Optimized Bioproduction of Resveratrol Using Genetically Modified *Escherichia coli*

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Introduction

Resveratrol is a phenylpropanoid with many promising health benefits as an antioxidant (1). It is produced naturally in various plant species such as grapes, peanuts, and some berries.

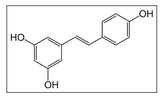

Figure 1. Resveratrol Structure

The production of resveratrol through plants can be a long and expensive process (2). Producing resveratrol via microorganisms could be a way to eliminate these problems and thereby lower the cost (3,4). *E. coli* can be used to produce this compound in several hours, compared to several months of growing plants.

This project includes the analysis of several major growth parameters in *E. coli* that were designed and tested to achieve the best optimization. These parameters included: media composition, temperature, induction methods, culture methods, and aeration.

These parameters were initially designed and tested using shaker flasks. After these initial tests were completed; the process was scaled up to a benchtop bioreactor to better test pH, dissolved oxygen requirement, and product production. From the data acquired during testing; scaled-up industrial size bioreactors for this process will be easy to design.

Design Criteria and Objectives

In order to design the optimal production of resveratrol; the following conditions were analyzed and tested:

- Growth Dynamics of *E. coli*
- Media types and composition
- Incubation stage temperatures
- Media concentration
- Elicitor concentration
- Product substrate concentrations
- Oxygen demand

Final objectives were:

- Adapt the production process from shaker flask growth to small-scale benchtop bioreactor
- Achieve an increased production yield of resveratrol from *E. coli* (target yield of 1500 mg/L)

Results

A growth curve for *E. coli* in 50 g/L TB broth was obtained using a spectrophotometer (Figure 2). Results from this test clearly show the lag phase, exponential phase, and stationary phase with their corresponding optical density (OD) measurements. From this it was determined that IPTG should be added between OD readings of 1.0-2.0.

The analysis of resveratrol production was completed using high-performance liquid chromatography (HPLC). The resveratrol peak can be observed around 14.5 minutes (Figure 3). Other peaks observed in the figure are impurities or remaining substrate found in the fermentation broth.

Media tests were performed using TB, LB, and K12 medias (Table 1). Results showed greater resveratrol production in TB as compared with LB. K12 had no resveratrol production.

Different stage temperatures were tested for resveratrol production in LB and TB broths at 25, 30, and 37 °C during the exponential phase (Table 2). Results in each broth showed better production of resveratrol at 30 °C. Therefore, this temperature was used for all later testing. Lag phase temperature was held at 37 °C to minimize lag phase time.

Carbon equivalent LB and TB media were analyzed for resveratrol production. Data confirmed that resveratrol production was higher in *E. coli* when grown in TB broth (Table 3).

Different concentrations of TB media were then tested for maximum resveratrol production (Table 4). Results showed the greatest yield of resveratrol using a broth containing 50 g/L of TB media.

The effects of the concentration of product substrate and elicitor were investigated (Table 5). It was determined that the addition of extra substrate did not increase product yield. A higher IPTG concentration may have increased resveratrol production in the shaker flasks.

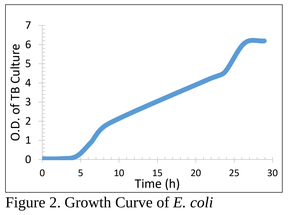

Figure 2. Growth Curve of *E. coli*

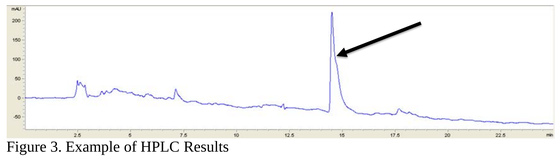

Figure 3. Example of HPLC Results

Table 1. Media Tests

Media:	Resveratrol (mg/L):
K12 Total #1	**NO RESULTS**
K12 Total #2	**NO RESULTS**
K12 Average	**NO RESULTS**
LB Total #1	**51.768**
LB Total #2	**41.100**
LB Average	**46.434**
TB Total #1	**108.306**
TB Total #2	**107.630**
TB Average	**107.968**

Table 2. Temperature Tests

Temperature (C)	Resveratrol (mg/L)
25	**109.803**
25	**70.331**
25 Average	**90.067**
30	**102.014**
30	**112.922**
30 Average	**107.468**
37	**62.268**
37	**20.326**
37 Average	**41.297**

Table 3. Carbon Equivalent Media Tests

Media:	Resveratrol (mg/L):
LB Total #1	**0.465**
LB Total #2	**0.520**
LB Total #3	**2.459**
LB Total #4	**1.020**
LB Total #5	**2.864**
LB Average	**1.4656**
TB Total #1	**8.345**
TB Total #2	**9.088**
TB Total #3	**11.605**
TB Total #4	**13.709**
TB Total #5	**20.613**
TB Average	**12.672**

Table 4. Media Concentration

TB Concentration:	Resveratrol (mg/L):
50 g/L Total	**22.463**
100 g/L Total	**13.531**
150 g/L Total	**12.475**
200 g/L Total	**9.477**

Table 5. Elicitor Concentration

Media:	Resveratrol (mg/L):
5 µL IPTG	**2.665**
5 µL IPTG + 0.05 g tyrosine	**4.407**
10 µL IPTG	**5.405**
10 µL IPTG + 0.05 g tyrosine	**4.430**
15 µL IPTG	**4.367**
15 µL IPTG + 0.05 g tyrosine	**3.719**

Final Design

The final design from the shaker flasks was applied to a benchtop bioreactor. TB broth was prepared with a concentration of 100 g/L and was used for the media. This was done to try and sustain more viable cells than in the shaker flasks with the bioreactor's aeration system. Temperature was set to 30 °C for the exponential growth phase. Impeller speed was set to 200 rpm. A pH probe was also used to track the change in acidity over the incubation period. Air was sparged into the broth, and dissolved oxygen (DO) was measured using a DO probe.

Four samples from the bioreactor were extracted and analyzed. Results show an average resveratrol production of 29.649 mg/L (Table 6). Media pH was maintained around a value of 7.0. Errors in the DO probe prevented accurate measurements of dissolved oxygen in the media broth. Therefore, oxygen demand for this process could not accurately be obtained.

Table 6. Bioreactor Results

Sample:	Resveratrol (mg/L):
Sample 1	**30.688**
Sample 2	**30.072**
Sample 3	**28.471**
Sample 4	**29.364**
Average	**29.649**

Figure 3. Benchtop bioreactor setup

Conclusions

Testing in shaker flasks determined the optimal parameters for resveratrol production in *E. coli*. It was determined to use an incubation temperature of 30 °C and TB broth at a concentration of 50 g/L as the media. It was also determined that the addition of extra product substrate had no effect.

Resveratrol production was achieved in a benchtop bioreactor with a yield of about 30 mg/L. This was significantly lower than our target production goal of 1500 mg/L.

Over time resveratrol production decreased. This was probably due to the loss of the original *E. coli* culture part way through the project. Additional *E. coli* cultures were grown, and transformed with plasmids with the resveratrol producing genes. Although resveratrol was produced by this culture; the production of resveratrol was greatly reduced. Probable causes of this may be due to genetic drift of the plasmid in the *E. coli* cells or incomplete transformation.

Recommendations

- Try to keep the same strain of *E. coli* throughout the project, and regrow the seed culture at least once a week
- Explore the viability of using L-tyrosine producing strains of *E. coli* to increase production of resveratrol
- Test using pyruvate instead of glucose as the carbon source
- Repeat bioreactor tests with 50 g/L of TB media
- Repeat oxygen demand test with a better DO probe

References

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Acknowledgments

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