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Editorial

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Modern Identification of Immunological Techniques

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Editorial

In the detection and identification of filamentous fungi and yeasts in foods, immunological techniques have become and will remain an important and widespread technology. This is especially possible due to the availability of monoclonal antibody (MAb) technology, which has revolutionised the identification and diagnosis of species in the development process. As many other microorganisms, fungi contain a variety of antigens that can be used with the desired specificity for identification. Isolate-, species- and genus-specific antibodies that are very sensitive and target-specific can be raised. The elevation of monoclonal antibodies from contaminated food products, however, causes problems with fungal antigen extraction. The advancement of in vitro technologies in which antibody genes are expressed in bacteria would simplify and allow the manufacturing process of monoclonal antibodies more widely available.

Immunological techniques

While both medical and plant pathology overlap, immunological diagnosis of food-borne fungi has resulted in many developments in the characterization of fungal antigens over the past decade in an attempt to characterise some of the essential immunodominant sites. Some common foodborne fungi, such as Aspergillus, Botrytis, Cladosporium, Fusarium, Geotrichum, Monascus, Mucor, Penicillium and Rhizopus, have been characterised by several antigenic sugars and proteins. The rapid identification using immunological techniques of popular food-spoilage flora in foods, however, is not well known.

In food mycology, modern detection assays are focused on the identification by unique immunoassays of fungal cell wall- and cell surface-associated or extracellular polysaccharides and many groups of cell surface protein antigens. It is possible to distinguish up to a dozen different antigens from any one animal. To acquire immune serum, these antigens may be used. Mannose, galactose, glucose, fucose and sometimes glucoronic acid are made from thermostable extracellular polysaccharides (EPS) from fungi. Many species release these EPS to the growth medium; however, the EPS yield of fungi can vary depending on species, a number of variables and conditions of culture. Animal antigenic EPS or cell surface proteins could be used to manufacture polyclonal rabbit immunoglobulin (Ig)G antibodies to be used directly and sensitively in a variety of immunoassays.

responses mediated by T cells are usually performed only on experimental animals and involves the preparation of these cells from blood or from the lymphoid organs, such as the spleen and the lymph nodes. Typically, any substance that has a distinctive structure or conformation that may be recognized by the immune system can serve as an antigen. A wide range of substances from simple chemicals like sugars, and small peptides to complex macromolecules and viruses can induce the immune system. Although the antigenic determinant of a test substance is usually a minor part of that substance called the epitope, a small antigen referred to as a hapten can rarely elicit an immune response on its own. It is not an immunogen and would therefore need to be covalently linked to a carrier in order to elicit an immune response. The induction of such a response to even large immunogenic antigen is not easy to achieve and the dose, the form and route of administration of that antigen can profoundly affect whether a response can occur. Especially the use of certain substances called adjuvants is necessary to alert the immune system and produce a strong immune response.

According to the clonal selection theory, antibodies produced in a typical immunization experiment are products of different clones of B-lymphocytes that are already committed to making antibodies to the corresponding antigen. These polyclonal antibodies are multi-subunit proteins that belong to the immunoglobulins family. They have a basic Y-shaped structure with two identical Fab domains, which form the arms and interact with the antigen, and one Fc domain that forms the stem and determines the isotype subclass of each antibody. There are five different isotype subclasses, IgM, Ig G, IgA, IgE, and IgD, which show different tissue distribution and half-life in vivo. They determine the biological function of the antibodies and appear during different stages of the immunization process. Knowledge about the biosynthesis and structure of these antibodies is important for their detection and use both as diagnostic and therapeutic tools.

Antibodies are highly specific for their corresponding antigen, and are able to detect one molecule of a protein antigen out of around a billion similar molecules. The amount and specificity of an antibody in a test serum can be measured by its direct binding to the antigen in assays usually referred to as primary interaction immunoassays. Commonly used direct assays are radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and immunoblotting techniques. In both ELISA and RIA, an enzyme or a radioisotope is covalently linked to the pure antigen or antibody. The unlabeled component, which most often is the antigen, is attached to the surface of a plastic well. The labeled antibody is allowed to bind to the unlabeled antigen. The plastic well is subsequently washed with plenty of buffer that will remove any excess non-bound antibody and prevent non-specific binding. Antibody binding is measured as the amount of radioactivity retained by the coated wells in radioimmunoassay or as fluorescence emitted by the product of an enzymatic reaction in the case of ELISA. Modifications of these assays known as competitive inhibition assays can be used that will allow quantifying the antigen (or antibody) in a mixture and determining the affinity of the antibody-antigen interaction by using mathematical models. Immunoblotting is usually performed in the form of Western blotting, which is reserved to the detection of proteins and involves an electrophoresis separation step followed by electroblotting of the separated proteins from the gel to a membrane and then probing with an antibody. Detection of the antigen protein antibody interaction is made in a similar way as in RIA

or ELISA depending on whether a radiolabeled or enzyme-coupled antibody is used.

Antibodies can also be monitored through immunoassays that are based on the ability of antibodies to alter the physical state of their corresponding antigens and typically involve the creation of a precipitate in a solid or liquid medium. The hemmaglutination assay used to determine the ABO type of blood groups and match compatible donors and recipients for blood transfusion is based on this assay. Currently, the most common application of this immunoassay is in a procedure known as immunoprecipitation. This method allows antibodies to form complexes with their antigen in a complex mixture like the cytosol, the nucleus or membrane complexes of the cell. The antigen-antibody complex is precipitated either by inducing the formation of even larger complexes through the addition of excess amounts of anti-immunoglobulin antibodies or by the addition of agarose beads coupled to a special class of bacterial proteins that bind the Fc region of the antibody. The complex can also be precipitated by covalently linking the antibody to agarose beads forming a special affinity matrix. This procedure will also allow the purification of the antigen by immunoaffinity, a special form of affinity chromatography. Immunoprecipitation is a valuable technique that led to major discoveries in immunology an all disciplines of molecular and cellular biology. It allows the precipitation of the antigen in complex with other interacting proteins and reagents and therefore gives an idea on the function of theantigen.

The T cell immune response is detected by using monoclonal antibodies, a specific family of antibodies that recognize surface markers that are expressed by lymphocytes upon their activation. These monoclonal antibodies are highly specific, and are produced by special techniques from single clones of B cells and are therefore, homogenous groups of immunoglobulins with the same isotype and antigen binding affinity. These antibodies are used to identify characterize cells by flow cytometry (FACS), immunocytochemistry, immunofluorescence techniques. The difficulty to isolate antigen specific T cells is due to the fact that these T cells recognize the antigen in the context of a tri-molecular complex involving the T cell receptor and the MHC molecules on the surface of specialized cells called antigen-presentingcells. These interactions aresubtle, havelowaffinity and are extremely complex to study. Novel and powerful techniques using tetramers of MHC molecules were developed in 1997 that are now used to identify and isolate antigen specific T cell clones. These tetramer-based assays are proving useful in separating very rare cells, and could be used in clinical medicine. In fact, virus and tumor specific T cells usually give a stronger response and are usually more effective in killing virus infected and tumor cells. Testing for the function of activated, antigen specific T cells known as effector T cells is routinely done in vitro by testing for cytokine production, cytotoxicity to other cells and proliferation in response to antigen stimulation. Local reactions in the skin of animals and humans provide information about T cell responses to an antigen, a procedure that is very used in testing for allergic reactions and the efficacy of vaccination procedures. Experimental manipulations of the immune system in vivo are performed to reveal the functions of each component of the immune system in vivo. Mutations through irradiation, or mutations produced by gene targeting (e.g., knock-out and knock-in techniques), as well as animal models produced by transgenic breeding, are proving helpful to researchers in evaluating this highly complexsystem.