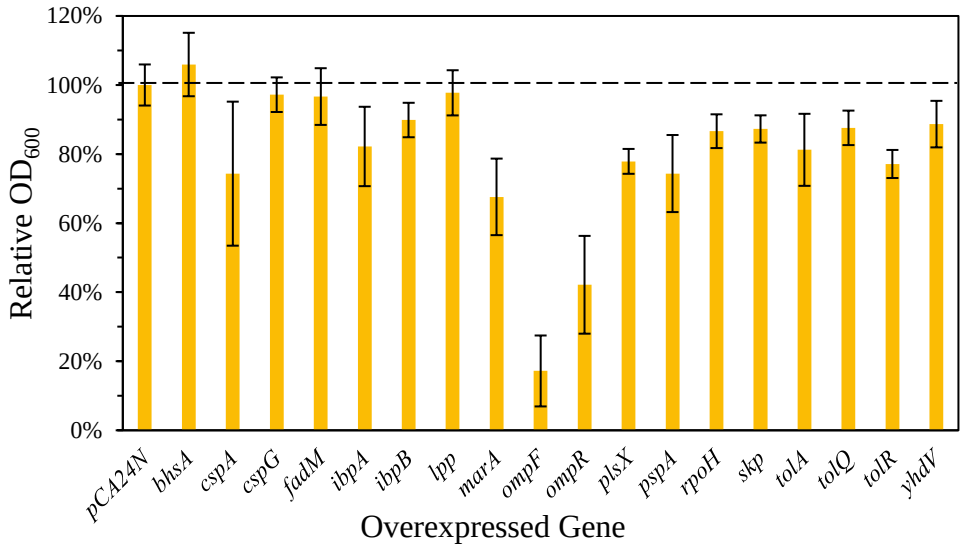

Figure 1.3.2: Relative OD_{600} of each gene induced with 10 µM IPTG to the control plasmid pCA24N under the presence of 100 mg/L styrene.

When the ASKA strains were induced with 10 µM IPTG and exposed to 100 mg/L styrene, most genes displayed similar growth to the control plasmid pCA24N, as seen in Figure 1.3.2. However, the genes that most significantly inhibited growth were *ompF* and *ompR*, at 17.2 ± 10.3% and 42.1 ± 14.2%, respectively. While the role of *ompR* was already mentioned, *ompF* is responsible for creating pores in the outer membrane for passive transport of small molecules.[3] These results are expected, as more pores reduce membrane integrity and expose the inside of the cells to potentially toxic molecules.

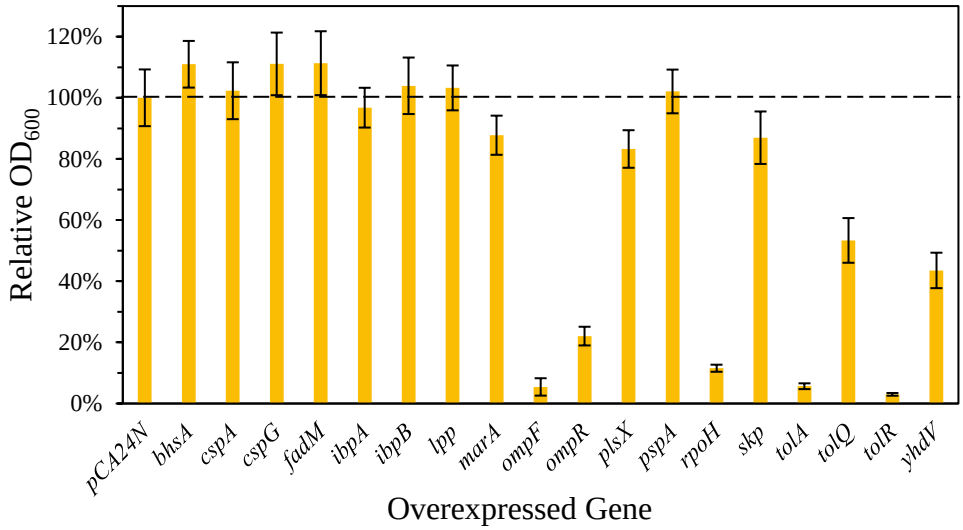

Figure 1.3.3: Relative OD_{600} of each gene induced with 100 µM IPTG to the control plasmid pCA24N under the presence of 100 mg/L styrene.

With the induction of the strains at 100 µM IPTG, Figure 1.3.3 shows significant growth inhibition for more genes. Again, *ompF* and *ompR* displayed very low relative growth (5.4 ± 2.8% and 22.1 ± 3.1%) as well as the Tol-Pal genes (*tolA*, *tolQ*, *tolR* at 5.7 ± 0.9%, 53.3 ± 7.3%, 3.0 ± 0.4%). However, an additional two genes, *rpoH* and *yhdV* displayed lower relative growth at 11.5 ± 1.2% and 43.5 ± 5.8%. The *rpoH* gene is involved in heat shock response while *yhdV* is predicted to be an outer membrane protein.[3]

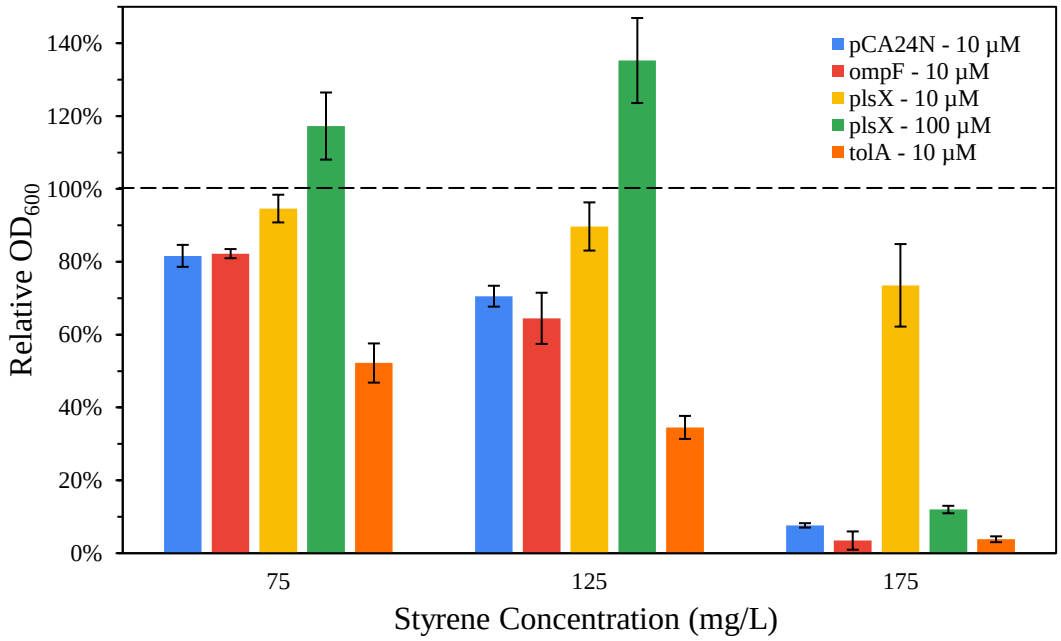

Figure 1.3.4: Relative OD_{600} of each gene at varying styrene concentrations to the same gene without styrene at varying IPTG.

As seen in Figure 1.3.4, the *plsX* strain with 100 µM IPTG performed better at 75 and 125 mg/L compared to the same strain with no styrene exposure. When compared to the control, *plsX* with 10 µM IPTG had better relative growth, *tolA* showed increased styrene sensitivity, and *ompF* showed no significant impact on styrene sensitivity. Most prominently, *plsX* at 10 µM IPTG was the only condition to have high relative growth at 175 mg/L. When standardized to the control vector at the same styrene concentration, shown in Figure A.4, the gene's relative OD_{600} is 706 ± 117%, which is a significant improvement compared to any of the other strains. This makes *plsX* a promising gene for engineering styrene-tolerant strains.

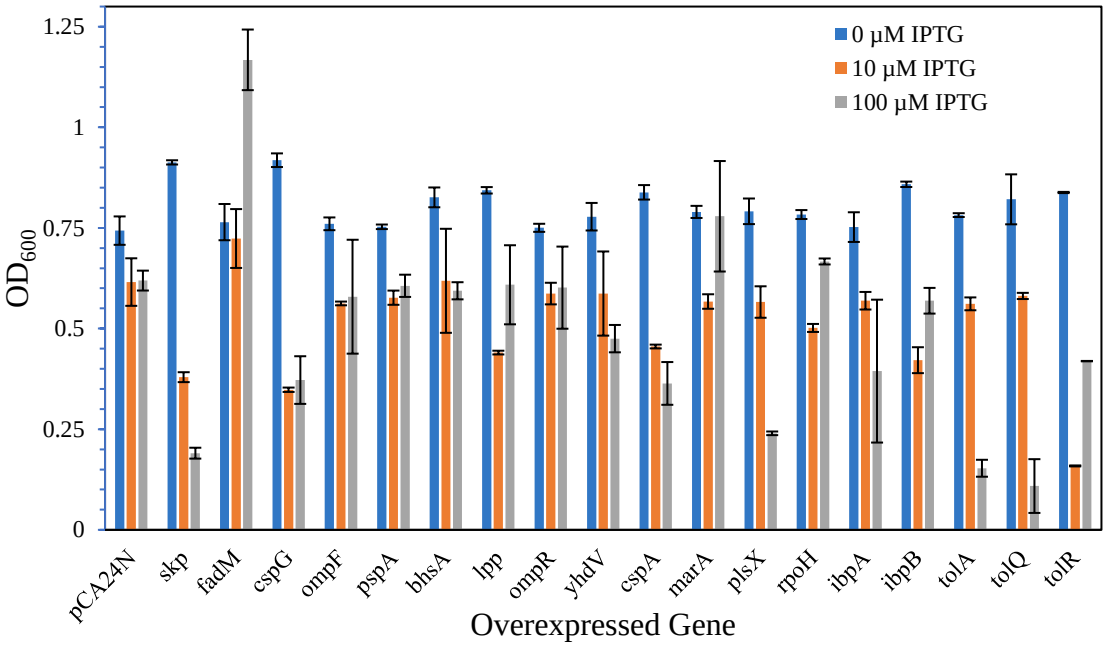

Figure 1.3.5: OD_{600} of overexpression genes without styrene at varying IPTG concentrations after 24 hours in a 96 well plate.

For every strain except for *fadM*, inducing cultures with IPTG without any styrene present inhibited growth after 24 hours of incubation. This is most likely due to the reallocation of resources towards the production of these proteins instead of growth. Additionally, since many of these genes are in some way related to membrane proteins and shock stresses, it may be limiting the transport of materials in and out of the cell.

1.4 Discussion and Conclusion

Based on the results, it can be seen that *plsX*, *ompR*, *ompF*, *tolAQR*, *rpoH*, and *yhdV* are most relevant to increasing styrene tolerance. The most promising gene, *plsX*, exhibited

slightly inhibited growth when compared to the pCA24N control plasmid at 10 µM for 75 and 125 mg/L styrene, but had a relative OD_{600} of 706% ± 117% at 175 mg/L styrene normalized to the control plasmid.

An important consideration when analyzing the results is that the growth behavior of cells may change over longer periods of times. Considering the styrene studies presented were run for only a period of 6 hours, it may not represent the full growth capacity of the strains. For example, the overexpression of genes may be inhibitory at the start as metabolites are invested in resource-intensive activities, but may pay off in the long run with increased cell densities through decreased cell toxicity and mutations. An example of this is *fadM*, as running it for 24 hours with 100 µM IPTG in a microtiter plate resulted in an almost 88.6% increase in growth compared to the control plasmid pCA24N at the same condition. Therefore, it may be beneficial to extend the styrene experiments for a few more hours or even days. Additionally, for these experiments, the headspace of the vials was not replaced with oxygen and therefore only contained ambient air. While it is unlikely that the air in the headspace does not provide enough oxygen during the 6-hour fermentation time, it would be important to maintain a high starting oxygen concentration to guarantee aerobic conditions in longer runs.

This study elucidated key genes that can be further investigated for styrene tolerance. Some genes such as *plsX* exhibit properties of a significant gene target to increase cell growth for aromatic-producing *E. coli* strains. With further experimentation and fine tuning of expression levels, cell growth can be increased and elicit greater production rates.

CHAPTER 2

ENGINEERING OVER PRODUCTION OF L-SERINE IN CYANOBACTERIUM *SYNECHOCOCCUS* SP. PCC 7002

2.1 Introduction

Fermentations for the production of biofuels and biochemicals are being developed as a more sustainable and renewable approach to fossil fuels. However, model organisms such as *E. coli* and *C. glutamicum* that are currently being used require a sugar feed such as glucose and xylose for production of desired compounds. Cyanobacteria are an attractive alternative, as they rely on sunlight and CO_2 for growth. In this study, a method for increased L-serine production is explored.

In A+ minimal media, L-serine is not present in the media nor after incubating PCC 7002 for 5 days.[4] This presents the opportunity to be the first to accumulate L-serine in cyanobacteria. Compared to heterotrophs like *E. coli* and *C. glutamicum*, cyanobacteria usually have lower titers and volumetric productivity because they can become light limited.[5] However, due to L-serine being only a few steps removed from the Calvin cycle, the amino acid's accumulation provides the opportunity to decouple production from growth. By using L-serine as an indicator for production, would simplify and help verify how changes in the Calvin cycle impacts production, specifically during the stationary phase where continued production is desired. Additionally, it would provide an alternative to current methods of L-serine production, which utilized resting cells to enzymatically produce L-serine using glycine and methanol, which are expensive feedstocks.[6]

High yield L-serine strains have been developed in *E. coli* and *C. glutamicum* following the isolation of feedback-insensitive copies of SerA; the entry point to the pathway used to convert G3P to L-serine.[7,8] Additionally, L-serine exporters are commonly over-expressed to facilitate removal of the amino acid from the cell, preventing its degradation and possible toxicity at higher concentrations. Finally, genes associated with L-serine degradation pathways (e.g., *sdaA, glyA*) are commonly deleted to maximize the accumulation of L-serine.

In an attempt to replicate this general metabolic engineering strategy in cyanobacteria, a strain of *Synechococcus* sp. PCC7002 was engineered to overexpress an IPTG inducible promoter with *serA* mutant known to deregulate feedback inhibition of L-serine while also overexpressing the exporter *eamA*, with plasmids found in Appendix C. However, based on preliminary experiments, no growth defects were found when inducing *serAmut* with 5 mM IPTG and *eamA* with varying ATC concentration, supplementing ATC to account for its light sensitivity and half-life. as seen in Figure 2.1.1. To confirm these results, they were derivatized and run on an HPLC using the method found in Appendix D, but no L-serine was detected. While this was initially only done for the final timepoint, L-serine has been seen to accumulate during growth before being consumed during stationary phase.[6] Therefore, the experiment was repeated, sampling every 24 hours before running them on the HPLC, but detectable titers were still not achieved. This is likely due to there being a significant driving force converting L-serine to another metabolite to better meet the organism's needs. Therefore, it is important to delete any major degradation enzymes of L-serine.

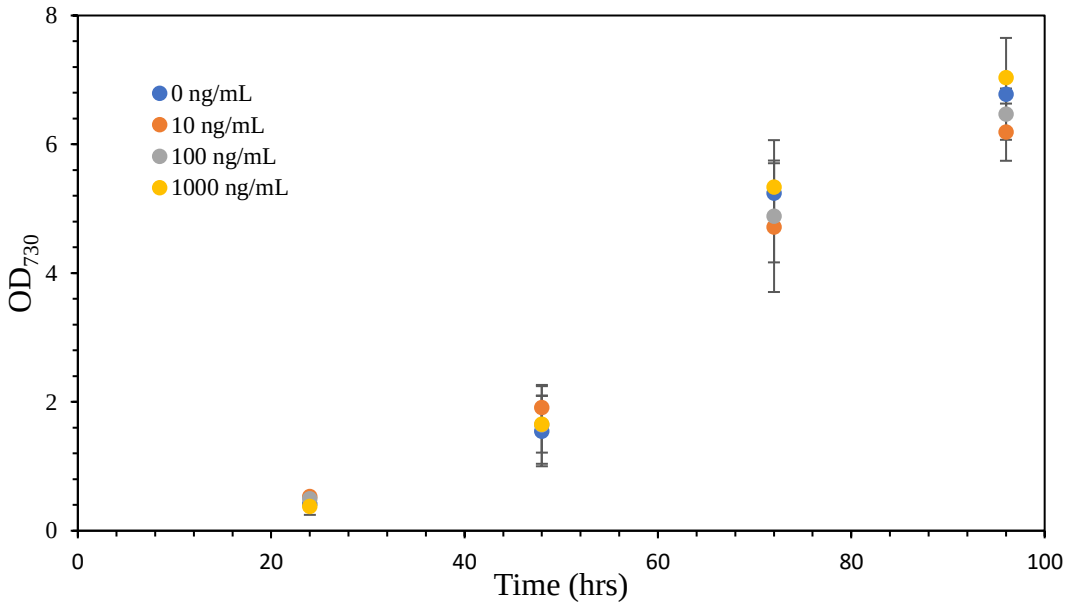

Figure 2.1.1: Growth curve for Synechococcus sp. PCC 7002 with serAmut plasmid induced with 5 mM IPTG and eamA plasmid induced at varying ATC concentrations.

One potential target is *ilvA*, encoding for L-threonine dehydratase, as it is responsible for the degradation of L-serine to pyruvate. This is especially a hindrance towards the accumulation of the amino acid because pyruvate is a core metabolite involved in many other pathways, so the flux would naturally prefer the degradation of L-serine. The issue, however, is that *ilvA* also plays a key role in another pathway. More specifically, the IlvA enzyme is responsible for initiating the conversion of L-threonine towards L-isoleucine, and, according to KEGG,[9] is the only know pathway in *Synechococcus* sp. PCC 7002 that is used to produce L-isoleucine. Figure 2.1.2 shows the many pathways involved in the biosynthesis of L-serine and other amino acids.

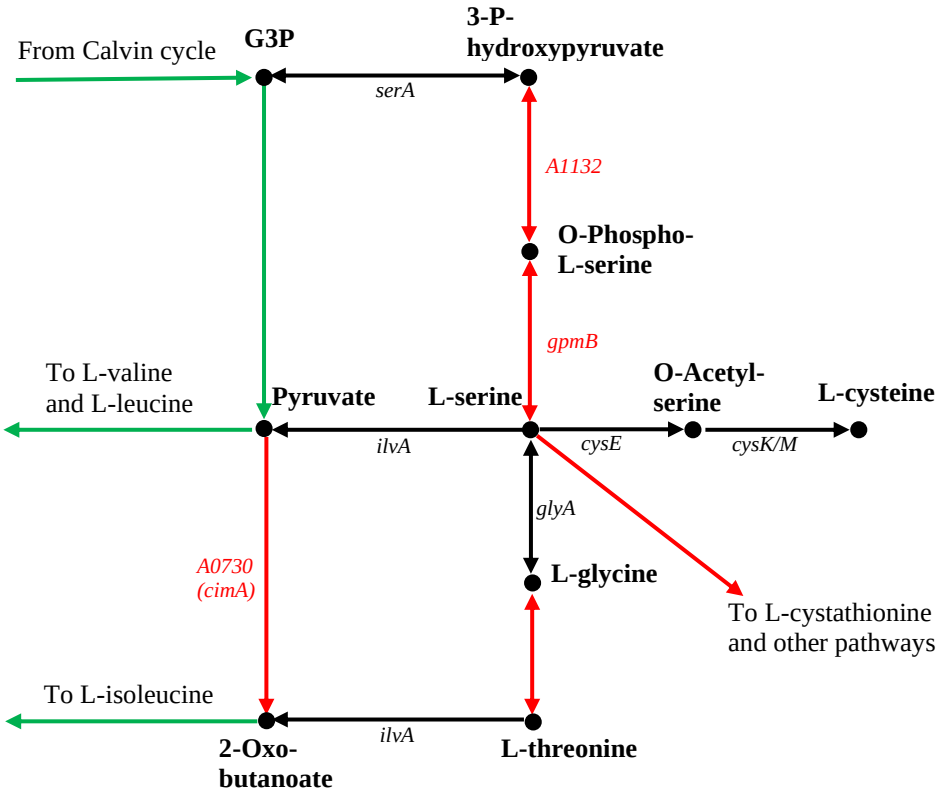

Figure 2.1.2: Metabolic pathway for L-serine production and its degradation pathways, adapted from KEGG.[9] *Green arrows denote multiple steps, red arrows/text for uncharacterized pathways, and black arrows for confirmed genes.*

Other organisms, however, use citramalate synthase to initiate the conversion of pyruvate towards L-isoleucine, and thus provides a potential alternative. Another cyanobacterium, *Cyanothece* sp. ATCC51142, was verified to have a homolog to *cimA* in *Geobacter sulfurreducens*, with only a 53% match according to pBLAST.[10] After running a pBLAST on *Synechococcus* sp. PCC 7002 comparing both organisms, it was found that its native gene *A0730* had a 68% and 51% similarity to those found in *Cyanothece* sp. ATCC51142 and *Geobacter sulfurreducens*, respectively, indicating high potential that it also exists in

Synechococcus sp. PCC 7002. Therefore, it is important to characterize the function of *A0730* in *Synechococcus* sp. PCC 7002 as this may serve as an important step towards creating an L-serine-accumulating strain of cyanobacteria.

2.2 Materials and Methods

In order to isolate and test the function of *A0730*, primers were designed to amplify the gene while also introducing digestion sites at both ends. This allows for the utilization of the multiple cloning site (MCS) region in the medium copy number plasmid pSTV28. Primer sequences can be found in Table 2.2, with BamHI and SalI chosen as common restriction sites.

Table 2.2.1: Primers used to PCR amplify A0730 with added restriction sites.

Name	Restriction Site	Sequence
OA009	BamHI	AAAGGATCCAGGAGGTTCCCCTATGGTTTCTCC
OA010	SalI	ATTGTCGACAGTCCGCAGAGTTAGAGTCACA

An annealing temperature of 60 °C and 2-minute extension time were used for the PCR reaction since the gene is ~1.7 kb. A Zymo Research DNA Clean and Concentration Kit was used to clean the PCR product, while the Zyppy™ Plasmid Miniprep Kit was used to purify pSTV28. These were then both digested by BamHI and SalI, heat inactivated the enzymes for 20 minutes at 65 °C, ran on an electrophoresis gel, and recovered using a QIAquick Gel Extraction Kit. The cut fragments were then mixed and ligated at 1:1 and 1:3 vector: insert molar ratios along with a control only containing digested pSTV28 before

being transformed into electro-competent *E. coli ΔilvA*. Since the *E. coli* strains and pSTV28 have kanamycin and chloramphenicol resistance, respectively, cells were plated on LB agarose with the aforementioned antibiotics.

2.3 Results

The *A0730* gene from *Synechococcus* sp. PCC 7002 was successfully inserted in the pSTV28 plasmid to create the final constructed plasmid pA0730, which can be seen in Figure 2.3.

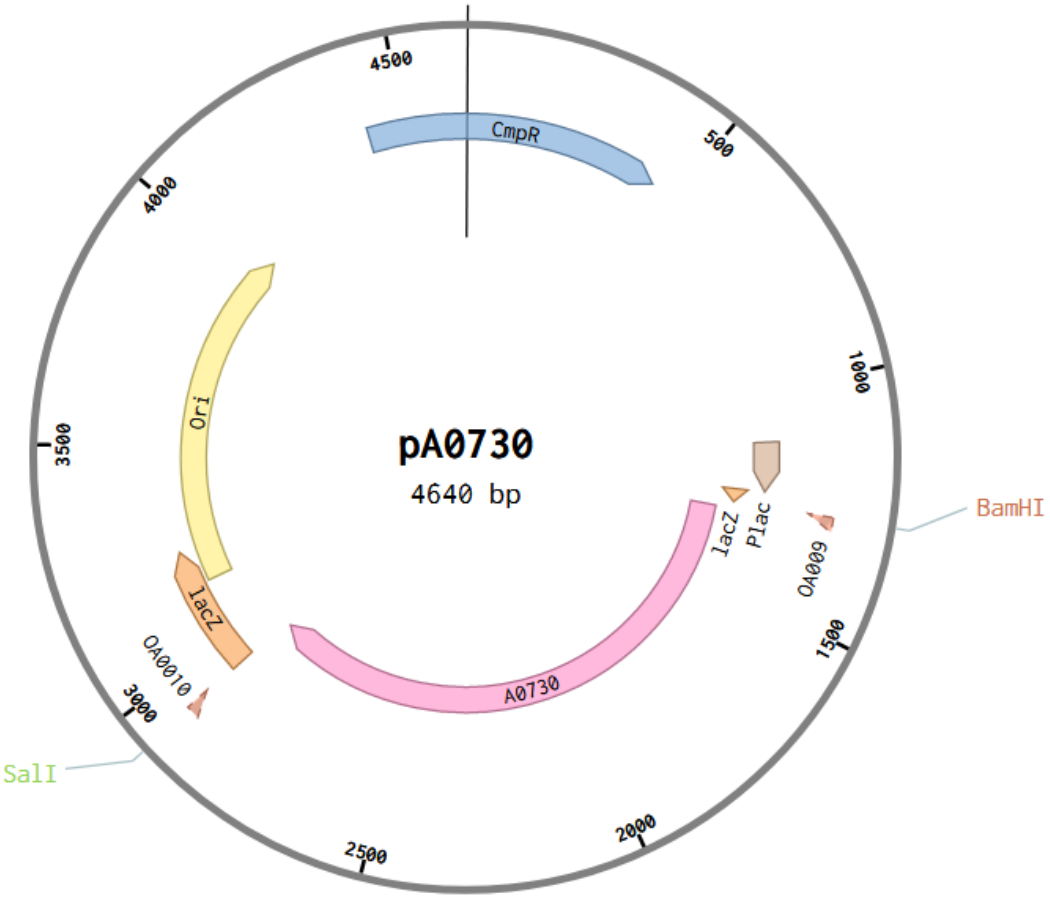

Figure 2.3.1: Engineered target plasmid with the insertion of A0730 into pSTV28.

Growth of the *E. coli ΔilvA* strain on LB-kanamycin-chloramphenicol plates was only found for the 1:3 vector: insert molar ratio, and only four colonies were seen. However, this should be sufficient for the identification and creation of the plasmid and further testing of the strain.

2.4 Discussion and Conclusion

In order to verify the correct formation of the plasmid, *E. coli ΔilvA* with pA0730 should be miniprepped and subsequently digested with AccI and EcoRI digestion enzymes. These can then be run on a gel alongside a ladder, and the band distances should match their expected sizes. Additionally, a miniprep of the plasmid needs to be sent for sequencing to authenticate the plasmid and ensure there are no mutations present. While the creation of the plasmid demonstrates progress towards engineering an L-serine overproducing cyanobacterial strain, more work needs to be done in order to ensure the *A0730* gene works as predicted. It has been shown that *E. coli* with an *ilvA* knockout cannot grow in M9 medium with 0.4% glucose or 1% glycerol at 37 °C.[11] To determine if *A0730* can replace the function of *ilvA* for the conversion of L-threonine to 2-oxobutanoate and ammonium, which initiates the reaction for the formation of isoleucine, the engineered *E. coli ΔilvA* with pA0730 must be run in these conditions against a control without the plasmid.

If the engineered strain grows in the M9 medium while the control does not, it indicates that the enzyme functions as expected and *ilvA* can safely be deleted from *Synechococcus* sp. PCC 7002 without any expected segregation issues. Deletion of *ilvA* in conjunction with the deregulated feedback inhibition of *serA* and expression with *eamA* is hypothesized

to be sufficient for the accumulation of L-serine. However, a few more targets have been identified if L-serine titers remain undetectable.

An alternative transporter in *C. glutamicum*, SerE, was identified as an L-serine exporter.[12] Previously, ThrE was the only identified exporter of L-serine. However, the Δ*thrE* strain had no increase in L-serine titers but Δ*serE* decreased titers by 56.5%, demonstrating the importance of the protein. The *serE* gene can therefore be taken from *C. glutamicum* and transformed into *Synechococcus* sp. PCC 7002 to potentially increase titers. The enzyme serine hydroxymethyltransferase, encoded by *glyA* and characterized in *Synechococcus* sp. PCC 7002, is responsible for the conversion of L-serine into L-glycine and may also be responsible for decreased titers. While *glyA* can be deleted in *E. coli* but not *C. glutamicum*, it is still unknown whether it can be removed from *Synechococcus* sp. PCC 7002. Assuming it is an essential gene in PCC 7002, an inducible promoter can replace the native promoter of *glyA* as was done in another *C. glutamicum* study.[5] The leakiness of the promoter may be sufficient to produce the required L-glycine for growth. These strains can then serve as a foundation to work towards decoupling production from growth in *Synechococcus* sp. PCC 7002 and increase its viability as a more sustainable route for the production of biofuels and biochemicals.

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APPENDIX A

STYRENE TOLERANCE IN DELETION AND OVEREXPRESSION STRAINS

In these graphs, the blue bars indicate the relative OD_{600} of a gene exposed to 100 mg/L styrene to the same gene without styrene. The red bars indicate the relative OD_{600} of a gene to the control strain, both without styrene. For deletion strains, the control is BW25113 while for the overexpression strains, it is BW25113 but with the control plasmid pCA24N. Dashed lines indicate 100% relative OD_{600}, and all error bars are relative standard deviations.

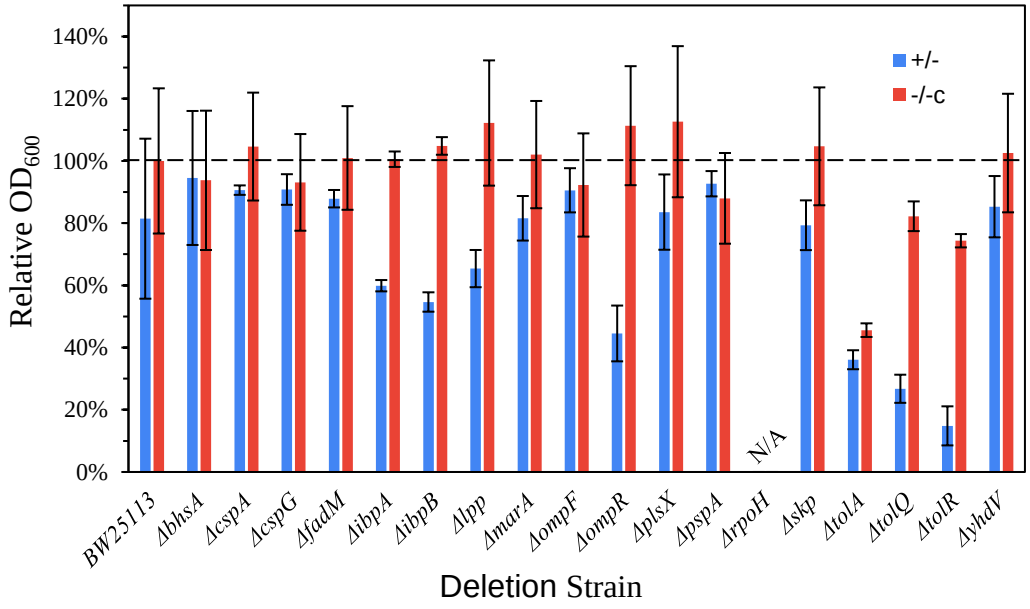

Figure A.1: Relative OD_{600} of each gene deletion to itself with and without 100 mg/L styrene, and the gene to control plasmid pCA24N without styrene.

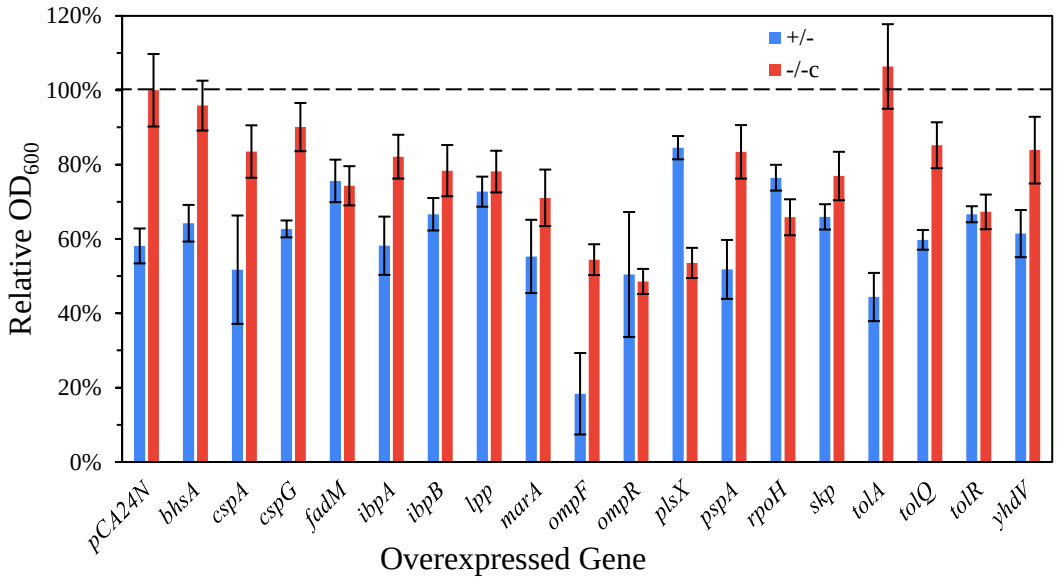

Figure A.2: Relative OD_{600} of each gene induced with 10 µM IPTG to itself with and without 100 mg/L styrene, and the gene to control plasmid pCA24N without styrene.

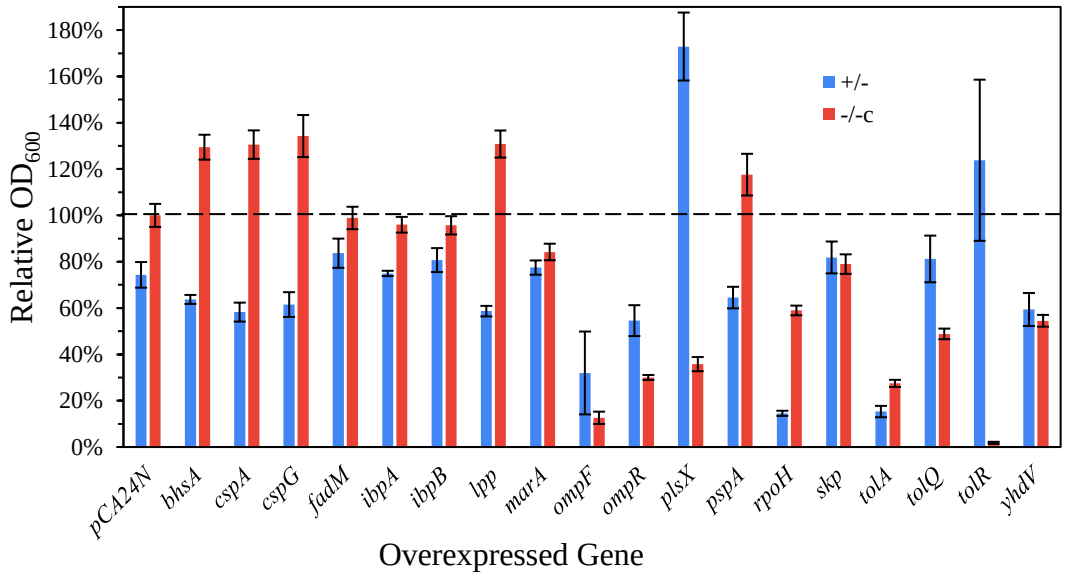

Figure A.3: Relative OD_{600} of each gene induced with 100 µM IPTG to itself with and without 100 mg/L styrene, and the gene to control plasmid pCA24N without styrene.

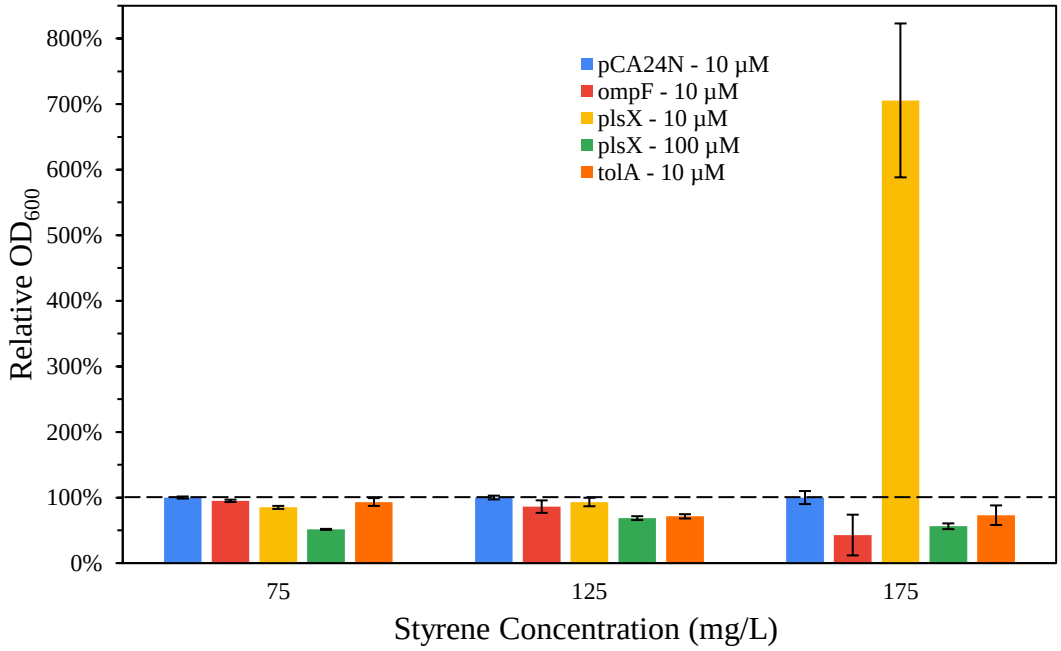

Figure A.4: Relative OD_{600} of each gene to the control plasmid at varying IPTG and styrene concentrations.

APPENDIX B

GROWTH CURVES FOR ENGINEERED STRAINS

Samples were run in 96-well plates for 24 hours in a plate reader with OD_{600} readings taken every 15 minutes. The conditions were set to 37 °C at 200 rpm, the same as the glass vial styrene experiments.

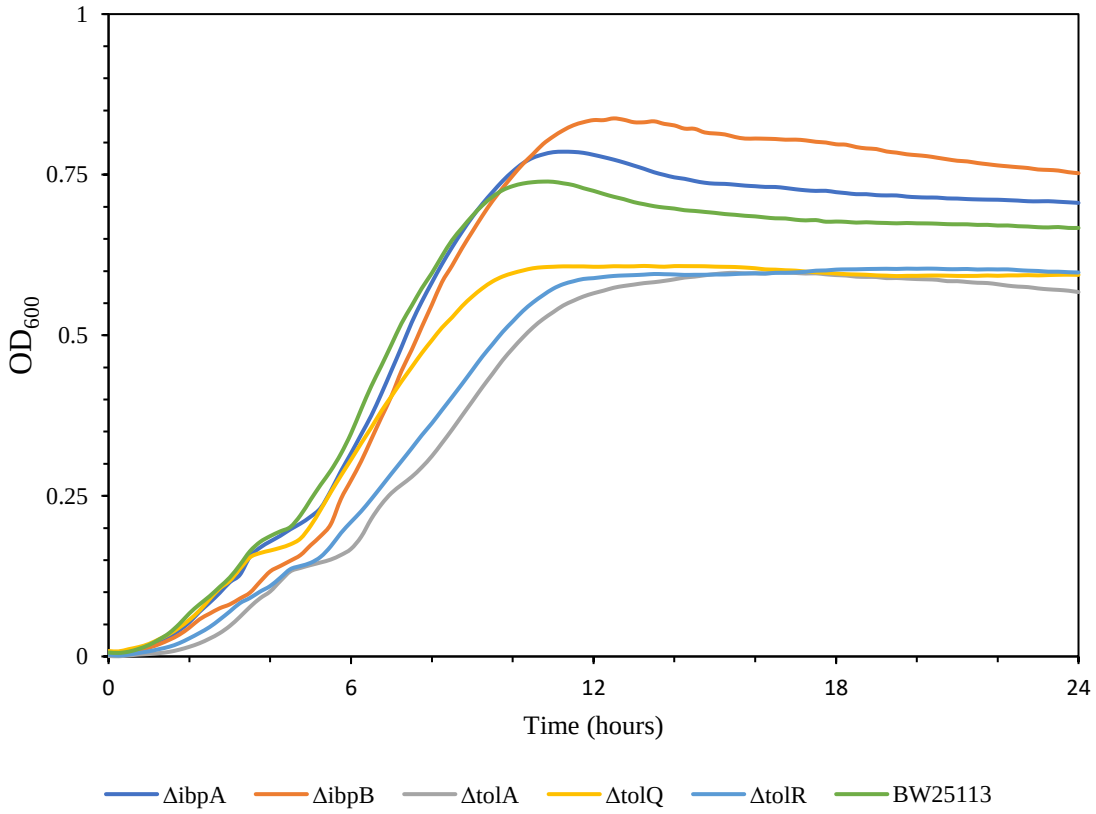

Figure B.1: Growth curves for deletion genes of interest.

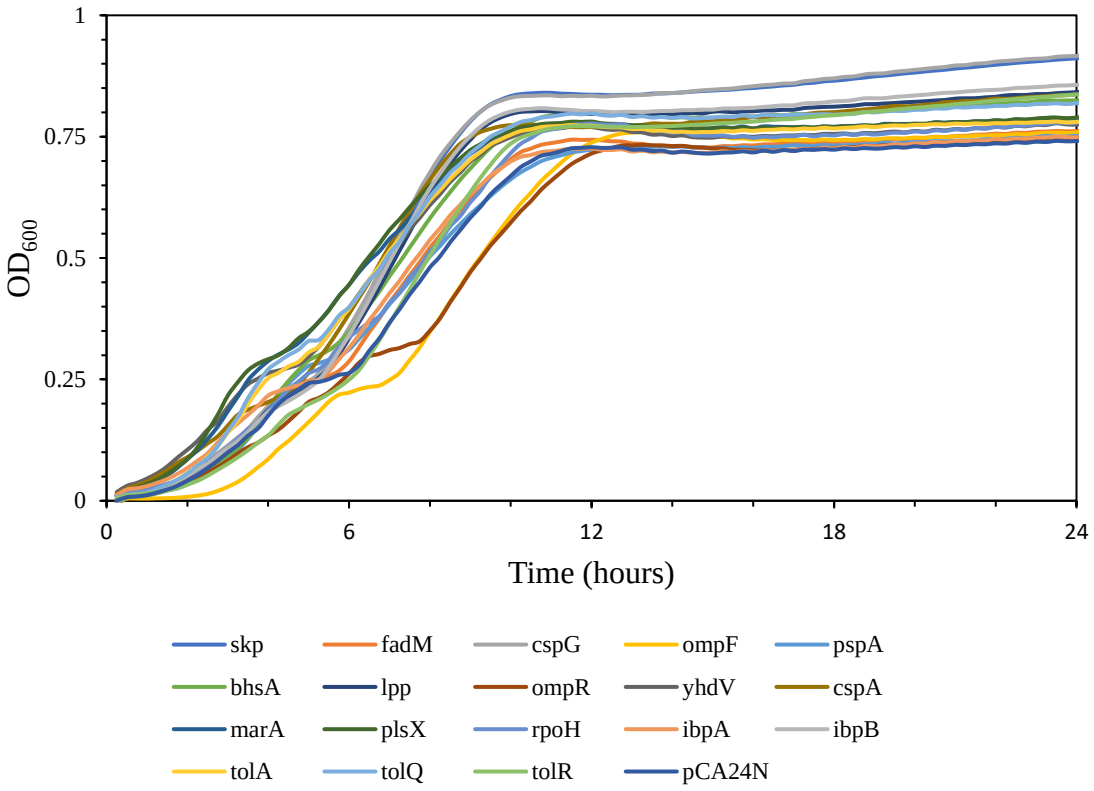

Figure B.2: Growth curves for each gene uninduced.

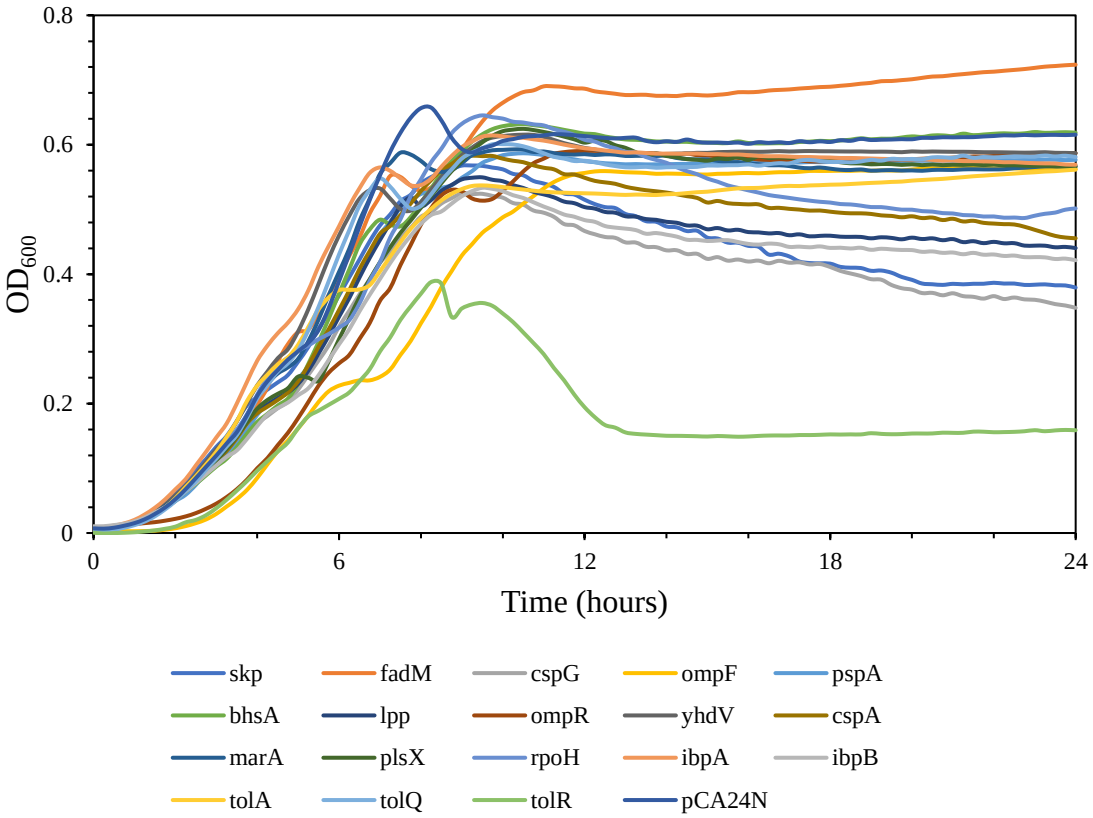

Figure B.3: Growth curves for each gene induced with 10 µM IPTG.

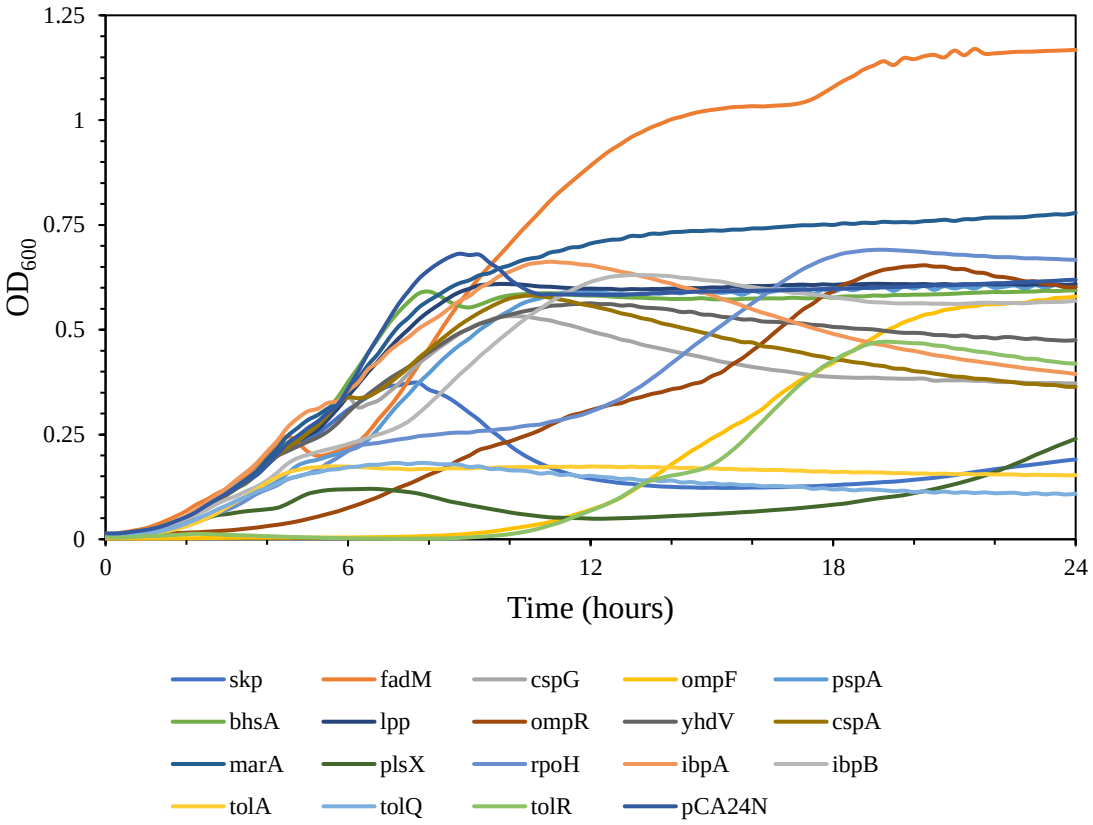

Figure B.4: Growth curves for each gene induced with 100 µM IPTG.

APPENDIX C

PLASMIDS CREATED FOR PREVIOUSLY ENGINEERED *SYNECHOCOCCUS* SP. PCC 7002 STRAIN FOR L-SERINE OVERPRODUCTION

Three point mutations needed to be made to *serA* to make the feedback-insensitive *serAmut* gene. The first plasmid, pCJ103, had a double point mutation, H334A and N346A, introduced during PCR amplification. The second plasmid, pCJ120, introduced the final mutation N364A. The *serAmut* gene had an IPTG inducible promoter, gentamicin resistance, and the *glpK* neutral site for integration.

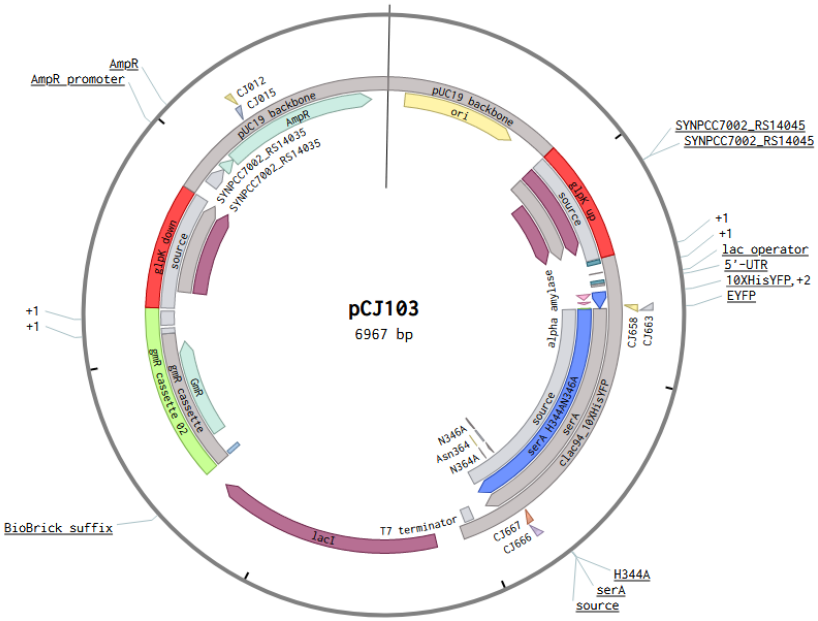

Figure C.1: Introduction of H334A and N3346A mutations into serA.

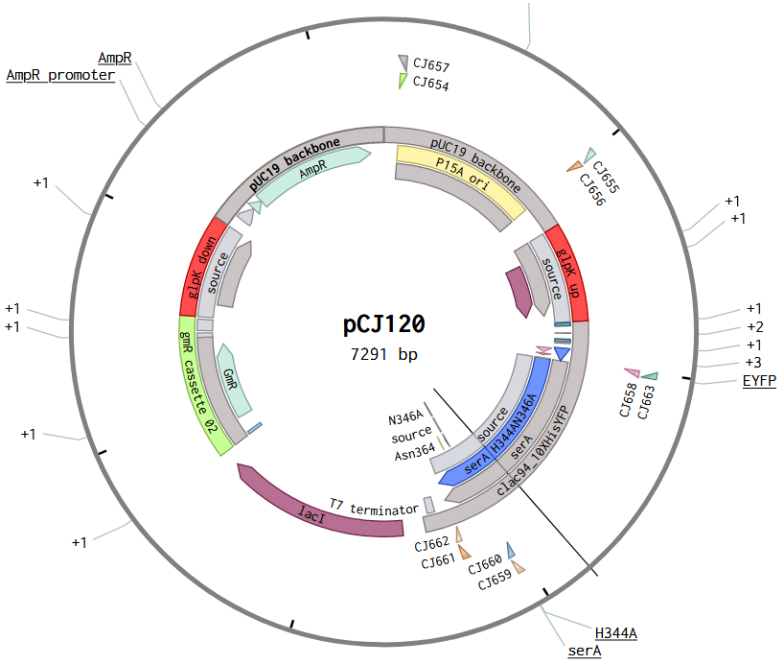

Figure C.2: Introduction of N364A mutation in previously mutated serA, creating the feedback-insensitive serAmut.

The plasmid pCJ104 was created to contain the *eamA* transporter gene with an ATC inducible promoter, zeocin resistance, and the *aquI* neutral site for integration.

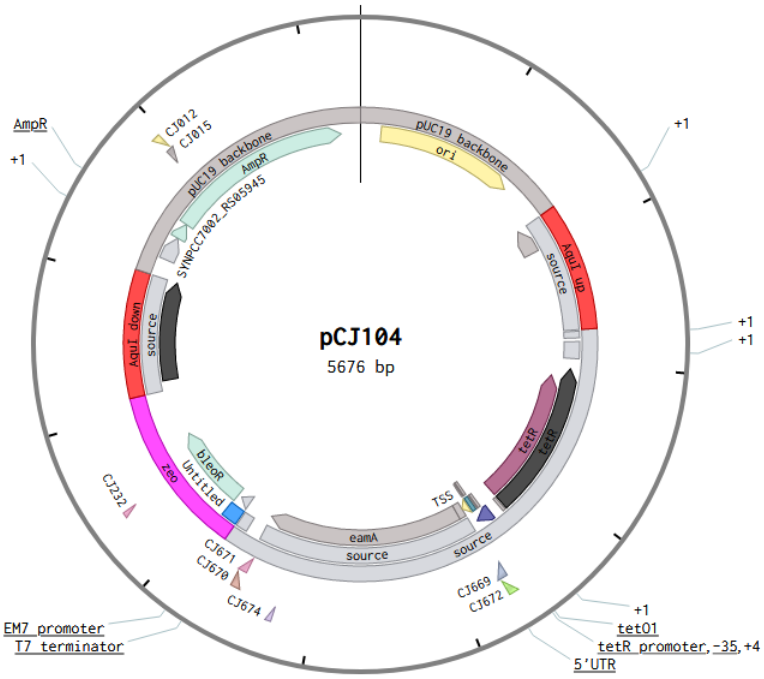

Figure C.3: Plasmid containing eamA with an ATC inducible promoter.

APPENDIX D

DERIVATIZATION AND HPLC METHOD FOR AMINO ACID QUANTIFICATION

A highly sensitive HPLC was adapted from Kim, et al.[13] and Rebane, et al.[14] To derivatized a sample, 18 µL of 0.5 M borate buffer at a pH of 9, 60 µL of 100% methanol, and 189 µL of distilled water must be mixed together in an HPLC vial. The culture sample needs to be centrifuged to pellet cells so 30 µL of the supernatant can be added. Finally, 3 µL of DEEM (diethyl ethoxymethylenemalonate) is added to the mixture. The vial is covered in aluminum foil to prevent light degradation and left for at least 2 hours before being run. For L-serine and most other amino acids, it can be left for at least 48 at room temperature without significant decrease in detection. The derivatized sample can also be frozen at -20 °C for similar results.[14]

The derivatized sample can then be run in a Hypersil GOLD C_{18} column for 30 minutes. This is run at a gradient using 25 mM aqueous sodium acetate buffer (4.8 pH) and acetonitrile, found in Table D.1, for resolved L-serine peaks.

Table D.1: Gradient HPLC method for L-serine quantification.

Time (min)	% Acetonitrile	% 25 mM aq Na Acetate Buffer
0-12	20-32	80-68
12-15	32-50	68-50
15-20	50-20	50-80
20-40	20	80

This method has been shown to work effectively for L-serine concentrations from 0.25-500 mg/L, as seen in Figure D.1.

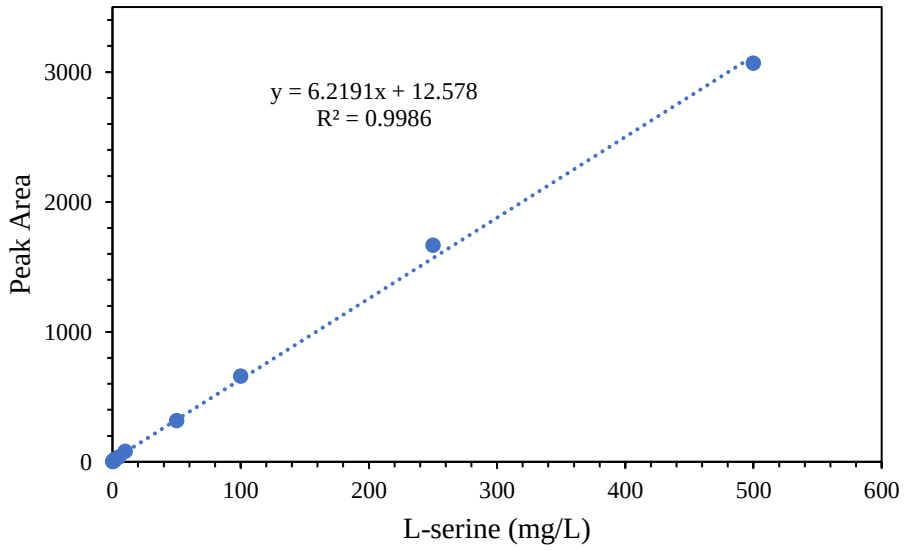

Figure D.1: HPLC calibration curve for L-serine

APPENDIX E

PROTEIN BLASTS OF GENES IN *SYNECHOCOCCUS* SP. PCC 7002

Table E.1: Protein BLASTS of genes-of-interest for L-serine overproduction.

Gene	Strain	Function	Similarity	Coverage	Gene or Accession # in PCC 7002
sdaA	*Escherichia coli (strain K12)*	L-serine deaminase	-	-	-
iclR	*Escherichia coli (strain K12)*	DNA-binding transcriptional repressor	-	-	-
arcA	*Escherichia coli (strain K12)*	DNA-binding transcriptional regulator	38.49%	95%	*A0851*
aceB	*Escherichia coli (strain K12)*	Malate synthase A	-	-	-
tdcB	*Escherichia coli (strain K12)*	Catabolic threonine dehydratase	38.44%	91%	*ilvA*
sdaB	*Escherichia coli (strain K12)*	L-serine deaminase II	-	-	-
tdcG	*Escherichia coli (strain K12)*	L-serine deaminase III	-	-	-
alaT	*C. glutamicum* SYPS-062	Putative aspartate aminotransferase	28.28%	87%	ACA98519.1
avtA	*C. glutamicum* SYPS-062	Valine-pyruvate aminotransferase	29.38%	98%	ACA98519.1
ilvN	*C. glutamicum* SYPS-062	Acetohydroxy acid synthase	43.87%	90%	ACB00680.1
sstT	*Escherichia coli (strain K12)*	Serine/threonine importer	35.14%	8%	WP_012308292.1
sdaC	*Escherichia coli (strain K12)*	Serine importer	-	-	-
Sll1559	*Synechocystis* sp. PCC 6803	PSTA or phosphoserine transaminase (like *serC*)	67.67%	95%	*A1332*
Slr1124	*Synechocystis* sp. PCC 6803	PSP or phophoserine phosphatase (like *serB*)	64.1%	100%	*gpmB*
slr0186	*Cyanothece* sp. ATCC 51142	Citramalate synthase (isoleucine pathway)	68.02%	99%	*A0730*
GSU1798	*Geobacter sulfurreducens*	Citramalate synthase (isoleucine pathway)	51.26%	97%	*A0730*