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Synthesis Of Chimeric Receptors Essential For Spore Germination

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Abstract

Various species of bacteria have been reported to form an endospore, a metabolically dormant cell, during times of nutrient deficiencies and extreme stress. Endospores are outnumbered by their exposure to harsh chemicals, high temperatures, and can reconvert to a metabolically active cells, through a process known as germination, when the necessary conditions are met. The rigid membrane of the endospore contains various germination (Ger) receptors which sense the external environment for necessary metabolites and germinants. Ger receptors are encoded by tricistronic operons that produce three distinct membrane proteins. A, B, and C subunits. Although the function of the Ger receptor has been established by genetics, no information is currently available for germination binding site. Bioinformatic and genetic approaches has predicted that the C-terminus of the II-subunit is the most likely candidate to contain the germination binding site. B. subtilis and B. megaterium, two species of the Bacilli genus, germinate in response to different germinants; B. subtilis germinates in response to 1-leucine by activation of the GerU receptor, while B. megaterium germinates in response to L-leucine by the activation of its GerU receptor. The focus of this study is to constructs chimeric genes in which fragments of B. subtilis GerA and B. megaterium GerB receptor are fused together. These B. subtilis: B. megaterium chimeric receptors will be introduced into the B. subtilis genome and the mutant B. subtilis strains will then be tested for the ability to germinate in order to establish the requisite binding site for GerU. During the initial pilot studies, the regions coding for the N-terminus of the GerA receptor from B. subtilis and the C-terminus of the GerU receptor from B. megaterium were amplified using polymerase chain reaction with primer ends complementary to each other in order to further produce the desired hybrid genes without the use of restriction enzymes.

Methods

Polymerase Chain Reaction (PCR)

The genomic DNA from B. megaterium QM1351 (ATCC 12872) and B. subtilis YB955 was extracted using the Wizard Genomic DNAs Purification Kit and used as a template for initial PCR reactions. Appropriate primers were designed to produce complementary ends, which would allow for the engineering of chimeric genes without the use of restriction enzymes (Horton, 1988). Primers 1 and 2, noted in fig. 2, amplify the front end of the B. subtilis GerA receptor. Primers 3 and 4 amplify the tail end of the B. subtilis GerA receptor. Primers 5 and 6 amplify the GerU receptor of B. megaterium. Samples containing the appropriate primers, PCR master mix (Promega), water, and template were subjected to 30 cycles of PCR amplification. Each cycle run with a denaturing temperature of 94°C, annealing temperature of 50°C, and elongation temperature of 68°C. The resulting products were amplified using overlamp PCR containing the appropriate primers.

Bacterial Transformations

Selected reactions containing the correct size PCR product were inserted into the blunt ITOPO vector (Invitrogen) following the manufacturer’s protocol. The vector was then transformed into One Shot TOP 10 Chemically Competent E. coli (Invitrogen) following the manufacturer’s protocol. Transformants were grown on Luria Bertani agar plates. Training a selective antibiotic and incubated overnight at 37°C. Resulting colonies grown in the presence of the selective media were screened for the desired insert. Colonies were picked and finally added into a PCR tube to act as the template for sequential PCR reactions as previously described.

Results

Discussion

The initial gene that codes for the proposed chimeric germination receptor was successfully generated and confirmed based on the addition of base pairs from subsequent PCR reactions when analyzed on an agarose gel. The synthesized chimeric product was then inserted into the PMutin4 vector. The PMutin4 vector was chosen due to its ability to integrate directly into the B. subtilis genome. The PMutin4 vector need to be subsequently cloned into a strain that would reproduce the vector, but with homologous recombination due to B. subtilis’ ability to first cleave circular DNA, then uptake it as a linear strand (Kauflenstein et al., 2011). B. subtilis has been transformed and successfully used to contain the insert. The general outline of introducing foreign germination receptor genes has been outlined in this project.

Future Direction

Having created only one mutant of wild type B. subtilis YB955, other mutants will be created using the same methods. Once this mutant has been tested for germination and produces desirable results, other genes coding for various other germination receptors can hopefully be integrated into the B. subtilis genome and then tested for. Using this strategy as a model species can be created as used in environmental probes, germinating only when inquired germinant or compound is present.

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