3.1. Rationale of Work

For more than 100 years, probiotic products based remedies have been used to fight against infections. Till date, Probiotics are categorized under different categories in different countries like natural health products, dietary supplements, drugs, medical food, live biotherapeutic agent, biological agent as per their intended use, functional food, food supplement, biotherapeutic/pharmaceuticals etc. in various countries. Present categorization in different countries has been depicted in Table 3.1 and seems to be a major challenge in having a harmonized probiotic regulation.

<table>
<thead>
<tr>
<th>Country</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark/Sweden/Finland</td>
<td>Food supplements</td>
</tr>
<tr>
<td>Canada</td>
<td>Natural Health Product</td>
</tr>
<tr>
<td>Italy</td>
<td>Functional supplements</td>
</tr>
<tr>
<td>Europe/Belgium/Germany</td>
<td>Biotherapeutic/Pharmaceuticals</td>
</tr>
<tr>
<td>Japan/India/China/Malaysia/Australia</td>
<td>Functional food</td>
</tr>
<tr>
<td>USA</td>
<td>Dietary supplements/Drugs/Live Biotherapeutic/Medical food</td>
</tr>
</tbody>
</table>

So there are different organizations regulating the probiotics under various categories across the globe. These organizations have covered related aspects in different manner and hence contributed in regulating probiotics by covering one or the other aspect of probiotics. Variety of parameters are being included in the currently existing guidelines which were observed after comparative study of various existing country guidelines which shows that some of the parameters are common in different countries whereas most of the parameters are either missing or not clearly mentioned as shown in Table 3.2.
Table 3.2: Comparative profile of parameters in existing guidelines for probiotic products

<table>
<thead>
<tr>
<th>S. No</th>
<th>Country</th>
<th>Organization</th>
<th>Parameters considered in existing guidelines of various countries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Identification</td>
<td>Evaluation</td>
</tr>
<tr>
<td>1.</td>
<td>---------------</td>
<td>FAO/WHO</td>
<td>✓</td>
</tr>
<tr>
<td>2.</td>
<td>Japan</td>
<td>FOSHU</td>
<td>X</td>
</tr>
<tr>
<td>3.</td>
<td>Europe</td>
<td>EFFSA</td>
<td>✓</td>
</tr>
<tr>
<td>4.</td>
<td>China</td>
<td>SFDA</td>
<td>X</td>
</tr>
<tr>
<td>5.</td>
<td>USA</td>
<td>DSHEA, USFDA, BLA</td>
<td>X</td>
</tr>
<tr>
<td>6.</td>
<td>India</td>
<td>ICMR/DBT and ILSI</td>
<td>✓</td>
</tr>
<tr>
<td>7.</td>
<td>Malaysia</td>
<td>FSQD, NPCB</td>
<td>X</td>
</tr>
<tr>
<td>8.</td>
<td>Canada</td>
<td>Health Canada</td>
<td>X</td>
</tr>
<tr>
<td>9.</td>
<td>Brazil</td>
<td>ANVISA</td>
<td>X</td>
</tr>
<tr>
<td>10.</td>
<td>Newzealand and Australia</td>
<td>FSANZ</td>
<td>X</td>
</tr>
<tr>
<td>11.</td>
<td>Italy</td>
<td>Ministry of Health</td>
<td>✓</td>
</tr>
<tr>
<td>12.</td>
<td>Philippines</td>
<td>BFAD</td>
<td>✓</td>
</tr>
</tbody>
</table>
At present, the status of probiotic based product is full of ambiguities because various regulatory agencies in different countries are defining and categorizing probiotics differently. Due to this, considerable confusion exists amongst regulatory bodies, producers and consumers, for the health claims of probiotic products.

As the concept of probiotics is spreading widely throughout the world, it necessitates an in depth investigation of probiotic traits for vital reinforcement of probiotic concept and is a pre-requisite for their rational development. It becomes evident from the careful analysis of regulatory aspects in different countries that all the leading nations worldwide are now recognizing the importance of probiotics and their beneficial impact on human beings. Each country is in process of addressing the problematic issues and regulating proper regulatory structure for the functional foods.

### 3.2. Need for Comprehensive Regulatory Guidelines for Probiotics

The importance of the functional foods, NHP and probiotic industry is reflected by its erroneous worldwide growth in the past few years which is continue to grow more abruptly in the near future. Such rapid growth in any sector will be accompanied by the need for appropriate policies and regulation to prevent the entry of misbranded, counterfeit and spurious drugs on to the market shelves and requires a thorough examination of issues and challenges related to consumers, manufacturing firms, market, government policies and regulations. The rapid growth, importance and complexity of this sector, leads to confusion amongst consumers, policy makers, regulators, manufacturers and health professionals around the world hence emphasizes the need for a comprehensive, internationally acceptable/harmonized and meaningful assessment. Probiotics are advocated by many health care professionals because of their evidence based health benefits in specific clinical scenarios. Probiotic therapy has already made its way in the treatment of number of diseases and hence the global market of these products is achieving a rapid pace but still the rational usage, selection and design of probiotics remain important challenges for the
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scientific community in concern with their safety factors. There is an urgent need to address the quality, safety and efficacy issues of probiotics. In the absence of any regulatory standards, most of the countries are regulating probiotics under the category of functional foods but these are marketed both as functional foods as well as pharmaceuticals. So a careful risk assessment for patients and proper handling of the probiotic during administration need to be conducted before using probiotics as drugs. So the government needs to take step forward to draft guidelines specifically for probiotic pharmaceutical preparations rather considering as food otherwise there would always be a possibility of spurious and ineffective products with false claims entering the market that can shatter the confidence of consumers in probiotic products. After making the desired regulations, if some products don’t meet the regulated criteria, they will surely be taken off the shelves. If the companies standardize the sale procedures and create the right kind of awareness, the players will surely be with a winning proposition. Excellent growth opportunities exist for domestic and foreign companies to capitalize the prevailing situation and produce resounding results.

Despite of resolving all the related issues, a common regulatory framework is required which to allow free exchange of products and to minimize confusion of different regulations. This will prevent the flooding of markets with misbranded products. Therefore proper regulatory framework and harmonization of regulations on probiotics with international standards are required to ensure the quality and safety for active utilization of probiotics across the globe, so well harmonized guidelines covering all aspects of manufacturing, packaging, approval process, evaluation, marketing and post marketing surveillance is the need of the hour. Taking into consideration all the above enlisted points, present work is proposed to draft comprehensive regulatory guidelines for probiotic formulations on points \( \text{viz.} \) probiotic definition, categorization, identification, evaluation, good manufacturing practices, approval process and health claims and labeling. Importance of all the above said points has been depicted in Table 3.3.
Table 3.3: Importance of parameters to be included in comprehensive guidelines

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Importance of parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition and categorization</td>
<td>To achieve adequate regulatory control for discussion of probiotic related issues among government, producers and consumers on international level.</td>
</tr>
<tr>
<td>Identification</td>
<td>To guarantee their accurate identity and thereby their safety in concern to therapeutic behavior.</td>
</tr>
<tr>
<td>Evaluation</td>
<td>To address the quality and to screen potential probiotics using various <em>in vivo</em> and <em>in vitro</em> tests.</td>
</tr>
<tr>
<td>GMP</td>
<td>To reduce lot–to–lot variations and to ensure quality.</td>
</tr>
<tr>
<td>Labeling and health claims</td>
<td>Indicates all the necessary information on product label.</td>
</tr>
<tr>
<td>Approval process</td>
<td>Lawfully commencement for granting status of safe and effective in its proposed use.</td>
</tr>
</tbody>
</table>

3.2.1. Proper Definition of Probiotics: The Need. Though probiotics were initially defined by FAO/WHO [FAO/WHO Guidelines, 2002] as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” but this definition is insufficient to describe probiotics as foods and pharmaceuticals. Apart it has considered health claims but these may have nutritional claims too. Moreover this definition does not address the inclusion of mono or mixed cultures in live or spore form. Since probiotic research is a relatively new area of scientific research and product development hence these probiotics need a holistic and internationally accepted definition which may describe impact of these products on general health *i.e.* nutritional claims and in case of preventing various diseases *i.e.* health claims. So in the new definition should be designed in précised language covering all the problematic issues included with all the necessary information.
3.2.2. Specified Categorization of Probiotics: The Need. Till date, probiotics are categorized into various categories in different countries as per their intended use. Although inappropriate categorization is not a direct obstacle for marketing of these probiotic based products but, the position of regulatory system for probiotics within existing categories becomes ambiguous. Hence a common terminology is necessitated to achieve adequate regulatory control describing separate categories i.e. ‘probiotics as food’ and ‘probiotics as pharmaceuticals’. This kind of new category might make sense which could be useful in future. In context to these issues, a common terminology should be enacted in the guidance draft.

3.2.3. Correct Identification of Probiotics: The Need. Bacterial identification is of prime importance while formulating any probiotic dosage form. This is so, because of the fact that the pharmacological actions which probiotics exerts on the host are very much strain specific. Probiotics products are aimed at delivering live bacterial cells for the benefit of humans to the gut ecosystem of humans and other animals. Most probiotic based products consists mixture of bacterial or sometimes fungal species also. Lactobacilli and Bifidobacteria are the most commonly used probiotic bacteria are most popular amongst bacterial species. So a probiotic may be composed of a single or combination of two or more species as well, but their continuous vigilant identification and cataloguing should be done before adding them to formulation. These all probiotic strains are rarely proved to be infectious in humans but still strain designation and identification is important:

- To describe new strains, whether having probiotic potential or not.
- To collect data for phylogenetic analysis, for differentiation of inter strain differences and discrimination of bacterial species at molecular level.
- To prove safety and natural therapeutic behaviour of probiotic bacteria without any kind of adverse effect.
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- To check alteration in genetic or phenotypic characteristics of particular microbe during the development of strain as live biotherapeutic or biological agent, before adding it to formulation.
- To detect possible rare cases of infection due to probiotics by molecular characterization and confirmation.
- To prevent mislabelling by identifying and accurately representing the content while labelling probiotic product.
- To ensure the quality of product by approving the strains after identification and to avoid health risks and misleading.
- To develop a new generation of probiotics for site and disease specific action.
- To develop better probiotic strains, which are able to counteract the observed deviations.

Consideration of the safety of probiotics and approval to the said safety is therefore a necessity for probiotics industry and that can be achieved by using good identification techniques. Pharmacological actions of probiotics are strain specific and hence it is strongly recommended to:

- Use of a combination of phenotypic and genotypic tests for identification of the genus and species of the probiotic strain as clinical evidence,
- In vitro testing access to delineate the mechanism of the probiotic effect and
- Clinical health benefits substantiation of probiotic agents with human trials.

3.2.4. Evaluation of Probiotics: The Need. Emergence of probiotics as drug products led to greater need for assessment of probiotic safety and efficacy as like drug products. This includes strain identification (i.e. determination of phenotypic and genotypic properties), safety evaluation (i.e. characterization of history of use safety contact, assessment of resistance to antibiotics, and evaluation of pathogenic properties in vitro and in animal models) and efficacy testing (i.e. functional characterization). To avoid consumer’s disillusionment and declining market, these claims must be scientifically substantiated. Data supporting scientific substantiation of
health claims generated through clinical trial studies involving the various *in vivo, in vitro* tests on human subjects should be labeled to provide consumers with correct and relevant information as any misleading information may lead to adverse effects. At a minimum, the manufacturer must have data to support the identity, potency (*i.e.* number of viable organisms to which a consumer will be exposed after consumption within the established time frame to expiration), purity and quality of the product as the onus of total safety is on the head of the manufacturer. Objectives of probiotic evaluation may vary according to the purpose of the research. For investigator-initiated research, the objectives might be (1) to ascertain safety and efficacy, (2) to identify adverse reactions, side effects related to use, and (3) to discover or verify clinical, pharmacokinetic, or pharmacodynamic effects including the possible mechanism of action. Conversely, for research initiated by manufacturers, the purpose may only be to validate or substantiate a proposed health related claim. For probiotic product approval results of evaluatory data containing both preclinical and clinical study are necessary as contents of INDA and NDA submission.

3.2.5. Good Manufacturing Practices for Probiotics: The Need. GMP (Good Manufacturing Practices) is a documented set of rules to be followed during the manufacturing/production of products and is of prime importance in case of pharmaceuticals. There are various types of procedures that a GMP facility can follow. The list of most common types of documents includes quality manual, policies, Standard Operating Procedures (SOPs), batch records, test methods, specifications and logbooks. Documentation of a manufacturing unit will help to build up a detailed picture of manufacturing function which had been done in the past and what is going to be done in present and, thus, it provides a basis for planning what it is going to do in the future. Clearly written procedures prevent errors resulting from spoken communication, and clear documentation permits tracing of activities performed. Maintaining proper documentation and records by the pharmaceutical
manufacturer constitutes the basic rules of GMP. Proper documentation helps to ensure:

- A detailed picture of what a manufacturing function has done in the past and what it is doing at present and, thus, it provides a basis for planning what it is going to do in the future.
- Products with a high degree of quality, safety and efficacy.
- Reduced risk of errors.
- Enhanced visibility of the quality assurance system.
- The correct specifications or production and control operations.
- The specifications regarding managerial responsibilities.
- The use of correct starting and packaging material.
- Whether all control on starting material, intermediate products, bulk products, other in process controls, calibrations and validations are carried out or not.
- The checking of processed and finished products correctly.

Effective documentation sounds better otherwise discrepancies in GMP systems leads to problems in traceability, ability to sufficiently prevent contamination.

3.2.6. Product Based Approval Process for Probiotics: The Need. At present, for approval of ‘new drug’ either chemical or biological, an Investigational New Drug Application (INDA) must be submitted and authorized by FDA before its administration into humans. The drug must be proven safe and effective for its intended use before marketing. In contrast to this, FDA pre-approval is not needed if a product is considered as dietary supplements. Only notification to FDA is required before marketing of such product on the other hand, if considered as live microorganisms, it must demonstrate safety, purify and potency. Although most of the probiotics have been used in food for centuries and therefore largely defined as “Generally Recognized as Safe” (GRAS) for foods, which should exclude probiotics from conventional approval pathway followed by drug/biologics. For marketing, a drug needs New Drug Application (NDA) approval while biologics require an
approved Biological License Application (BLA) for the same. Hence in order to clear this ambiguity, there is urgent need of clearly defined separate regulatory process for approval of probiotics irrespective of its category. Such approval will also increase acceptance by the medical community to be sold on prescription hence better commercial acceptance of probiotics. Furthermore certifications regarding approval process for microbes included in GRAS list, novel probiotics, probiotics with nutritive claim/therapeutic claim is very much necessary. Approval process will definitely ensure:

- Proper use of the term ‘probiotics’ under different categories.
- Well established proposals for granting status of safe and effective in its proposed use, proposed labeling is appropriate, and methods used in manufacturing the drug and the controls used to maintain the drug's quality are adequate.
- Awareness about the fact that, all clinical research involving probiotics is not reasonably falls within the requirements of the INDA to all concerned authorities.
- Accurate specifications for proper use of the term INDA, NDA for specified categories of probiotics.
- If any category is exempted from the conventional approval pathway, it should be clearly mentioned in the document and concerned should be aware about the law.

3.2.7. Well Defined Health Claims and Labeling of Probiotics: The Need. Labels are mute but major resource of the story behind a product and market, which are affixed to depict contents, nature, ownership and destination. Labels are required to:

- Assist consumer in making product choices.
- Understand nutrition principles when relying on particular probiotic based products.
- Highlight nutritional properties, nutritional quality, functions or health benefits of certain nutrients their product.
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- Describe physiological role of the nutrient in growth, development or normal functions of the body or role in reduction of disease risk claim in a disease related condition
- Assure whether they are factual and appropriate as per the regulations or not.

So the monitoring of probiotic products via authorized authority regarding a claim specifically the health claim as a label is necessary. It will certainly reduce the false claiming and entry of misbranded probiotic products in the market, which in return will assure the increased consumer acceptance and rapid growth in the market.

3.3. Aim and Objective of Study

Common terminology and universally accepted regulations for probiotic products has become a necessity to achieve adequate regulatory control for discussion of probiotic related issues among government, producers and consumers. This inconsistency across the globe leads to legal uncertainty and confusion instead of being a direct obstacle for development of a mature market for probiotics. Due to wide therapeutic benefits and their use in treatment and prevention of clinical disorders, harmonized as well as well defined regulatory guidelines including proper definition, categorization, identification, evaluation, labeling, manufacturing and approval process for sale of probiotics in the market must be there. The major emphasis of the proposed work will be:

1. To review the terms: probiotic, prebiotic and biotherapeutics.
2. To review the regulatory definition of drug, biologic, dietary supplement and GRAS w.r.t. probiotics.
3. To discuss the regulatory difference between a dietary supplement and a drug/biologic
4. To focus on the regulations of probiotics as GRAS/ biologics/pharmaceutical substances.
5. To focus on regulatory categorization of probiotics by considering all the related reported categories.

6. To discuss regulatory considerations in the development and evaluation of probiotics for clinical indications.

7. To study all existing regulatory guidelines in different countries and common point selection.

8. To draft harmonized regulatory guidelines for probiotic formulations, w.r.t.
   a) Identification,
   b) Evaluation,
   c) Good Manufacturing Practices for production, pre-clinical and clinical trials,
   d) Investigational New Drug Approval (INDA) and New Drug Approval related documents and process,
   e) Packaging and labeling of probiotic products

9. To validate selected marketed formulation on the basis of proposed guidelines for identification.

Hence the destined work was carried out as per following plan of work.

3.4. Plan of Work

The proposed work will be carried out on following lines:

3.4.1. Literature Survey.

- Review of literature on probiotic microorganisms.
- Probiotics-as food supplements.
- Therapeutic claims associated with probiotics.
- Current scenario of categorization and regulatory guidelines on probiotics in different countries.
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- Literature survey of existing guidelines for probiotics across the globe including countries like US, China India, Japan, Canada, Europe, Korea, Malaysia and Brazil.

3.4.2. Common Point Selection from Existing Regulatory Guidelines.

3.4.3. Identification and Selection of Parameters to be Included Based on Existing Guidelines, Product Characteristics and Therapeutic Applications.

3.4.4. Drafting of Comprehensive Regulatory Guidelines.
- *Guidance Document for Identification of Probiotics* including:
  - Techniques for probiotic identification.
  - Level of discrimination achieved by each technique.
  - Primers used in molecular/genotypic methods of identification.
  - Use of polyphasic approach as a preferred method of choice.
  - Recommendations.

- *Guidance Document for Evaluation of Probiotics* including:
  - Methods for pathogenicity and viability check.
  - *In vivo* and *in vitro* methods for assessment of safety and efficacy of probiotic formulations.
    - Resistance to gastric acidity
    - Bile acid resistance
    - Adherence to mucus and/or human epithelial cells and cell lines
    - Antimicrobial activity against potentially pathogenic bacteria
    - Ability to reduce pathogen adhesion to surfaces
    - Bile salt hydrolase activity
    - Resistance to spermicides (applicable to probiotics for vaginal use)
    - Recommendations.
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• **Guidance Document for Good Manufacturing Practices Guidelines for Probiotic Formulations** including requirements for:
  - Personnel.
  - Building and premises.
  - Equipment and utensils.
  - Production area.
  - Documentation and records.

• **Guidance Document for Approval of Probiotic Formulations** including:
  - Approval process for probiotics used both as food and drugs.
  - Contents of INDA and NDA submission.

• **Guidance Document for Health Claim, Labeling and Packaging of Probiotic Formulations** including:
  - Labeling and packaging requirements for probiotic formulations and health claims.

3.4.5. **Validation of a Marketed Formulation on the Basis of Suggested Guidelines for Identification.**