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Article · December 2021

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e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:03/Issue:12/December-2021 Impact Factor- 6.752 www.irjmets.com

MOLECULAR TECHNIQUES FOR IDENTIFICATION OF MICROORGANISMS OBTAINED FROM DIFFERENT SOURCES REVIEW BASED RESEARCH UPON LACTIC ACID BACTERIA

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ABSTRACT

When we come across the nature we seen the huge biodiversity in organisms. Present variation amongst species it help to shape up evolution of life variety due to which there is repairs of living systems of natural entities of biosphere. a variety of techniques used now days as molecular tool for identification and detection of new genetic sequences the techniques like Restriction analysis of chromosomal DNA, , amplified fragment length polymorphism, Restriction fragment length polymorphism, Pulse Field Gel Electrophoresis, Variable number of tandem repeats, randomly amplified polymorphic, 16-S etc have been used to study genetic diversity. Present tools given accurate information about new variation, and ultimately support for investigation of new genetic makeup of that organism. New genetic makeup surely has some new treats than that of previous one.

Keywords: 16S Rrna, LAB, Molecular Identification.

I. INTRODUCTION

PCR- polymerase chain reaction. It's a test to detect genetic material from a specific organism and DNA sequencing, comparison of the gene sequences of bacterial species showed that the 16S ribosomal RNA is greatly preserved inside a species and amid species of the alike genus and so that its taking spotlight for use as new tool for determination of the species of micro organisms. it help to indicate phylogenetic tree, which is the base of variation among species, so that the micro organisms put into into new genera and re classification can be achieved (1, 2,)

Micro organisms play a significant function in lots of fermentation and food industries. Separation and viewing of microorganisms from innate habitats, it's the most powerful way for finding proficient cultures for applied purposes. This is certainly holds true for lactic acid bacteria, which are widely used in the construct of ample variety of established fermented food. Since they are concerned in fermentation of variety of food products and do not cause health risk to man, they are generally recognized as safe organisms.

Lactic acid bacteria belong to a wide range of genera with extensive number of species. Which shows positive stain of gram, non spore forming ,and anaerobic micro-organisms. The genera of lactic acid bacteria are *Lacto-bacillus*, , *Lacto-coccus, Pedio-coccus* and *Leuconostoc* etc., LAB genus marked as the leading group in Lactobacteriaceae family , which contain over more than 110 various species (3,4). Lactobacilli are nutritionally fastidious and associated with a variety of plants and animals. They are the part of normal gastrointestinal (GI) micro biota of human and animals. The probiotic properties, implying 'living micro-organisms which upon ingestion in certain numbers exert health benefits beyond inherent nutrition has been added further incentive to detailed microbiological, biochemical and genomic studies of this group (5) use of molecular techniques (16S rRNA gene sequencing) has been found more useful and supportive for identification of microorganisms (6) The genes indoctrination for 16S rRNA in prokaryotes and 18S rRNA in eukaryotes are most widely used in molecular identification of microorganisms. among the initiation of molecular techniques, RAPD- randomly amplified polymorphic DNA technique used for finding type and recognition of closely associated species of micro organisms and evaluation genetic relationships (7) In the RAPD short oligonucleotide primers that anneal haphazardly all through the genome are used. The result is a characteristic set of intensification products of dissimilar banding patterns of single species or large population. RAPD analysis is quite quicker;

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the technical aspect of this method is quite easy and more inexpensive than the other type determination methods like RFLP and AFLP. distinct conventional polymerase reaction (PCR), data on DNA sequence of the organisms are not a pre-requisite for RAPD analysis. Though there is huge research on segregation and classification of Lactobacilli rich sources some fruits, vegetables also good source of LAB. segregation of Lactobacillus commencing various fruits plants and vegetables as a natural source has always been the most powerful means for obtaining efficient cultures for commercial purposes.

II. METHODOLOGY

- **Sources Of Segregation Of Lactobacillus SPP**

The LAB strains segregated from natural source viz. plats ,grain crop, gut region ,gizzard, ileum and caeca of chicken. fishes and various animals Decimal dilution of the contents of these segments were mixed with MRS medium and incubated for 48 hours at temperature ranges 37°C in anaerobic condition. More common identification of culture based upon characteristics of Lactobacilli as presented in the Bergey's Manual of Determinative Bacteriology. Morphological tests like Gram staining growth occurred at temperature ranges from 15 °C, 45°C, fermentation occurred at variable carbon containing sources. Based upon criterion, Lactobacillus fermentum (LF) was recognized and used as probiotic for chickens (8)

LAB (Lactic acid bacteria) separated from milk of camel it shows the abundance of lactic acid producing micro organisms viz. Streptococci - S. cremoris ,S. lactis Lactobacilli -L. *acidophilus*. Capability of each strain were studied. consequence of chilled was also traced. (9)

- **16s RRNA Gene Sequencing**

Phylogeny is specially meant history of evolution of living world. The genes encoding for 16-S ribosomal RNA (prokaryotes), 18S (eukaryotes) were predominantly used molecular phylogenetics. Smaller sub unit 30-S ribosomal RNA (SSU rRNA) employed widely for evolutionary analysis (based on gene sequencing) by considering the characters like distributed across the world, constant, conserved, sufficient length to give a sight of evolution encircling living micro -organisms (10)

Illinor University professor Carl woese said to be pioneered of utilization of small subunit of ribosomal RNA (SSU- Rrna) in phylogenetic experiments, the study carried out by him founded the 3 domains in life form viz. Bacteria, Archaea and Eucarya. The chromosomal (database) project- II holds a assortment of sequences, numbering more than 440,000 and give a range of analytical programmers (10) The 23-S, large ribosomal RNA (LSU rRNA) is also informative with its great sequence leads to give additional information.

- **Amplification Of 16s Ribosomal RNA Sequences**

Phylogenetic examination by means of DNA sequencing relies upon PCR (polymerase chain reaction) for the purpose of to find ample copies of genes for proficient sequencing. oligonucleotide primers planed to binds at the ends of genes of attention, which permits DNA polymerase to make copy of genes.

- **Sequence Alignments:**

When DNA sequence is obtained, further it allow for sequence alignment. The network (web) based BLAST (Basic Alignment Search Tool) of the National Centre for Biotechnology Information (NCBI) do this mechanically which useful for identification of genes homologous to a new sequence from among the many thousands of genes already sequenced.

Selective culture media and phenotypic experiments facilitate Lactobacilli to be distinguished among morphologically comparable bacteria. The perfect recognition of Lactobacillus species can be achieved by 16S r RNA gene sequence.

Molecular techniques for the complete identification of Bifido bacterium species yet not presented. (11) designed the Lactobacillus specific group PCR primer (12) (S-G-Lab-0677-a-A-17), to selectively amplify 16-S ribosomal DNA (rDNA) from Lactobacilli and related lactic acid bacteria, including members of the genera like Pediococcus, Leuconostoc, , Amplicons, Weissella. produced by PCR from a variety of GI- (gastrointestinal) tract, counting those originate from feces and cecum, consequences mostly in Lactobacillus-similar to sequences, in which 28 % were the majority similar to the 16S r-DNA of Lactobacillus ruminis. Carlos (13) prepared a phylogeny based on entire-genome alignments which shows that Lactobacillus salivarius related to

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Lactobacillus plantarum than that of the Lactobacillus sakei, which was adjoining to E. faecalis, in contrast to 16-S r-RNA gene closeness. Species relatedness based on this protein set was largely concordant with genome synteny-based relatedness. The great deviation of the Lactobacillus genomes examined supports the identification of new sub generic division. Elizete (14) segregated Lactobacillus reuteri (LPB P01-001) from the gut of wild swine and was distinguish by biochemical test , sequencing of gene 16-S r- RNA. L. reuteri (LPB P01-001) act as acid-uric bacteria because it grows superior lesser pH medium with no pH control. Though, the lactic acid manufacture yield was half (9.22 $g.L^{-1}$) of that get beneath a steady pH of 6.5, which attain 30.5 $g.L^{-1}$ past 28 h. of fermentation. The acetic acid formationtion was high under controlled pH.

- **Molecular Markers**

The molecular markers the resourceful gear for research in the field of molecular biology, animal nutrition and genetic engineering. With the beginning of molecular markers, latest developed production of markers has been introduced in last 2 decades, which has transformed the entire picture of life sciences. From their expansion, continually being customized to improve their usefulness and automation in the procedure of genome analysis. The discovery of PCR -polymerase chain reaction serve as pointer in this attempt and confirmed to be a distinctive procedure due to which new class of DNA markers was introduced. Molecular markers distinguish living entity up to level of DNA and are inherited in Mendelian manner. (15) There is dissimilar kinds DNA marker and further there are discovery of many markers. There are 2 significant kinds of markers like markers based upon hybridization (Restriction fragment length polymorphism RFLP) and markers based upon PCR (RAPD, , SSR, AFLP , ISSR, SCAR, SNP, CAPS)

- **Random Amplified Polymorphic DNA (RAPD)**

RAPD - Randomly amplified polymorphic DNA engaged the use of randomized primers in PCR reactions (16, 17.) Predominantly now a day's used rousingly to distinguish closely linked organisms. (18,) based on polymorphism among RAPD merchandises. RAPD tools is a helpful, easy, quick and instructive. In additional, the technique gives a chance to get information about the variations in a group of segregates to distinguish them from one another. Anjali (19) carried out research on different PCR based DNA fingerprinting techniques for recognition and prejudice of microbiol strains. In randomly amplified polymorphic DNA. 14 strains of Salmonella alienated into 9 factions farther than which maximum number of strains i.e., 5 presented in one group. In box PCR technique primer with highly preserved repeated series which was longer than the used in RAPD created 6 different groups which demonstrated the occurrence of frequent band. The RAPD- random amplified polymorphic DNA technique foundation of polymerase chain reaction (PCR) used as most promising molecular techniques to develop DNA markers. RAPD markers are intensification inventions of unsigned DNA sequences by solitary, tiny and random oligo nucleotide primers, hence no requirements of prior information DNA sequence. Low cost, competence in increasing a great number of DNA markers in less time and necessity for less classy tools it made RAPD technique valuable (20) Gupta (21) secluded the 16 segregate of Xanthomonas oryzae at various geographical locations in India and 2 isolates from country Philippines, were analyzed using polymorphic RAPD with seven primers viz., (22)

III. CONCLUSION

Current decade is developing science, now days a number of pathogens have turn into significant and harmful to animal health. Recent data and research shows the spreading of pathogens in animal group. Utilization of molecular techniques for detecting and typing of pathogens,as well as to identify the beneficial micro organisms which further attack upon pathogens . it provide trustworthy epidemiological data for segregating the source of animal infections. The help we taking to achieve this goals by using molecular techniques like pulse field gel electrophoresis, random amplified polymorphism multi- locus sequence typing, etc. Helpful for consider, form, classification of micro organisms and pathogens. In brief its more accurate, detect correct proteins genes of interest.

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