Alkaline Phosphatase: Assessing Streptomyces Griseus as a Model Organism

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Abstract

Hypophosphatasia is a disease resulting from decreased alkaline phosphatase activity. Alkaline phosphatase activity is controlled by zinc levels because of zinc's catalytic effect upon binding to the enzyme. Streptomyces Griseus was identified as a model organism based on BLAST and Zinc 1 binding sites located at nucleotides 326, 330, and 412. Researchers were targeted as possible areas of mutation that would cause hypophosphatasia. Primers were designed around the binding sites and PCR was performed. Gel electrophoresis confirmed the primers were accurate, allowing for replication of this segment of DNA in future studies. Experimental mutations of these sites in S. griseus compared to known mutations causing hypophosphatasia in the human ALPL gene will lead to more knowledge of the disease and possible treatments.

Introduction

1. Streptomyces Griseus is a filamentous bacterium that is a well-known organism in the production of antibiotics. S. griseus is alkaliphilic, meaning it lives in basic conditions, the prime environment for alkaline phosphatase.

2. Hypophosphatasia is a genetic disease that is characterized by demineralization of the bones and teeth. The disease is caused by a mutation in the ALPL gene.

3. The ALPL gene is the gene coding for Alkaline phosphatase in the human liver, bone and kidney tissue.

4. Alkaline phosphatase is a zinc-containing and zinc-dependent metallo-enzyme, meaning that in cases of zinc deficiency alkaline phosphatase cannot function properly, causing build up of substances normally processed by the enzyme.

5. Alkaline phosphatase has two zinc binding sites, we focused on the zinc 1 binding site while designing our primers.

II. Streptomyces Griseus as a Model Organism

I. Assessing Alkaline Phosphatase Activity in Lobsters

Alkaline phosphatase activity was found to be significantly more active in hepatopancreas tissue versus heart tissue in lobsters. The hepatopancreas consists of digestive tissue while the heart is a contractile muscle. This is expected because Alkaline Phosphatase is known to be prevalent in tissue that transport nutrients.

II. Binding Site Determination

The hepatopancreas contains high concentrations of zinc ions to help catalyze reactions, making for easy interaction with the Zinc molecule compared to a helix because it is more flexible and available.

III. Secondary Structure

The secondary structure at the two conserved sites is a coil, while the varying site is part of a β-sheet. This is expected because it is more flexible and available.

IV. Dilution and Cell Concentration

Figure 5: Dilutions of S. griseus versus E. coli to test for cell concentration. Dilutions from an original stock of assumed concentration, 2x10^9 cells per 1000 µL, were made to dilute the stock to the given expected concentrations. Based on dilution 2 for E. coli it was calculated that the original stock was actually 2.2x10^9 cells per 1000 µL. This is an acceptable range of the predicted value, ensuring our methods and calculations were accurate. The S. griseus colonies did not grow as expected, this is assumed to be caused by the inability for the cells to clump.

V. Designing Primers

Figure 7: Primers were determined around the zinc 1 binding sites. The expected amplification product encompassed 386 base pairs. The primers were tested for cell competence and were found to have good results. A BLAST was also performed to look for highly similar sequences in other regions of the S. griseus genome, and it was found that one primer had a similarly matched sequence, the other was sufficiently unique.

VI. Gel Electrophoresis

Figure 6: Gel Electrophoresis of the DNA acquired from PCR with our experimental primers. Our experimental DNA showed the expected band at ~386 bp, while the control was a blank band at ~1000 bp. Our positive control met its expected band length. The negative control showed no DNA.

Conclusions

- Alkaline Phosphatase activity in lobster hepatopancreas tissue is greater than in heart tissue.
- S. griseus StrK gene and Human ALPL gene have many conserved regions, including all but one zinc 1 binding site.
- The secondary structure at the two conserved sites is a coil, while the varying site is part of a β-sheet.
- An original stock of E. coli was determined to have a concentration of 2.2x10^9 cells/mL. It was not possible to determine cell concentration of S. griseus because of its clumping nature.
- Based on our gel electrophoresis, our primers successfully replicated our target region of DNA (~386 bp).
- The presence of the second band (at ~1000 bp) could be caused by the similar primer sequence found by the BLAST elsewhere in the genome.
- S. griseus is a good model organism for examining alkaline phosphatase.

Future Studies

- Knowing that these primers work, we can isolate the target region and make mutations at and around the zinc 1 binding sites and introduce them to the S. griseus genome and examine the effects.
- We can compare our mutations to known mutations causing hypophosphatasia, as there are many that vary in severity of symptoms.
- We can work to improve the purity of our PCR to eliminate the unwanted bands.

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