Optimization of nitrogen sources for protein expression in *Escherichia coli*

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Introduction

As *Escherichia coli* is one of the most widely used production organisms for active pharma ingredients and enzymes we decided to start a program to screen our current product portfolio and at the same time explore new options for the future (specifically focusing on the development of animal component free option). Furthermore, we were very interested to find out more about the interactions between peptones and yeast extracts. For this screening we decided to use two very different *E. coli* Model strains. One growing relatively slowly, constitutively expressing a cytoplasmic enzyme and one growing considerably faster, expressing a IPTG inducible periplasmically protein. Using the approach of testing for growth and expression of two very different model strains as well as actual production strains we develop solutions for "*E. coli* based expression" rather than a solution for one particular case.

Materials and Methods

All Sheffield™ peptones are dissolved in our proprietary chemically defined base medium at a concentration of 20 g/l when tested separately, or at of 13.33 g/l in combination 6.66 g/l of our Hy-Yest™ yeast extracts. In all experiments the *E. coli* are pre-cultured overnight in the final medium. All experiments are conducted at 37 C in a shaking incubator (100 to 115 RPM), growth is measured as optical density at 620 nm. Growth and Bacterial Alkaline Phosphatase (BAP) expression in *E. coli* BL21: The media are inoculated at an OD_{620} of 0.08 from the corresponding pre-cultures. Cells are induced by 1 mM of IPTG at OD_{620} 0.3 to 0.4. Samples were tested for growth and expression at 4 and 5 hours after induction. The periplasmically expressed BAP was released from the cells by a combination of freezing and Fast Break reagent (Promega) and was quantified by measuring color formation (OD_{620}) over a 40 minute time period, after addition of Alkaline Phosphatase Blue Microwell Substrate (Sigma). Growth and Luciferase expression in *E. coli* DH10B: The media are inoculated at an OD_{620} of 0.2 from the corresponding pre-cultures. Growth and expression was followed for 10 hours, the average values over this 10 hour period are depicted. The cytoplasmically produced Luciferase-EYFP fusion gene product was quantified using the Luciferase Assay System (Promega) after the cells were broken by a combination of lysozyme and Cell Culture Lysis Reagent (Promega).

Results

The results depicted in the graphs are selection of a larger screening program. Only results of individual peptones and peptone yeast extract combinations where the "traditional LB ratio" of 2/3 peptone and 1/3 yeast extract was used are depicted here. Also samples that were not tested at least in triplicate were omitted (error bars in graph represent the standard error of mean). N-Z-Soy™BL4 (a enzymatic digest of soy isolate – animal component free) is taken as an example in these graphs of a peptone that when tested as a separate product gives poor growth and gene expression in both of our model strains. However when tested with the right yeast extract it is actually a very good animal free option.

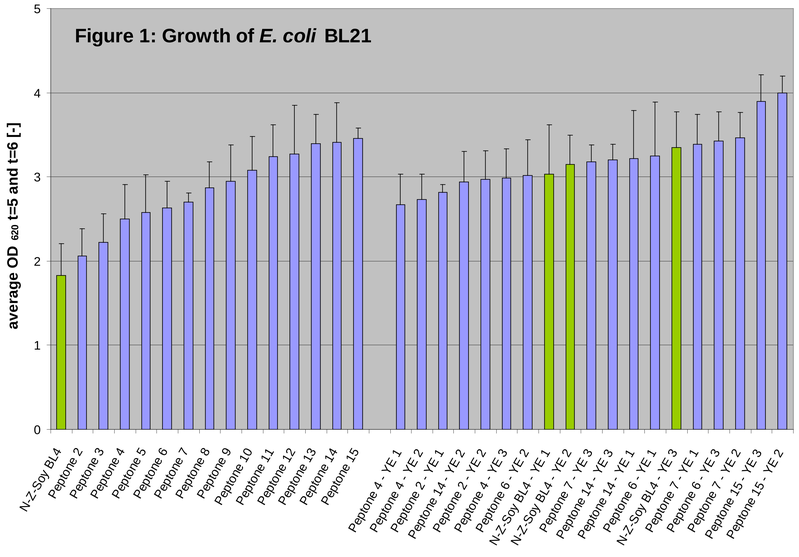
Figure 1: Growth of *E. coli* BL21 (OD_{620} of the samples also used for the BAP activity measurement taken 5 and 6 hours after induction). Whereas as a separate product N-Z-Soy™BL4 results in the lowest biomass formation; in combination with Yeast Extract 3 is actually one of the best performing samples. Especially considering that the best two samples for biomass actually are the worst for gene expression (Fig. 2).

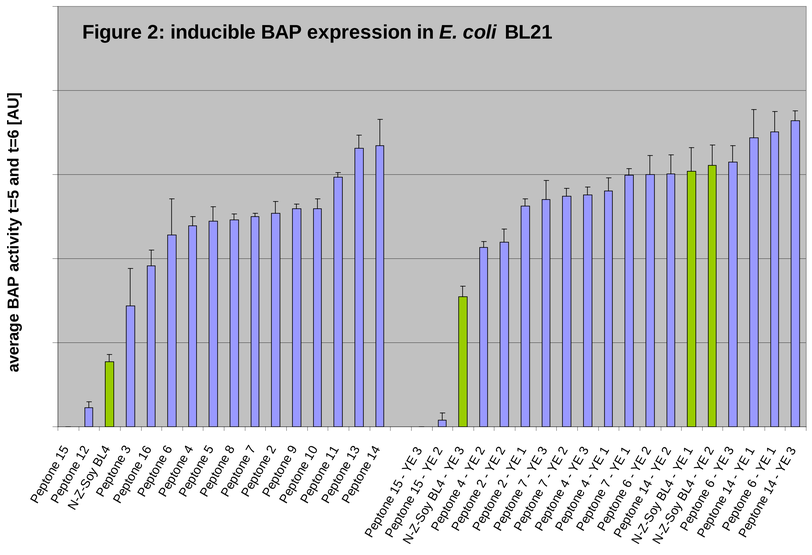
Figure 2: Bacterial Alkaline Phosphatase (BAP) activity measured in the same samples used to obtain figure 1. Also for product formation N-Z-Soy™BL4 as a separate product is one of the worst and in combination with the right yeast extract (YE1 and YE2) it performs very well.

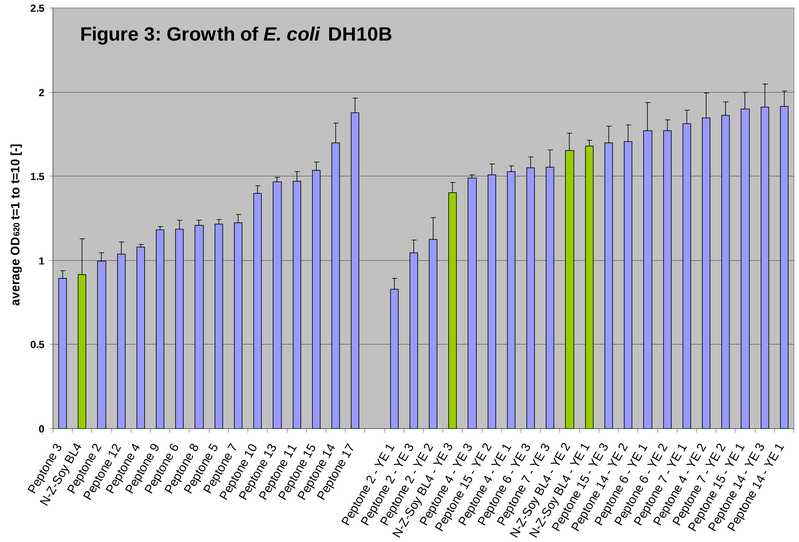
Figure 3: Growth of *E. coli* DH10B (OD_{620} of the samples also used for the Luciferase activity measurement, average of samples taken from 1 to 10 hours). Also for this strain N-Z-Soy™BL4 as a separate product results in more or less lowest biomass formation, in combination with the right yeast extracts (YE1 and YE2) it is however quite acceptable.

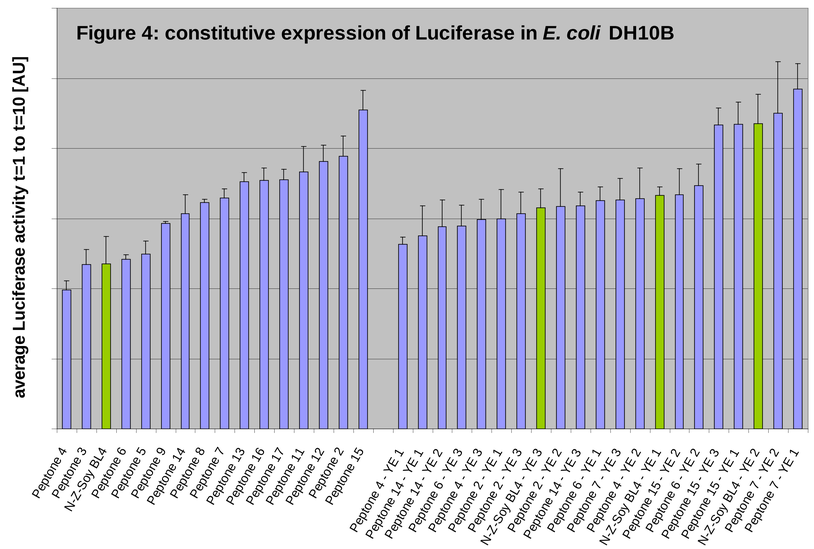
Figure 4: Luciferase activity measured in the same samples used to obtain figure 3. Also for product formation N-Z-Soy™BL4 as a separate product is one of the worst but in combination with the right yeast extract (YE2) it performs very well.

The results clearly show that to find the optimal nitrogen source it is crucial that the peptones and yeast extracts are screened together.









Summary

As the global microbial application lab we support both the yeast extract and peptone business of Sheffield™ Bio-Science. During projects with the end-users of our nitrogen sources we frequently find that initial material qualifications are performed on the separate products, ignoring the interactions. We set out to substantiate our claim that in order to fully explore the potential of both the yeast extract and the peptone it is essential to co-test them, taking into account how they influence each other.

To accomplish this we tested a selection of yeast extracts and peptones both separately and in combination, for growth and expression of two E. coli model-strains. That there is some merit in testing ingredients separately is demonstrated in Figure 2 where combining the least performing peptone (Peptone 15) with yeast extracts (YE2 and YE3) also resulted in the lowest expression level. However, this is not always the case and potentially great combinations can be overlooked as demonstrated by the example of N-Z-Soy™BL4. In both model systems this non-animal peptone was one of the least performing products for growth and expression, if tested as the sole source of nitrogen. However, in combination with the right yeast extract it actually became one of the best performing nitrogen sources.

By screening combinations of peptones and yeast extracts, both for growth and expression in the two different model-systems as well as in *E. coli* strains provided to us by customers, we were able to formulate our new Hy-Express™ line of non-animal nitrogen sources that have a very high probability of working for various *E. coli* based expression systems.