Comparing the Effect of Different Peptone Media Formulations on Growth of Various Strains of *Escherichia coli*

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INTRODUCTION:

Peptone is a hydrolysate of a commodity protein typically broken down to short polypeptides and free amino acids through enzymatic digestion followed by purification to yield a dry powder. The processing and purification increases the bioavailability of the nutrient content of the protein making it suitable for use in bacteriological media formulations to provide a source of nitrogen and carbon (Michiels et al, 2011). The source of protein, the enzyme(s) used to digest the protein, as well as the method of hydrolysis used in production may differ among peptones. Companies that produce peptones must meet consumer needs and provide peptones to fit varying demands. The protein used to produce the peptone may be animal-free, kosher, non-GMO, or a non-allergen source (see Table 1). Peptones can be tailored to meet the specific needs of a process or end application while retaining its effectiveness in media for bacterial growth.

*Escherichia coli* is one species of bacteria that is commonly found in the microbial community of the lower intestine of mammals. Some strains of *E. coli* are harmless while others are pathogenic, and can cause illness, or in rare cases, death. Furthermore, there are natural strains of *E. coli* as well as strains that have been developed purely for laboratory use. Because some strains can cause serious illness, billions of dollars are spent each year in hospital visits and research (Rasko et. al, 2008). *E. coli* is of particular interest for a variety of reasons; it’s easily culturable in a laboratory setting, has a well researched and documented genome, and the genes are conveniently easy to manipulate, making it an easy choice for a workhorse bacteria in the lab, expressing foreign DNA and making proteins that may have otherwise been difficult to produce, characterize, and study.

The experiments in this study were run to compare how peptone source and processing impact the growth rate and yield of different strains of *E. coli* propagated in liquid media. Strains of *E. coli* that were studied in this experiment include BL21, EPI300, DH5α, and JM109. Peptones tested in the experiments were sourced from soy, pea, or a mixture of peptone sources. The results show that the processing of the soy and pea peptone significantly impacted the growth rate and yield of *E. coli*. 
Table 1: Peptones used in this study and their percent total Nitrogen and Amino Nitrogen to Total Nitrogen ratio. The percent total nitrogen (%TN) and the ratio of amino nitrogen to total nitrogen (AN/TN) for the soy and pea peptones in this study. The HSP-A soy was also provided as a blend with yeast extract (YE) preparations. A higher AN/TN ratio is consistent with greater fragmentation of the source protein.

<table>
<thead>
<tr>
<th>Peptone</th>
<th>% TN</th>
<th>AN/TN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy, Nu-tek HSP</td>
<td>7.6</td>
<td>0.41</td>
</tr>
<tr>
<td>Soy, Competitor A</td>
<td>9.2</td>
<td>0.21</td>
</tr>
<tr>
<td>Soy, Competitor B</td>
<td>9.2</td>
<td>0.25</td>
</tr>
<tr>
<td>Soy, Competitor C</td>
<td>10.2</td>
<td>0.24</td>
</tr>
<tr>
<td>Pea, Nu-tek HPP</td>
<td>12.4</td>
<td>0.40</td>
</tr>
<tr>
<td>Nut-tek Soy + YEa</td>
<td>9.1</td>
<td>0.48</td>
</tr>
<tr>
<td>Nut-tek Soy + YEb</td>
<td>8.1</td>
<td>0.42</td>
</tr>
<tr>
<td>Nut-tek Soy + YEc</td>
<td>8.9</td>
<td>0.46</td>
</tr>
</tbody>
</table>

METHODOLOGY:

Peptone samples were obtained from Nu-tek Bioscience and included their products as well as common competitors within the industry. In a standard growth experiment, cultures of *E. coli* are grown in a liquid medium that consists of a 0.1% total nitrogen solution of peptone that varies by source protein/preparation, M9 salts, and 0.5% glucose. One day prior to the growth experiment, single colonies grown on nutrient agar plates were used to inoculate a 5 mL pre-growth culture in the corresponding media formulation used for the growth study. A volume of o/n pre-growth was inoculated into 30 mL of pre-warmed media contained in a 50 mL sterile Erlenmeyer flask to an 0.05 OD<sub>600</sub> starting culture density. The 30 mL cultures were grown statically in an incubator at 37°C. Culture density was measured spectrophotometrically using a CARY300 UV-Vis (Agilent Technologies, Santa Clara, CA). The OD<sub>600</sub> of a representative aliquot of each culture was taken every hour. When the culture densities exceeded 1.00 OD<sub>600</sub>, appropriate dilutions were made to preserve the accuracy of measurements. Triplicate measurements were averaged and plotted using Microsoft Excel to generate growth curves.
RESULTS:

*Strain dependent variability in growth performance.* Comparison of peptones and four strains commonly used laboratory strains of *E. coli* is shown in figure 1. Each peptone used in the common media formulation revealed some manner of strain specific performance. Nu-tek HSP and HPP peptones showed the least strain dependent variability in growth performance (rate and stationary phase OD$_{600}$). In contrast, the papaic digest of soy (competitor C) was consistently and significantly the poorest performing peptone. The soy peptone from competitor A performed as well as HSP and HPP except for DH5α, where as soy peptone from competitor B performance was relatively poor in DH5α and BL21.

![Figure 1. Comparative growth curve of *E. coli* in growth media with varying peptone compositions.](image)

All media formulations used in this study were based on: 0.1% TN from peptone; 1X M9 salts; 0.5 % Glucose. Pre-growth cultures of *E. coli* were inoculated from single colonies into 5 mL of 0.1% TN peptone media. The volume of inoculum for each o/n culture (11 hr) was determined by OD$_{600}$ to obtain a starting OD$_{600}$ of 0.05 in 25 mL of prewarmed media. Cultures were grown at 37°C without shaking. Triplicate samples were drawn from each culture.

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Soy peptone and yeast extract blends. Blending HPP with a weakly performing peptone, competitor C in this case, supports growth of DH5α that is not significantly different to HSP alone (figure 2.). The stationary phase yield was significantly greater for the 1:1 HSP:comp. C peptone blend over the comp. C peptone alone as the sole peptone source (figure 3.).

**Figure 2. Comparative growth curves of E. coli DH5α in growth media with varying peptone compositions.** All media formulations used in this study were based on: 0.1% TN from peptone or yeast extract; 1X M9 salts; 0.5 % Glucose. Pre-growth cultures of E. coli were inoculated from single colonies into 5 mL of 0.1% TN peptone media. The volume of inoculum for each o/n culture (11 hr) was determined by OD_{600} to obtain a starting OD_{600} of 0.05 in 25 mL of prewarmed media. The YEI-A, -B, -C samples are 1:1 HSP:yeast extract blends prepared from different YE sources. Cultures were grown at 37°C without shaking. Triplicate samples were drawn from each culture.
Figure 3. Viable Plate Count of stationary phase *E. coli* DH5α. A comparison of the peptones HSP (Soy A), Competitor 3 (Soy D) and 1:1 blends with HSP. The yeast extract and soy peptone displayed in the histogram. The VPC shows that each media formulation contained viable CFU’s at the end of the growth study, with the only formulation being significantly lower than the others being Soy D (Soy Competitor 3).

**GROWTH STUDY FINDINGS:**

The Nu-Tek Soy HSP-A peptone had the most consistent performance in the media formulations for all strains of *E. coli* tested here. A 1:1 blend of HSP with a poorly performing peptone was as nearly effective in supporting the growth rate, early stationary phase culture density and viability to the HSP alone. Not surprisingly blending HSP with a variety of yeast extracts had the greatest overall performance. The media formulation with Nu-Tek pea peptone, HPP, supported similar growth rates and stationary phase optical densities to HSP. We conclude that HPP is an effective substitute for soy or animal based peptone in media formulations where high-density growth is desired.

**LITERATURE CITED:**

