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Probiotics: A Study on Its Sources, Separation, Characterization, and Evaluation

Abhishek Kumar[1]* Archana Singh[2]

[1] Research Scholars, Department of Zoology, Sai Meer Degree College, Uttar Pradesh

[2] Supervisor, Department of Zoology, Sai Meer Degree College, Uttar Pradesh

Abstract – Probiotics, which are living cells with various beneficial qualities, have been widely investigates and economically used in a variety of goods across the globe. The use of genetic techniques to understand the functional functions of probiotics has increased as a result of this work, which looks at completely sequenced genomes. However, evidence from carefully performed clinical research may not essentially support the favorable benefits of probiotics.

Keyword – Probiotics, Sources, Separation, Characteristics, Evolution

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INTRODUCTION

By increasing the absorption of magnesium and calcium from milk proteins, digesting lactose, and generating folate and B vitamins, probiotics have a substantial impact on the bioavailability of nutrients in the human body. Lactobacillus and Enterococcus species are Gram-positive and non-toxic lactic acid bacteria that are often ingested as probiotics. Lactic acid bacteria are often thought to be non-harmful bacteria. The large number of probiotics includes the Lactic acid bacteria group that is taken from safe sources like fermented dairy products and may be utilized as probiotics.

Probiotic is a relatively recent term that means "for life," and it refers to bacteria that have been linked to beneficial effects on humans and animals. These bacteria contribute to the microbial equilibrium in the intestine and have a role in overall health. Probiotic bacteria are predominantly Lactobacillus and Bifidobacterium strains, though Bacillus, Pediococcus, and yeast strains have also been discovered to be acceptable options. They work mutually to defend the organism from dangerous microorganisms while also strengthening the immune system of the host. Antibiotic treatments (for various ailments) eliminates the microbial flora in the digestive system, thus they're normally ingested after that (both the useful and the targeted harmful microbes). Food containing probiotic bacteria should be consumed on a regular basis to maintain a positive balance of helpful or helpful germs in the gut flora.

Probiotic components, supplements, and food were valued at $14.9 billion globally in 2007, with forecasts of 15.9 billion in 2008 and 19.6 billion in 2013, imply a compound yearly rate of 4.3 percent. The novel microorganisms discovery for potential probiotic bacteriotherapy uses has tremendously aided extensive study of probiotics. The purpose of this study is to look at probiotics' definition, history, uses, manufacturing, technology, and future developments.

Lactobacillus and Enterococcus species are Gram-positive and non-toxic lactic acid bacteria that are often ingested as probiotics. LAB thrives in comparable environments. As a consequence, established phenotyping and biochemical approaches, such since sugar fermentation at the genus level, were unable to identify and classify these bacteria, as these procedures do not provide unambiguous classification findings. However, when these procedures are used in isolation, it's not provide reliable clustering results at the strain level, hence a combination of them should be investigated. As a result, this work used crucial morphological and biochemical tests with three molecular methods: 16S rDNA sequencing, REP-PCR (GTG-PCR), and ARDRA to screen traditional dairy products of Iran microbiota west to identify novel strains with high probiotic activity.

Regulation of Probiotics

Probiotic products are sometimes misrepresented as being uncontrolled. Clearly, the FDA controls probiotic products and producers' duties, including labeling and safety, whether in food, supplement, or medication form. The FDA published rules on August 24, 2007, requiring that current good manufacturing standards for dietary supplements

be phased in over the following three years. Despite the fact that these standards do not address the verification of efficacy claims, they should aid in improving the compositional quality (identity, purity, and strength) of probiotic supplements on the market in the United States. Food and supplement makers, conversely, are not obliged to get premarket clearance for claims of effectiveness or safety. In practice, the FDA has never questioned a probiotic product's labeling or safety, except when the product is marketed as a medication (i.e., to treat, cure, prevent, mitigate, or diagnose illness) but does not have FDA clearance. In most instances, inaccurate product labeling by manufacturers who intended to promote a dietary supplement is to blame. Consumers may find it difficult to discern between probiotic products that are properly prepared and labeled and those that are not due to a lack of effective FDA regulation of effectiveness claims.

Use of suggestions issued by a working group co-organized by the United Nations' Food and Agriculture Organization and the World Health Organization, which include the following, is a suitable strategy for manufacturers marketing a product that includes a probiotic.

1. Identification of all probiotics in the product to the strain level, with all strains deposited in an international culture collection

2. Each strain is characterized for features that are crucial to its safety and function.

3. Human studies to verify health benefits, including determining the amount of microbe necessary to deliver the benefit.

4. Accurate and non-misleading effectiveness claims and content labeling till the end of the shelf life

Role of Strain, Dose, and Product Format in Probiotic Function

Although identical evidence in people is unusual, strains of the same probiotic species may be diverse, as has been established both in vitro and in animals. As a consequence, clinical findings from one research are solely relevant to the strain or strains tested in that research. However, various strains may have the same effects, and various strains have been exposed to have comparable immunological responses. Certain species- or genus-specific characteristics are expected to be discovered as study proceeds. Identification of genes or gene systems may make in vivo function possible to anticipate, and documenting of gene expression may be sufficient proof of in vivo function. However, for the time being, studies specific to the strain or strain combinations should be employed to back up claims of physiological advantages. Furthermore, all probiotic microorganisms in a product should have their strain, species, genus, and designations mentioned.

Probiotic doses should be based on amounts that have been shown to be effective in human trials. For all strains, a single dosage level cannot be expected to be effective. Bifidobacterium infantis 35264, for example, has been shown to be effective at 10 8 cfu/day, but the recommended dosage of VSL#3 (VSL Pharmaceuticals) is (1.81012) cfu/day, a 4-log cycle difference.

The effect of product format on probiotic function has not yet been thoroughly investigated. The commonly used quality-control measurement of colony-forming units per gram may not be the primary indicator of final product effectiveness. Additional aspects that affect a product's efficacy include probiotic growth during manufacturing, enteric coating, preservation technology, probiotic metabolic state, and the inclusion of other functional components in the final product. More study is required to find out the extent to which such variables impact in vivo effectiveness.

METHODOLOGY:

The study aim is to look at where probiotic strains come from, how they're isolated, and how they're characterized and evaluated. Figure 1 indicates the multiple processes required to characterize a bacterial strain as an unique probiotic.

We wanted to do a literature review of the origins, isolation, characterization, and assessment of probiotic strains in this work. The current study summarizes a total of 1500 publications from the PubMed database, with the aim of providing historical background and current status of the subject.

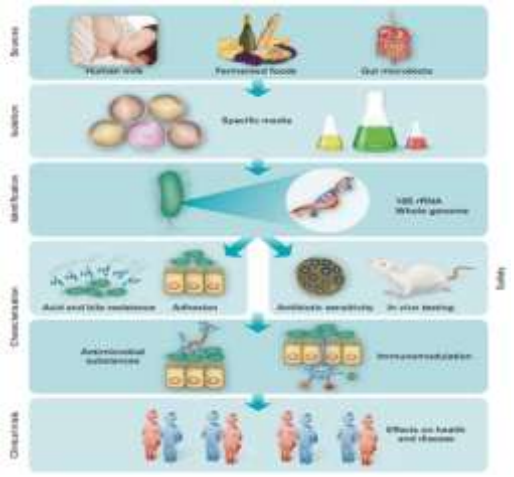

Figure 1: There are many procedures that must be completed before a bacterial strain may be classified as a new probiotic. rRNA stands for ribosomal RNA.

Isolation, identification, characterisation and safety

Cultivation-based techniques to microbial ecology are widely acknowledged to give an inadequate microbial diversity image. Ecological niches are complicated interrelationships between microorganism species that can't be replicated using regular culture techniques. As a way of determining the microbial diversity of diverse sources, molecular techniques that skip the culturing stage have become popular. These techniques have yielded valuable information on microbial communities, especially probiotic sources. Isolation of ecosystem members is the first and most significant stage in researching it. When we wish to evaluate in vivo activities related with good impacts on human health, we don't need to identify organisms, particularly in probiotic microorganisms.

RESULTS AND DISCUSSION:

Sources

Lactic acid bacteria, bifidobacteria, and other species derived from fermented milks have been used in this context for a long time. In Mongolia and Africa, spontaneous milk fermentation has a long history, and the utilization of beneficial bacteria in fermented dairy products has been done for millennia (1).

Traditional fermented milks include a wide range of LAB species, making them a valuable source of probiotic bacteria. Probiotic yeasts and lactobacillus strains have also been detected in kefir grains, Masai milk, and Koumiss, a fermented milk beverage; these bacteria may affect immune responses (2).

Traditional fermented foods have lately been investigated as potential natural probiotic microbe sources. The bulk of bacteria obtained from fermented foods belong to the Lactobacillus genus (3).

Skin or faecal contamination has long been assumed to be the source of germs in breast milk. Despite the fact that the lactobacilli found in human milk are genotypically distinct from those found on the skin and that the LAB strains found in breast milk were also found in the feces of the corresponding infants, it was only recently recognized that breast milk is a valuable source of probiotic LAB and bifidobacteria for use in infant formulas and foods (4).

Furthermore, breast-fed newborns have less allergies and gastrointestinal illnesses than formula-fed newborns; hence, the intestinal microbiota of the breast-fed newborn may be regarded to be perfect (5).

Two Lactobacillus strains obtained from human breast milk were also shown to boost natural and acquired immune responses by activating natural killer and T-cell subsets and increasing regulatory T cells (6).

Probiotics may also be found in the human gastrointestinal tract. There are around 500 different bacterial species in the adult human stomach. Many of today's probiotic strains, like Lactobacillus gasseri and Lactobacillus reuteri, were isolated from this source (7, 8).

It's a frequent misperception that probiotics must colonize the digestive system in order to work. In reality, certain probiotics (such as B. longum and Bacteroides thetaiotaomicron) are found in the human gut microbiota, whereas others (such as Lactobacillus casei and Bacteroides animalis) are absent. The majority of the probiotic strains, such as B. longum and L. acidophilus RY2, were isolated from healthy adults' and babies' feces, respectively. Several studies have documented the isolation of probiotics from breast-fed newborn feces, which is consistent with breast milk (9-11).

Isolation

Before incubation in selective medium, the first step in the isolation of probiotic bacteria is to keep the sample in ideal conditions. Because most probiotics are anaerobic or facultatively anaerobic, they should be handled in anaerobic conditions as soon as feasible (3h). Several mediums for selective or elective isolation of bifidobacteria and lactobacilli have been developed. With a propionic acid based on the Columbia agar, Rogosa et al. established a selective medium. Bacteroides and Eubacterium species, which are prevalent in human feces, are inhibited by the low pH of this medium, which is tolerated by lactobacilli and bifidobacteria (12, 13).

For the development of bifidobacteria and other anaerobic species, the plates are incubated at 378C for 48–72 hours in an anaerobic environment, or in a CO2-rich environment for the development of lactobacilli. Colonies are then isolated and transferred to broth or a fresh agar plate.

Identification:

The initial stage in the selection of possible probiotics is to identify bacteria in the GI tract or dietary sources. The process of cataloging biodiversity using a polyphasic approach that includes genotypic and phenotypic approaches is known as taxonomic categorization. For many decades, the taxonomy was mainly based on the kind of sugar fermentation and the fermentation products produced. As a result, probiotics are generally classed as LAB. The technique of choice now is 16S RNA gene analysis. Microbiologists have employed this conserved segment for phylogenetic categorization for the last two

decades, and the relatedness of species is calculated by comparing their sequences in publicly accessible databases (DDBJ, ENA, GenBank). To detect bacterial communities from gutorecological sources, 16S RNA gene analysis was integrated with other approaches.

In comparison to the bacterial genome (30 000–40 000 bp), the 16S DNA segment is exceedingly tiny (1500 bp). Complementary information is often required owing to a lack of base sequence variety that allows strains of a particular species to be distinguished. There is a lot of sequence and length diversity in the 16S to 23S intergenic spacer region. The investigation of the bacterial genome is without a doubt the most valuable tool for identifying and characterizing the mechanisms underpinning prokaryote diversification and evolution. Genome sequencing, on the other hand, is still a time-consuming and costly procedure (14).

Characterisation

Biliary salts aid lipophilic compound digestion while simultaneously acting as an antibacterial agent by affecting the formation of the gut flora. Human bile concentrations that are physiologically relevant vary from 0.3 to 0.5%. (88,89). In vitro tests are carried out in 0.3–0.7% bovine bile (Oxgall) for 60–180 minutes. Probiotics have a wide range of acid and bile salt resistance, which is depending on both species and strain. Bifidobacteria have been shown to be very sensitive to low pH levels in many investigations. At pH 2.0 for 90 minutes, some species had survival rates of 0%, less than 1% at pH 3.0 for 2 hours, or higher percentages at pH 3.0–5.0 for 3 hours. Certain bifidobacteria have been shown to have the greatest survival rates. A number of Lactobacillus strains have shown a high level of resilience to low pH. At pH 3.0 for 1 hour, survival rates of 2–100% were recorded in a research including twenty Lactobacillus strains. In 0.3 percent Oxgall for 90 minutes, several bifidobacteria showed a survival rate of 1–70%. At pH 2.0 and 3.0, two L. plantarum strains demonstrated better than 50% survival, and 1.0 percent survival in 73–180 percent bile salt (15).

Surprisingly, these properties are strain-specific qualities that may vary greatly within the species or genus. As a result, in vitro models must be used to pick the most promising strains. As a result, human clinical studies are the gold standard for determining probiotic activity.

CONCLUSION:

In a range of physiological situations and pathologies, such as allergies, intestinal and liver disorders, urinary and upper respiratory infections, AIDS, and metabolic illnesses, probiotics have been demonstrated to induce a range of biological effects. Probiotics are most often found in the genera Lactobacilli and Bifidobacteria. Traditional fermented foods, as well as human breast milk, gastrointestinal tract, and feces, are the main sources of these microbes. Culture in selective medium is used to isolate probiotics. Currently, probiotic strain identification is aided by the sequencing of their 16S RNA genes.

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Corresponding Author

Abhishek Kumar*

Research Scholars, Department of Zoology, Sai Meer Degree College, Uttar Pradesh