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Production of recombinant fusion protein 'fliglo': fliH gene of Escherichia coli O157:H7 tagged with GFP gene

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Escherichia coli O157:H7 is a very common food borne pathogen. Without the detection at the right time, it could turn fatal. It is not only diagnosis that is important, but also the trials to make it non-virulent are also important. Detection of this in humans is also important, since appropriate therapy can be administered. Here we demonstrate a system appropriate for E. coli O157:H7 that can be extended to other bacteria and viruses such as HIV, MRSA detection. The gene fliH, in E. coli O157:H7, which is one of the major genes that produce the protein necessary for the association of the flagella, was targeted here.

A recombinant protein (for fliH) that tagged with GFP protein was produced using the plasmid. Green fluorescent protein (GFP) obtained from GFP gene is known for its fluorescence. This makes it very useful in molecular biology to detect the target gene when it is tagged to GFP. The fliH gene, which plays an important role in flagellar association in the bacteria, was used here. This when tagged with GFP is an important diagnostic tool for detecting the presence of E. coli O157:H7 in the samples. For preparing this system, E. coli O157:H7 DNA was isolated and restriction digested and by using other techniques, the fliH gene was introduced into the pGLO plasmid. This plasmid was expressed to produce the fusion protein 'fliglo'. The isolated fliglo fusion protein has different spectral signature than the original GFP. GFP shows an emission peak at 509 nm, where as the fusion protein shows it at 461 nm. This is useful in isolation of pure fusion protein. The fliglo protein will be used in detection assay to be developed by Nano Science Diagnostics, Inc. for their NanoFluro Device for the detections of E. coli O157:H7. Further the fusion protein system will be utilized for other bacteria and virus detection in blood samples and also the production of highly specific antibodies for medical uses.

Keywords: Escherichia coli O157:H7, fliH, GFP, pGLO, restriction digestion, emission, fliglo.

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Biological control of Echinochloa crus-galli (Barnyard grass): A major weed in rice (Oryza sativa L)

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Echinochloa crus-galli (Barnyard grass) is one of the most invasive paddy (Oryza sativa L.) weed in Andhra Pradesh. In this study, a survey was conducted in the paddy fields of eight southern districts of Andhra Pradesh to document the fungal pathogens of barnyard grass. Pure cultures were isolated from the naturally-infected barnyard grass leaves collected from different paddy growing areas. A total of 51 fungi were isolated and two isolates Alternariaalternata(SBT#21) and Curvularia lunata (SBT#30) exhibited more pathogenicity (>80%) under greenhouse conditions on Echinochloacrus-galli. These two strains were confirmed at species level by molecular characterization and further used for mass production by both submerged and solid state fermentation on cheaper and readily available sources. The conidia were collected and developed the powder formulations. Bioassays were conducted with formulated products onto both weed and rice seedlings under greenhouse conditions at different concentrations. 100% mortality of weed seedlings was observed at 10g/ lit concentration containing spore load of 10^7conidia/g. The phytotoxins were also isolated from both the pathogens, purified by column chromatography and subjected to HPLC analysis. The obtained fractions were tested for phytotoxic effect under laboratory conditions and found the complete wilting of weed leaves after 48 hr. This study concludes that these two fungal pathogens can be used as bioherbicides for the control of weeds in paddy.

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