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Construction of broad host range vector for expression of heterologus protein in Brucella abortus strain 19

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Brucellosis, a major zoonotic disease causing abortion and infertility in cattle, is controlled mainly by vaccination with strain Brucella abortus strain 19. It is a smooth, attenuated and live vaccine candidate which can induce a strong humoral and cellular immune response and therefore, it is an attractive vector platform for the delivery of heterologus antigens. The objective of this study was to construct a broad host range vector for expression of heterologus protein in B abortus strain 19. For this, promoter sequence of Brucella groE gene was cloned into broad host range vector pBBR 122. For one step purification and easy detection of the expressed protein, six histidine tag was added in to the reverse primer of groE promoter. To check promoter activity and expression status of the construct, a promoterless GFP gene was cloned into the vector. The construct pBBR GroE_ GFP was then electroporated in to strain 19. The expression of GFP was induced by heat shock at 42°C and treatment with H_2O_2. Then the recombinant protein was purified by using urea lysis method and followed by Ni-NTA affinity chromatography. Further, the purified protein was dialysed and the specific reactivity of the recombinant protein was checked by western blotting using anti histidine antibody. A single specific band could be detected at 28kDa confirming the expressed recombinant protein. Based on the present study, it is concluded that B abortus strain 19 can be used as vector for expression of foreign protein and further experimentation is required study its vaccinal potential.

Keywords: Brucella abortus strain 19, broad host range vector, expression vector, recombinant DN.

Biography

Justin Davis K is the native of Kerala, India. He has completed his MVSc degree from Indian Veterinary research institute in veterinary epidemiology and has procured PhD from the same institute on Veterinary Bacteriology. He had received IVRI fellowship for both MVSc and PhD. He had also received PTF grant for young scientists for presenting a paper at Estonia conference.

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Transfer and expression of tobacco osmotin (Tbosm) conferred fungal disease resistance in Indian soybean

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Soybean is an important vegetable protein and oil crop throughout the world. India ranks fifth in soybean production with an annual production of 10.12 million metric tons. Soybean productivity is negatively influenced by several diseases and pests. Among the several diseases, fungal diseases causes significant lose in soybean production. To overcome the negative impact of fungal diseases in soybean, we transformed soybean cv. Pusa 16 with biotic and abiotically induced and apoplastically secreted pathogenesis related tobacco osmotin (Tbosm) gene using Agrobacterium-mediated genetic transformation. Integration and expression of Tbosm in transformed soybean was confirmed by polymerase chain reaction (PCR), southern hybridization and western blotting. Totally 5 transformed soybean plants were obtained and all the transformed soybean plants showed significant resistance against four fungal pathogens Microsphaera diffusa, Septoria glycines, Phakopsora pachyrhizi, and Rhizoctonia solani. The transformed soybean plants accumulated significant levels of proline, glycinebetaine, chlorophyll, APX, CAT, SOD, DHAR, and MDHAR than non-transformed plants during fungal disease analysis. The present investigation clearly shows that expression of Tbosm protects the soybean plants against fungal diseases.

Biography

K. Subramanyam is pursuing Doctoral degree in Biotechnology under the guidance of Dr. A. Ganapathi. Bharathidasan University, Tamil Nadu. He standardized Agrobacterium mediated genetic transformation for chilli, banana, and soybean. At present he is concentrating on genetic improvement of soybean against salinity stress and fungal disease resistance. His findings were published in well reputed journals like Planta, Plant Cell Reports, Plant Cell Tissue and Organ Culture, and Acta Physiologiae Plantarum.

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