ICMR-DBT

GUIDELINES FOR EVALUATION OF PROBIOTICS IN FOOD

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(Department of Health Research, Ministry of Health & Family Welfare)
Department of Biotechnology
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CONTENTS

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FOREWORD

Probiotics are bacteria that help maintain the natural balance of microflora in the intestines. The normal human digestive tract contains about 400 types of probiotic bacteria that reduce the growth of harmful bacteria and promote a healthy digestive system. Experiments into the benefits of probiotic therapies suggest a range of potentially beneficial medicinal uses for probiotics. Recent research on the molecular biology and genomics of *Lactobacillus* has focused on the interaction with the immune system, anti-cancer potential, and potential as a biotherapeutic agent in cases of antibiotic-associated diarrhoea, travellers' diarrhea, paediatric diarrhoea, inflammatory bowel disease and irritable bowel syndrome.

The increasing globalization of food trade has resulted in India being a fast emerging market for probiotic products. With the availability of these products increasing exponentially and the multiple claims made regarding the beneficial health effects, there is the need to put in place sufficient safeguards to protect the consumers from any adverse effects, ensure standardization of commercial products and their efficacy.

The present ICMR-DBT guidelines comprehensively address the various concerns regarding safety, efficacy and reliability as well as labeling of probiotic products being sold in India.

I hope the scientific community, the regulatory agencies and the public at large will be benefited by these guidelines.

-sd-
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PREFACE

The concept of probiotics was introduced in early 20th century, however they gained importance in recent years with the emerging scientific evidences suggesting their role in digestive and immunological functions.

During the last decade there has been increased influx of probiotic products in Indian market. However, there was no systematic approach for evaluation of probiotics in food to ensure their safety and efficacy.

Being the apex body in India for the formulation, coordination and promotion of biomedical research, Indian Council of Medical Research (ICMR) along with Department of Biotechnology (DBT) took the initiative to formulate the guidelines for evaluation of probiotics in food in India. A Task Force was constituted to examine various guidelines available in different parts of the world and deliberate on the relevant issues keeping in view the local conditions. The guidelines formulated and presented in this document define a set of parameters required for a product/strain to be termed as 'probiotic'. These include identification of stain, *in vitro* screening for probiotic characteristics, animal studies to establish safety and *in vivo* animal and human studies to establish efficacy. These also include requirements for labeling of the probiotic products with strain specification, viable numbers at the end of shelf life, storage conditions etc which would prevent misleading the consumer.

These guidelines have been developed for scientific purpose with the main aim to guide the regulatory authority for evaluating probiotic products in our country. I hope that these will also stimulate thinking among scientists interested in developing this area in India.

-sd-
(V.M Katoch)

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We also acknowledge the inputs received from the NGOs/Industry representatives present in the scientific deliberations and the suggestions received from others following posting of draft guidelines on ICMR website.

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ABBREVIATIONS

BMS	:	Basic Medical Sciences
CFU	:	Colony Forming Units
DBT	:	Department of Biotechnology
DHR	:	Department of Health Research
DNA	:	Deoxyribonucleic Acid
FAO	:	Food and Agricultural Organization
GCP	:	Good Clinical Practices
GMO	:	Genetically Modified Organisms
GMP	:	Good Manufacturing Practices
GRAS	:	Generally Recognized as Safe
HACCP	:	Hazard Analysis and Critical Control Point
ICMR	:	Indian Council of Medical Research
ICPS	:	International Committee on Systematics of Prokaryotes
JIPMER	:	Jawaharlal Institute of Post Graduate Medical Education and Research
PCR	:	Polymerase Chain Reaction
PFGE	:	Pulsed Field Gel Electrophoresis
PGIMER	:	Postgraduate Institute of Medical Education & Research
PI	:	Publication & Information
RNA	:	Ribonucleic Acid
RHN	:	Reproductive Health & Nutrition
SOPs	:	Standard Operating Procedures
WHO	:	World Health Organization

ICMR-DBT GUIDELINES FOR EVALUATION OF PROBIOTICS IN FOOD

1. INTRODUCTION

The concept of probiotics (which means, "for life") was introduced in early 20th century by Elie Metschnikoff (1), it however gained momentum only recently with considerable and significant advances in functional and health food market across the world. India is also fast emerging as a potential market for probiotics in food. The global probiotic market generated US $15.9 billion in 2008 and is expected to be worth US$ 32.6 billion by 2014 with a compound annual growth rate of 12.6% from 2009 to 2014 (2). On the other hand the probiotic product industry in India was estimated to be around Rs 20.6 million with a projected annual growth rate of 22.6% until 2015 (3).

Probiotics, especially *Lactobacillus* and *Bifidobacterium* have been suggested to be associated with alleviation of lactose intolerance (4); prevention and cure of viral, bacterial and antibiotic or radiotherapy induced diarrhoeas (5,6.7); immunomodulation (8); antimutagenic (9) and anticarcinogenic effects (10); and even blood cholesterol reduction (11). The optimism associated with probiotics is, however, counter-balanced by skepticism as many “probiotic” products in the market are unreliable in content and unproven clinically (12-15).

Also, Lactobacilli and Bifidobacteria have been rarely associated with human clinical infections which are likely to be a result of opportunistic infections especially in immunocompromised individuals (16-17). Many probiotic strains in use for several decades have been validated for their safety and efficacy and are therefore, safe to use (18,19). Any new strain used as a probiotic should be evaluated for safety and efficacy.

However, *Enterococcus* is emerging as an important cause of nosocomial infections and isolates are increasingly becoming vancomycin resistant (20). Some side

effects, though rare with probiotics are i) systemic infections ii) deleterious metabolic activities iii) excessive immune stimulation in susceptible individuals and iv) gene transfer (21). The absence of pathogenicity and infectivity thus is an essential pre-requisite of probiotic safety (22).

International guidelines (23,24) on probiotics in food broadly specify the kind of tests that may be required to determine the safety and to assess the health claim of a probiotic product in food. Such tests are based on the current understanding of the subject.

The regulatory mechanism for probiotics differs from country to country and also even within a country (23,25).

In India there are no regulatory guidelines for probiotic foods. In the absence of any such standards and guidelines, there is great scope for spurious products with false claims being marketed. It therefore, becomes imperative that these products fulfill some essential prerequisite conditions before being labeled as a 'probiotic product'. A holistic approach is therefore needed for formulating guidelines and regulations for evaluating the safety and efficacy of probiotics in India which should be in consonance with current international standards.

Keeping in view the above, a Task Force was constituted by ICMR, comprising of experts from varied fields to develop guidelines for evaluation of probiotics in food in India. The Task Force took into consideration the guidelines available in different parts of the world (20, 26-31) and deliberated on the various aspects to be covered (32-36). The guidelines set forth in this document are meant to be followed for a strain or food to be termed as 'probiotic' for marketing in India.

2. GUIDELINES AND REQUIREMENTS FOR PROBIOTIC PRODUCTS

2.1. Scope: The guidelines deal with the use of probiotics in food and provides requirements for assessment of safety and efficacy of the probiotic strain and health claims and labeling of products with probiotics.

Note: These guidelines are not meant for probiotics which by definition would come under drugs, beneficial microorganisms not used in foods or genetically modified microorganisms (GMOs).

2.2 Definition of Probiotics: Probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2002) (20).

2.3 Genus, species and strain identification: Effects of probiotics are strain specific. Strain identity is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies. Both phenotypic and genotypic tests should be done using validated standard methodology. Nomenclature of the bacteria must conform to the current, scientifically recognized names as per the International Committee on Systematics of Prokaryotes (ICPS) (available at http://www.the-icsp.org/).

The current molecular techniques used for identification include PCR based techniques, 16S rRNA sequencing and DNA finger printing techniques like ribotyping and Pulsed Field Gel Electrophoresis (PFGE).

It is recommended that probiotic strains in use in India should be deposited in an internationally recognized culture collection/repositories.

2.4 *In vitro* tests to screen potential probiotic strains: The following *in vitro* tests* with standard methodology are recommended for screening putative probiotic strains:

2.4.1 Resistance to gastric acidity.

2.4.2 Bile acid resistance.

2.4.3 Antimicrobial activity against potentially pathogenic bacteria (acid and bacteriocin production)

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* Adherence to mucus and/or human epithelial cells and cell lines has in the past been used to screen candidate probiotics. The Committee debated this and concluded that there are probiotics which are not adherent. Hence this should not be used as a mandatory criterion or to claim superiority of one strain over another in terms of probiotic attributes and functionality.

2.4.4 Ability to reduce pathogen adhesion to surfaces

2.4.5 Bile salt hydrolase activity

These tests are based on the hostile gut environment which they mimic under in vitro conditions. The cultures evaluated as probiotics based on these tests should be subjected to preclinical validation in appropriate animal models before clinical trials are conducted in human subjects.

2.5 *In vivo* safety studies in animal models: Assessment of the acute, subacute and chronic toxicity of ingestion of extremely large amounts of probiotics should be carried out for all potential strains. Such assessment may not be necessary for strains with established documented use.

2.6 *In vivo* efficacy studies in animal models: To substantiate *in vitro* effects, appropriate, validated animal models must be used first, prior to human trials.

2.7 Evaluation of safety of probiotics for human use: In recognition of the importance of assuring safety, even among group of bacteria that are Generally Recognized as Safe (GRAS)**, probiotics strains needs to be characterized at a minimum with the following tests:

2.7.1 Determination of antibiotic resistance patterns. It should be ascertained that any given probiotic strain is not at significant risk with regard to transferable antibiotic resistance.

2.7.2 Assessment of undesirable side-effects.

2.7.3 If the strain under evaluation belongs to a species that is a known mammalian toxin producer or of hemolytic potential, it must be tested for toxin production and hemolytic activity respectively.

** As defined by Food and Drug Administration (FDA), USA

Assessment of lack of infectivity by a probiotics strain in immunocompromised individuals would be an added measure.

2.8 Evaluation of efficacy studies in humans: The principal outcome of efficacy studies on probiotics should be proven with similar benefits in human trials, such as statistically and clinically significant improvement in condition, symptoms, signs, well-being or quality of life, reduced risk of disease or longer time to next occurrence or faster recovery from illness. Each of the parameter should have proven correlation with the probiotics tested.

Probiotics delivered in food may not be tested in Phase 3 studies (effectiveness), unless the product makes a specific health claim wherein it becomes imperative to generate the required evidence necessitating carrying out Phase 3 studies.

If a probiotic food has a record of documented long and safe use outside the country, the data regarding this could be reviewed and deemed as sufficient to allow its marketing within the country. However, labeling of health benefits may require evaluation in a different manner. While taking into account studies done abroad, efficacy studies of probiotics (which are of proven benefit in 'other' populations) should also be conducted on Indian subjects. It is recommended that such 'bridging' human trials should comply with the principles laid down by the Drug Regulatory Authority. Adverse effects, if any, should be monitored and incidents reported to the appropriate authority.

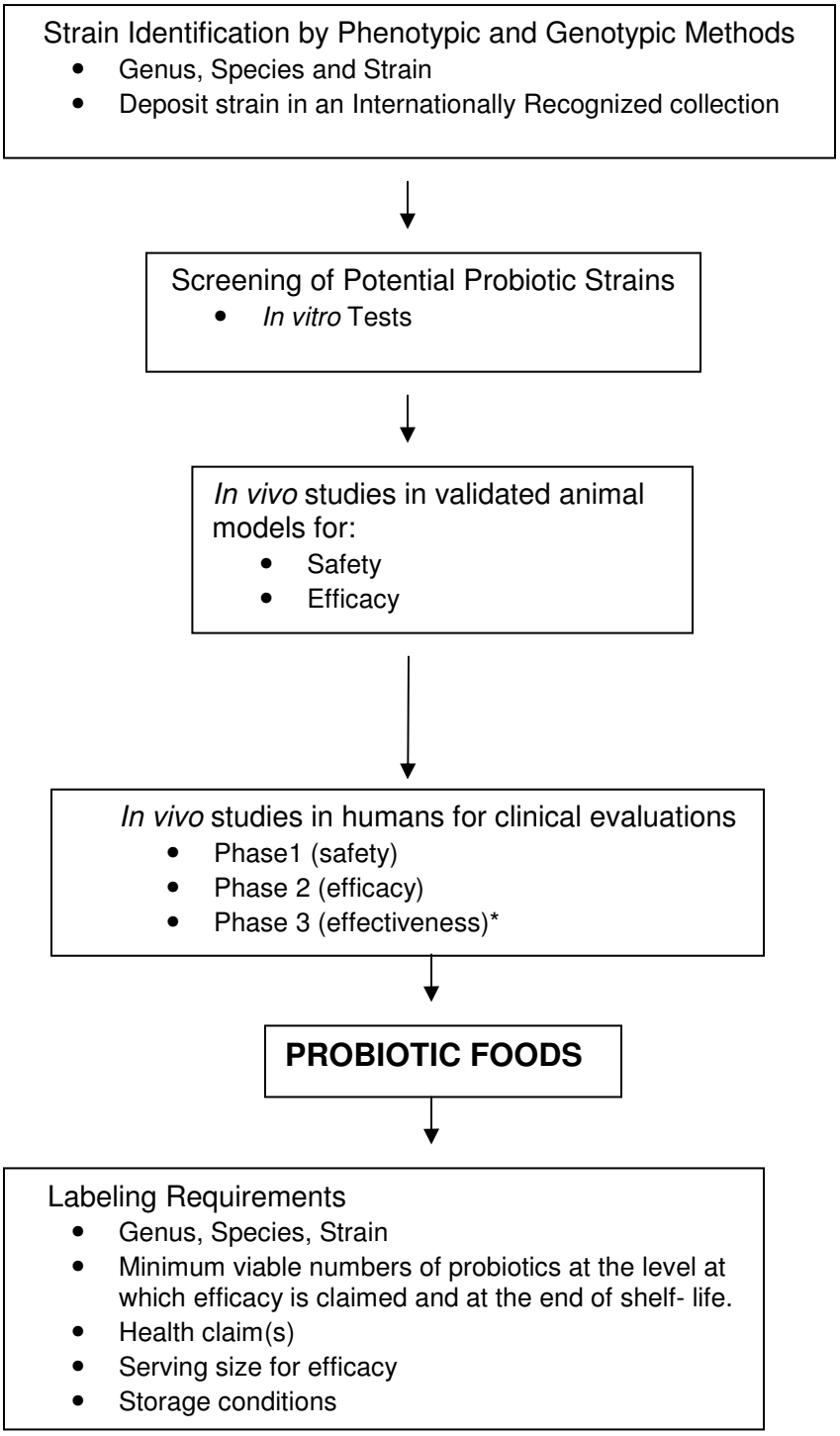
2.9 Effective dosage of probiotic strain / strains: The minimal effective dose or the level of viable cells of the probiotic strain in terms of cfu/ml/day in the carrier food that demonstrates general health promoting functions or well being or specific health claims in target population should be clearly indicated.

2.10 Labeling Requirements: In addition to the general labeling requirements under the food laws, the following information should also be mentioned on the label (23,37):

- Genus, species and strain designation following the standard international nomenclature.
- The minimum viable numbers of each probiotic strain should be specified at the level at which efficacy is claimed and at the end of shelf- life.
- Evidence-based health claim(s) should be clearly stated.
- The suggested serving size to deliver the minimum effective quantity of the probiotic related to the health claim.
- Proper storage conditions to be mentioned.

2.11 Manufacturing and handling procedures: Adequate quality assurance programmes should be in place. Good Manufacturing Practices should be followed in the manufacture of probiotic foods. The Codex General Principles of Food Hygiene and Guidelines for Application of Hazard Analysis and Critical Control Point (HACCP) should be followed.

Figure 1: Guidelines for evaluation of candidate probiotic strains



*** Only required if a specific health claim is made**

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4. GLOSSARY

Probiotics: Probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'

Shelf-life: Survival of sufficient viable organisms ($\geq 10^8$ CFU/gram) to confer health benefits to the host when stored at a specified temperature.

Health Claims: A statement, which characterizes the relationship of any substance to a disease or health-related condition, and these should be based upon well-established, generally accepted knowledge from evidence in the scientific literature and/or recommendations from national or international public health bodies with clinical validation of safety and efficacy.

HACCP: Hazard Analysis and Critical Control Point is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product**.**

Codex Alimentarius: The Codex Alimentarius is a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety. The Codex Alimentarius Commission was created in 1963 by FAO and WHO with the purpose of protecting health of the consumers, ensuring fair trade practices in the food trade, and promoting coordination of all food standards work undertaken by international governmental and non-governmental organizations.

GMP: Good manufacturing practice is that part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.