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MODERN BIOTECHNOLOGICAL TOOLS FOR DETECTING MICRO/MACRO ORGANISMS

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Different biochemical procedures used for the analysis of chemical structure or properties of microbial cells have been described in the communication.

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Every organism, including all prokaryotic and eukaryotic pathogens, is characterized by the particular biochemical activities that are necessary for its survival. The biochemical characterization of bacterial nutrition is well established. Similar differences in the utilization of nutrients by filamentous fungi can also assist in their taxonomy and identification. The range of biochemical procedures used for the analysis of chemical structure or properties of microbial cells includes the following technique.

Chromatography

In chromatography, an adsorbent layer is used to separate mixtures; several techniques available for the separation of components of mixtures of metabolites require a considerable amount of equipment and a high level of expertise[1].

Thin layer chromatography (TLC) : TLC is popular for clear, quick, simple, sensitive, and cheap and can be visualized under UV light with corrosive sprays or high temperature[2]. In TLC, a layer of an adsorbent powder such as silica gel, aluminium oxide or cellulose is placed on a glass, aluminium or special plastic plate. These sheets can be subdivided by cutting. After the mixtures are applied, the sheets are stood in the selected solvent in a glass tank. Sometimes several solvents are used to run chromatograms. The plate is allowed to dry once the solvent has reached nearly to the top of the plate and the solvent front has been marked. Spots are detected by several methods depending on their chemistry, then the ratio of the distance moved by the spot relative to the solvent front (Rf value) is measured and compared with similar known standards.

Gas chromatography (GC) and gas liquid chromatography (GLC)

GC and GLC are very sensitive technique for analyzing complex mixtures of volatile substances and so can be employed to identify characteristic volatile substances found in micro-organism. In these methods of chromatography, the various components of the sample pass at different relative speeds through a long narrow tube packed with inert material coated with a non volatile liquid. Columns packed with low porous materials are used to separate gases and low molecular weight materials. The speed that the various components pass through the coloumn depends on their partition coefficients. Although GLC is not suitable for general routine use in diagnosis, it is probably more widely used than the TLC.

ELECTROPHORESIS

In electrophoresis, substances in a mixture, which are ionizable, can be separated from others that are not, by subjecting the mixture to an electric field. Polyacrylamide gel electrophoresis (PAGE) is probably now the most commonly used method for studying protein differences between species previously classified on the

basis for their morphological characters. Polyacrylamide gel is prepared in a layer usually 1-1.5 mm thick with a suitable buffer on a glass plate, with air extracted. The acrylamide monomer is neurotoxic and should be handled strictly as advised by the manufacturer. Test substances are introduced near one end, usually by pipette. Wicks of lint connect the gel to a buffer solution at each end and an electric current is applied. The cathode is applied at the insert end of the gel to produce a potential drop of 6-10 volts per cm of gel and the time required for separation is usually determined by placing a spot of a suitable dye at the point of insertion of the sample, or by the use of a discontinuous buffer system, when a brown line will pass along the gel masking the interface between the gel and the buffers. Once the run is completed, the gel can be stained for proteins with Coanassie Blue or silver, and may be sliced horizontally in order to stain for different components[3].

ELECTRON MICROSCOPY

Electron microscopes rely on a beam of electrons produced from a hot filament (e.g. Tungsten), rather than light as the source of illumination and their power of resolution is consequently very much greater as it is not limited by the wavelength of light. Electron microscopes are of two types, transmission electron microscopes (TEMs) or scanning electron microscopes (SEMs). While a TEM is analogous to a compound light microscope, an SEM is more similar to a binocular stereoscopic microscope. Computer processing of electron microscope images is also a potentially valuable technique. When using a TEM, a beam of focused electron is passed through a thin section of the specimen. Electromagnetic lenses magnify the image formed in the beam before the final image is projected onto a fluorescent screen where it can be seen and photographed. Computer processing of electron microscope is also a potentially valuable technique[4].

IMMUNOLOGICAL TECHNIQUES

Immunoassays have been revolutionized by the introduction of highly specific monoclonal antibodies and enzyme linked immunosorbent assays (ELISA) as routine methods to detect only the molecules (antigen) or antibody binding sites (epitopes) that are unique to the infecting organism, or its metabolites. Diagnosis can be completed in several hours instead of the days or even weeks taken if cultures are made.

ELISA

Enzyme linked immunosorbent assay (ELISA) has been extensively deployed in clinical diagnostics and for the detection of pesticides[5, 6]. ELISA consists of immobilization of antigen to a microtitre plate and it's binding with antibody confugated to an enzyme. The enzyme hydrolyses the substrate resulting in a coloured reaction. The possible application of immunoassays for the detection of insect filth in stored blood commodities is based on the principle that myosin, a muscle protein is present in all life stages, except eggs of insects. The myosin content increases as the larva becomes older, decreases at pupal stage and again increases in the adult stage of insect. Myosin is not found in food grains and processed foods. The muscle protein is extracted from infested grains and the mass of insect material (myosin content) present is correlated to the number of insects in the infested grains. Antibodies to insect myosin

developed are allowed to bind with the insect protein extracted from sample confugated to an enzyme in microtitre plate. The intensity of colour proportional to quantity of myosin is measured in colorimetric ELISA reader at a fixed wavelength. The assay gives an indication of the amount of insect biomass accumulated over the period of storage. Currently, ELISA technique is applicable for detecting a majority of stored product insect pests in different types of food commodities, raw and processed.

Two types of ELISA commonly used are (i) Double antibody sandwich ELISA (DAS-ELISA). Here antigen is trapped by antibody and detected using enzyme labelled antigen specific antibody. (ii) Direct antigen coated ELISA (DAC-ELISA). Here the microtitre plate is first coated with antigen, followed by reaction with homologous antibodies, which are then detected by the enzyme labelled second antibody against antibodies of the first.

ELISA is a sensitive, accurate, quick and well adopted for small and large scale use in pathogen detection[7].

Western Blotting

Western blotting involves the transfer of proteins or glycoprotein on polyacrylamide gels electrophorectically onto a membrane or solid phase and the probing of such membrane bound antigens with a specific antibody serum. This technique is especially useful for detecting antibody against antigen in complex mixtures, such as on the surface of fungal hyphae or bacterial cells.

NUCLEIC ACID BASED METHODS

Polymerase chain reaction (PCR)

Many of the inherent disadvantages of the hybridization method can be overcome by the use of PCR technique. The PCR is a comparative new method, which relies on two specific DNA primers, a thermostable DNA polymerase and temperature cycling to amplify discrete regions of DNA. The PCR is a biochemical method of amplification that multiplies the concentration of the target sequence by up to a million fold. Thus single DNA molecules can be amplified to levels that are easily visualized on agarose gels[8]. Very closely related strains of pathogen can be distinguished by PCR, without prior knowledge of the nature of polymorphic region[9].

NMR and NIR Techniques

Nuclear magnetic resonance (NMR) and near-infrared spectroscopy (NIR) have been commonly deployed for the determination of components such as water, proteins and oil in food grains. Since insect body contents like haemolymph, lipids and chitin, insect infested grain can be differentiated from uninfested grains utilizing NMR and NIR techniques[10]. NMR can detect *Sitophilus granarius* in wheat. NIR in the reflectance mode has been used as a rapid method for detecting both internal *(Sitophilus granarius)* and external (*Oryzaephilus surinamensis)* infestations. The NIR analysis has been widely used for detecting mite infestation in animal feed by measuring the difference in the absorption of haemolymph in mites to the absorption of water in feed.

RADIOGRAPHY

X-ray technique was developed for infestation detection in 1950. It is an offical method in USA. Both living and dead insects are detected by radiographic method. However, the X-ray technique is not sensitive for egg and early larval stages. An accuracy of 80% and above has been reported for the X-ray method and the accuracy of detection increases, as the insect matures as the differences between background (grain) and foreground (insect) increases. This technique is especially useful for screening packed foods and in food processing facilities, where large number of samples are to be checked to meet quality control standards.

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