Poster

Optimizing production of a single-chain antibody (scFv) against the 33-mer peptide in bacteria.

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ABSTRACT

Motivation: Celiac disease is a permanent intolerance to gluten from wheat, barley, rye and, in some patients, oats. Partially digested gluten peptides produced in the digestive tract cause inflammation of the small intestine. Monoclonal antibodies were developed against a highly immunotoxic 33-mer peptide to facilitate their detection in food. One service that gives Biomedal, is the diagnosis of gluten in food, and for this, they have developed single chain antibodies from monoclonal antibodies. Single chain antibodies (scFv) are merging the variable regions of the heavy and light chain joined by a linker. Single chain is powerful tools in research and clinical settings for the relative ease of produce them in large quantities, at low cost in bacteria. The main objective of the project it has been improved the production of ScFv in different strains of bacteria and optimize the purification and production of functional antibody.

Methods: Various induction test it has conducted at different concentrations of inducer and temperature in different strains of Escherichia coli which have been transformed with different vectors with the ScFv coding region using different expression systems to identify which is the right to produce the most functional antibody. It also has optimized antibody purification by affinity chromatography IMAC, It has been found that the antibody is active by ELISA, Western blotting and It has been quantified by Bradford.

Results and Conclusions: It has been able to purify in an ideal way the single chain antibody and has been seen that remains functional because it recognizes gliadin peptides and 33-mer in grand part because there is a fraction which is not active. Furthermore, it has not been able to improve the conditions of expression of the ScFv in any of the strains tested, we think that the basal production of ScFv is able to kill the bacteria.

REFERENCES

Comino Isabel, Real Ana,Vivas Santiago, et al. Monitoring of gluten-free diet compliance in celiac patients by assessment of gliadin 33-mer equivalent epitopes in feces. The American Journal of Clinical Nutrition. 2012;95:670-677.

Morón Belén, CebollaÁngel , Hamid Manyani, et al. Sensitive detection of cereal fractions that are toxic to celiac disease patients by using monoclonal antibodies to a main immunogenic wheat peptide. The American Journal Clinical Nutrition. 2008;87:405-414.

Morón B, Bethune MT, Comino I, et al. Toward the assessment of food toxicity for celiac patients: Characterization of monoclonal antibodies to a main immunogenic gluten peptide. PloS one. 2008;3(5).

Segovia Menacho Patricia, Revuelta González Matilde, Arévalo Rodríguez Miguel. Producción en bacterias de minianticuerpos anti- gluten recombinantes. BIOSAIA 2015;4.