CONSTRUCTION OF FUSION PROTEIN DECTIN-FC AGAINST β –GLUCAN ON FUNGAL CELL WALL

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Abstract

In recent decades there has been an increase in the incidence of invasive fungal infections (IFIs) . The IFI display a high morbidity and mortality, and their causes have been attributed to a increase in organ transplantation,and the increasing number of immunocompromised patients. Thus , there is a need for the development of new therapies for the control of IFI. Recent studies have demonstrated that passive immunization with monoclonal antibodies can protect mice from fungal infections.In an attempt to generate a immunobiological against all fungal infections, our group developed a strategy to using the C-type lectin dectin -1, with affinity to a structure common to many fungi , the β (1,3) -glucana , and fused it to the effector Fc portion immunoglobulins .In our work we used the obtained coding sequence of dectin -1 from GenBank which was amplified by PCR.The product was used further digested with two restriction enzymes, one for each end of the product . Plasmids pFUSE - IgG1Fc , IgG2aFc , IgG2bFc and IgG3Fc containing the coding sequences for the IgG1, IgG2a , IgG2b and IgG3 Fc portions were used in another reaction digestion with the same enzymes used in the PCR fragment . The PCR product which had been previously digested was cloned into pFUSE for construction of plasmids dectin - FcIgG1 , dectin - FcIgG2a , dectin - FcIgG2b and dectin - FcIgG3.After ligation of the plasmid sequences , these were used to transform chemically competent E. coli by heat shock. About eight colonies from each transformation were picked , grown in liquid medium and had their plasmid extracted. An screening was conducted by PCR of the plasmid samples.The sequence of the insert in dectin pFUSE plasmids was confirmed by PCR and currently from each transformation a plasmid is being sequenced for confirmation..

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