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Examination of Germination Receptors of B. subtilis and B. megaterium

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Abstract

Many bacterial species including those in the Bacilli group form spores as a mechanism to survive harsh conditions such as extreme temperature, radiation, chemicals, and nutrient starvation. By forming spores, they can remain metabolically dormant for an extended period and revert to their vegetative form when environment becomes favorable. This resumption of metabolism and growth is marked by a process called germination that is triggered by exogenous nutrients such as amino acids, sugars, and nucleotides. The Ger (germination) receptors that are postulated to respond to these germinants, in the case of B. subtilis and B. megaterium, are a complex of at least three different proteins (the A-, B-, and C-subunits) transcribed from the same operon. While similar in gene arrangement and protein complex formation, these two Bacilli sp. respond to different germinants. This experiment investigates the Ger receptor of B. subtilis and the GerU receptor from B. megaterium. Gear of B. subtilis is activated with L-proline, while GerU of B. megaterium is activated with L-alanine. In order to determine the location of the binding site, different fragments of the GerAB gene and the GerUB genes encoding for protein A and B from each operon were amplified and fused together in frame to make a chimeric gene product. Receptors from B. subtilis mutant strains expressing chimeric protein complexes will be tested for germination in the presence of L-proline and/or L-alanine. These studies will provide insights into how bacteria sense their environment and possible strategies to control and prevent growth.

Methods

Designing Chimeras and Primers

GerAB primers: F (1-171 aa) R (172-275 aa) 312 bp fragment GerUB primers: F (2-269 aa) R (270-367 aa) 294 bp fragment

Amplification and Fusion

Using E. coli subunit shuttle vector pGEMI, fusion inserted in the lacI gene

Ligation

Transformation

Plasmid Extraction and Verification

Integration

PCR product of GerAB

Fusion Individual Fragments

Hypothesis

Although all three subunits are necessary for the receptor complex to function, we hypothesize that subunit A and C are not involved in germinant recognition and that the unique germinant recognition site is located on the B-subunit. Different chimeric B-subunit receptors from B. subtilis and B. megaterium will respond differently to germinants and will identify the region that recognizes the germinants.

Results

Conclusions

The resultant bands from the individual PCR fell between 250 and 500 bp indicating successful amplification of GerAB and GerUB fragments. The fusion product fell between 500 and 750 bp which validates the expected length of 606 bp. We were able to amplify and fuse B-subunit fragments from B. subtilis and B. megaterium. We have also developed a protocol to test these chimeric receptors in B. subtilis.

Future Directions

• We will design new constructs that fuse together three different fragments and test them similarly
• We will integrate them in B. subtilis to create mutant strains producing chimeric receptors
• We will test mutant strains with different germinants.

References


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