''बेटी बचाओ, बेटी पढ़ाओ''

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Program	:	5^{th} sem.
Course Name	:	BMLT
Session No. & Name	:	2023

Academic Day starts with –

- Greeting with saying **'Namaste'** by joining Hands together following by 2-3 Minutes Happy session, Celebrating birthday of any student of respective class and **National Anthem**.

Lecture Starts with- Review of previous Session- Introduction of enzyme.

Topic to be discussed today- Today We will discuss about the isoenzyme and the method application of the isoenzyme.

- University Library Reference- satyanarayan, godkar..

  National song' Vande Mataram'

TOPIC :- ISOENZYME

Isoenzymes (or *isozymes*) are a group of enzymes that catalyze the same reaction but have different enzyme forms and catalytic efficiencies. Isozymes are usually distinguished by their electrophoretic mobilities. All living systems apparently require multiple molecular forms of certain enzymes in order to maximize biological capacity.

Isozymes arise from gene duplications and/or different epigenetic modifications of a gene product(s). In this sense, most of the recombinant enzymes with deletion, insertion, and/or other mutations at the genetic level fall into the category of isozymes.

In a restricted definition, "isozymes" are different in genetic origins. An example is the human alkaline phosphatases which have at least three different genetic origins, i.e., for placental, intestinal, and liver/bone/kidney enzymes (see Section I,B, Chapter 5). The enzymes that have epigenetic differences due to differential precursor processings, covalent modifications, and/or tissue distributions are then called *isoforms*. Examples of isoforms are the liver/bone/kidney alkaline phosphatases which are encoded by the same gene but differentially modified in a tissue-specific manner.

The five "classical" isozymes of lactate dehydrogenase (LDH) arise from combinations of the two restricted definitions described earlier. LDH isozymes consist of two genetically distinct polypeptide chains, A (or M for muscle type) and B (or H for heart type), which form varying combinations of tetrameric structures.

METHODOLOGY

A. SAMPLE SELECTION AND PREPARATION Sample selection and preparation are the most critical steps in isozyme analysis. The quality of genetic information obtained from an experiment is only as good as the

sampling of isolates from which the data are derived. The number of isolates and their geographic and host range will all affect data interpretation. Care in sample preparation is also essential for a successful study. Poor resolution, faint staining or absence of bands, and irregular banding patterns can be caused by the incorrect choice of sample buffer or improper extraction techniques. Sample preparation has been thoroughly discussed elsewhere.

B. ELECTROPHORETIC TECHNIQUES Different electrophoretic techniques can be used to separate isozymes, including starch gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), isoelectric focusing, and two-dimensional electrophoresis. Advantages, disadvantages, and protocol references for different electrophoretic procedures have been summarized.2 Traditionally, isozyme analysis was performed with starch or PAGE, but isoelectric focusing is now being used more commonly. Isoelectric focusing and two-dimensional electrophoresis resolve larger numbers of isozymes than do the other techniques, but the electrophoretic banding patterns may not lend themselves to a genetic interpretation due to their complexity. The identification and visualization of individual enzymes using specific activity stains requires the presence of active, nondenatured enzymes. Dissociating procedures, such as SDS-PAGE (polyacrylamide gel electrophoresis in which the proteins are denatured with the detergent sodium dodecyl sulfate), cannot be used for isozyme analysis.

C. STAINING Isozymes are visualized on the electrophoretic gel by reaction with specific activity stains. Detection of specific enzymes is possible because the appropriate substrates and cofactors required for activity are provided in the staining solution. The enzymatic reaction forms a colored product, either through direct activity with a dye or by involving other enzymes in a series of reactions with the generation of a colored product as a final result. For some enzymes, such as superoxide dismutase, isozymes

are seen as white bands on a dark background. Fluorescent products can be detected with ultraviolet light. Conversely, non fluorescent products can be visualized as "negatively stained" by reacting the starch with a fluorescent compound. The biochemistry of the different staining reactions has been discussed,6,10 and stain "recipes" for many different enzymes have been described.

APPLICATIONS OF ISOZYME ANALYSIS

Many aspects of plant pathology, both applied and basic, can be studied with isozyme analysis.2,27 Most applications have involved fungal pathogens, but the technique has also been used for nematodes28 and bacteria. 29,30 Isozymes are frequently used by plant geneticists and breeders as genetic markers for resistance. This application is very important for plant pathology, but it is beyond the scope of this paper. Readers interested in using isozyme analysis to study host plants are referred to Conkle et al.,9 Cheliak and Pitel,31l Conkle,32 and Tanksley and 0rton.

A. TAXONOMY Isozyme analysis is frequently used for taxonomic purposes, especially when a taxon is morphologically diverse or plastic. In most cases, fungal species are easily differentiated by electrophoresis. The technique is commonly used to make recommendations on the separation or combination of species.34–39 Subspecies, varieties, and intersterility groups have also been separated.

B. IDENTIFICATION OF UNKNOWN ORGANISMS The ability of isozyme analysis to differentiate species and subspecies leads to its application in the identification of plant pathogens. Isozyme analysis can be used both to identify unknown pathogens and to "fingerprint" commercially important strains. The correct, rapid identification of an unknown pathogen may allow early implementation of control measures

that will prevent large economic loss. State and federal agencies also need to be able to identify pathogens of regulatory significance, often from very small samples. Industry must be able to identify commercial strains that have been developed and patented. Of all the applications of isozyme analysis, pathogen identification is the one most important economically. This topic has been recently reviewed.

C. GENETICS Genetic information about a pathogen can be derived from isozyme analysis, including the amount of genetic variability (i.e., the percent polymorphism) of a species or population, the amount of heterozygosity, the linkage of specific loci, and genetic maps of the chromosomes. As genetic markers, isozymes are useful for studying population structure, tracing epidemics, establishing the origins of new pathogenic forms, and analyzing crosses.

Enzyme Activity

The presence of an enzyme is generally recognized by the occurrence of the chemical reaction that it catalyses, and the amount of enzyme present may be determined by measuring the rate of this reaction.

Enzyme Nomenclature: Recommendations 1964 of the lnternational Union of Biochemistry [l] defined a standard unit of enzyme activity as that amount which will catalyse the transformation of 1 micromole of the substrate per minute under standard conditions ; this definition was taken from the Report of the Enzyme Commission in 1961 [2]. The Commission on Biochemical Nomenclature, in Enzyme Nomenctature, Recommendations 1972 [3], recommended that, in order to adhere to SI units, reaction rate should be expressed in moles per second. At the same time, it proposed to define a new unit of enzyme activity (katal) in line with the Sj,stkme International, and to

give it a name, to be used instead of the earlier, unnamed enzyme unit. The katal, being related to the second as unit of time, fits much better with the rate constants used in chemical kinetics than does the earlier enzyme unit. The 1972 version identified the enzymic activity with the rate of reaction, but these are now distinguished.