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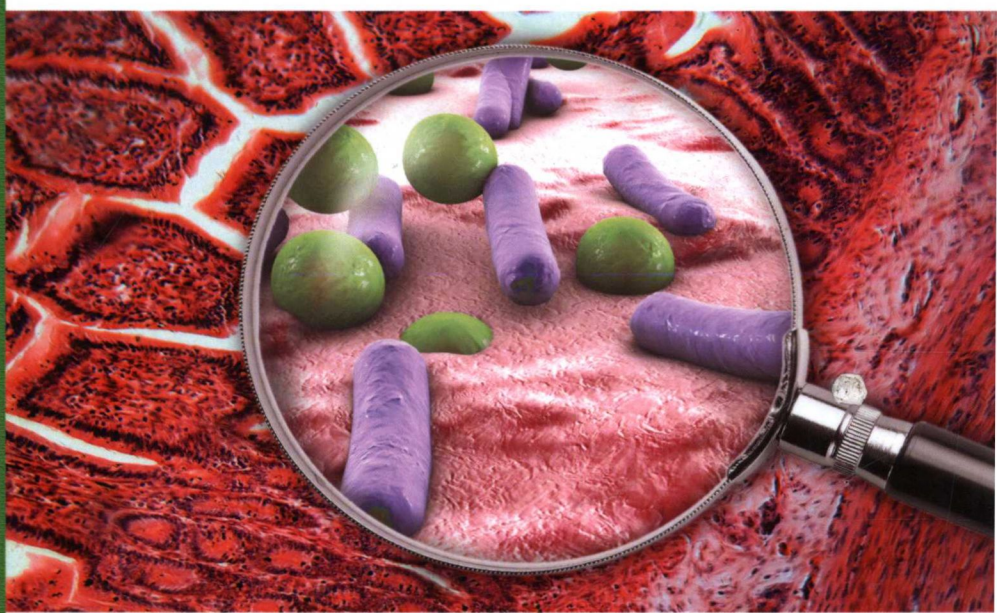
Cellular Interactions of Probiotic Bacteria with Intestinal and Immune Cells



Sarah Moore • Kasipathy Kailasapathy

BACTERIOLOGY RESEARCH DEVELOPMENTS

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Professor Kasipathy Kailasapathy is an internationally leading researcher in the field of probiotic micro-organisms, particularly in protecting them through novel bio techniques in encapsulation. He was awarded the most prestigious Danisco International Dairy Science Award by the American Dairy Science Association in 2010 acknowledging his outstanding contribution to his world-leading research in probiotics and microencapsulation. He graduated in 1982 with a PhD in Food Sciences from Penn State University, University Park, Pennsylvania, USA. He was a Professor in Food Sciences at Western Sydney University, Australia and at Taylor's University, Malaysia. He has published many books and scientific refereed articles in probiotics and microencapsulation. He is a Fellow of Australian Food Science and Technology and a professional member of the Institute of Food Science and Technology and the American Dairy Science Association.

Sarah Moore completed her Bachelor of Science degree (Biological Science) and her honours thesis in bioactive peptides at the University of Western Sydney. Sarah received her PhD from the University of Western Sydney in 2013 for researching the responses of intestinal and immunological cells to probiotics. A unique synergy was achieved between confocal microscopy and microbiology through this research. Through this research she pioneered and developed imaging techniques for microencapsulated probiotic bacteria using confocal microscopy, fluorescence dyes and image analysis software. These techniques have direct applications for the probioics industry and broader applications for research applications in human and animal nutrition, physiology and health.

"Immunomodulation is one of the strongest claims made by many probiotic organisms and hence it is one of the most interesting fields of research in science. In this context, the present book is most appropriate as it will significantly contribute to the existing knowledge on the application of probiotics. Professor Kailasapathy is a world-leading expert researcher in probiotics and microencapsulation and this book is a testament to his knowledge in this field."

J. B. Prajapati
Professor of Dairy Microbiology, Principal & Dean
Faculty of Dairy Science, Anand Agricultural University, India

"Professor Kailasapathy is one of the foremost and outstanding experts in the microencapsulation of probiotic bacteria and their incorporation into foods and feeds. One of the established benefits of probiotics is stimulation of the immune system. This book presents valuable information on the effect of microencapsulation on the functionality of probiotic bacteria on immune cells of the gastro-intestinal system. In particular it sheds light on the mechanisms by which probiotics exert their beneficial effects on the porcine immune system. It is therefore recommended reading for students and scientists involved in improving animal welfare through probiotics."

Dr. Claude P. Champagne
Agriculture et Agroalimentaire Canada
Saint-Hyacinthe, Quebec, Canada

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CELLULAR INTERACTIONS OF PROBIOTIC BACTERIA WITH INTESTINAL AND IMMUNE CELLS

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PREFACE

The significant health benefits including immune-modulation, associated with the regular intake of probiotic bacteria, have paved a strong commercial outlet on the worldwide multibillion dollar consumer market for probiotic food and drink products. Much of the published literature has evaluated the immune-modulatory properties of probiotic bacteria to identify the type of immunological response, including anti-inflammatory, regulatory and maturation of immune cells. The interactions between probiotics and intestinal cells have also been investigated by researchers, primarily the alleviation of intestinal inflammation by probiotics to ameliorate the symptoms of diseases such as Crohn's disease.

An understanding of the immune cell molecular behaviour and responses to soluble factors produced by probiotic bacteria and intestinal/probiotic co-cultures will contribute to scientific knowledge and the commercial development of probiotics as therapeutic supplements. Such understanding will allow scientists to identify new probiotic strains with enhanced immunomodulatory effects, ascertain minimal probiotic dose requirements, distinguish the type of immunological response to probiotics and identify potential probiotic soluble factors that induce immunological reactions.

This study involved the use of adaptive immune cells with intracellular-expressed fluorescent proteins acting as bio-indicators of

responses to soluble factors produced by probiotic strains and intestinal/probiotic co-cultures. Real-time fluorescence microscopy and image analysis software (Raster Image Correlation Spectorscopy, or RICS) was applied to determine the diffusion rates of the fluorescent proteins in response to the probiotic treatments. Immune cell proliferation and gene expression of cytokines and CD markers were also investigated. In addition, immune cell responses to microencapsulated probiotic bacteria were explored. Microencapsulation has been proposed to protect probiotic bacteria from food processing and harsh gastrointestinal environments. However, the behaviour of probiotics in the confined encapsulated environment has not been described. The immune cell response to the microencapsulated probiotics was used as a downstream indicator to determine changes in the behaviour of the confined probiotics.

Many studies have described immunological enhancement from probiotic bacteria treatments including *in vivo*, *in vitro* and *ex vivo* experiments. However, the immune intracellular mechanisms underlying such responses have rarely been described. In this research, the porcine progenitor immune cell lines, L45 and L23 (T- and B-cells) were used to determine intracellular responses to probiotic bacterial-produced soluble factors. Porcine immune cells were chosen for these investigations due to the close relationship with human cells and also are applicable to animal cellular studies.

Recent developments in fluorescence microscopy have provided the opportunity to obtain real-time information from viable cells. Fluorescent proteins have been widely used as bio-indicators of cellular responses. The porcine progenitor immune cells were transfected with the fluorescent proteins, phMGFP and pCIneo-DsRed2. Changes in fluorescence and diffusive properties of these fluorescent proteins in response to the probiotic bacterial-produced soluble factors were imaged by LSCM and FCS, and analysed using the RICS program. These changes were compared to the immune cellular responses to pathogen-produced soluble factors (*Streptococcus pyogenes*). The intracellular responses of the immune cells to the probiotics and pathogen were compared to determine if the probiotic strains exhibited similar effects on immune cellular responses.

As mentioned previously, microencapsulation has been investigated as a delivery tool for probiotics to maintain cellular viability and to release the cells at sites of interest within the GI tract. Changes in probiotic bacterial-activities from the microencapsulated environment have rarely been described and were evaluated in this research using the transfected porcine progenitor immune cells as a downstream indicator. To date, very few studies have described any form of immunological responses (innate or adaptive) to microencapsulated probiotic bacteria. These results were compared to the responses of immune cells treated with non-microencapsulated probiotic bacteria.

Initially, mammalian cell culture media was conditioned by free and microencapsulated bacterial-produced soluble factors and applied to the transfected porcine progenitor immune cells. The immune cellular proliferation, cytokine response and intracellular fluorescent protein diffusion rates were analysed. To gain an understanding of the responses of immune cells within the gastrointestinal (GI) tract, porcine fibroblast intestinal cells (IPI-1) were used as an intestinal cell barrier to determine if the porcine immune cells responses to the free and microencapsulated bacterial-produced soluble factors differed. The proliferation, cytokine response and intracellular fluorescent protein diffusion rates were also analysed and compared to the treatments without an intestinal cell barrier.

This book contains eight chapters:

Chapter One provides a detailed literature on various topics related to probiotics and immuno-modulation.

Chapter Two investigates the effect of microencapsulation on the viability of probiotic bacteria and develops a standard curve for microencapsulated bacteria using LSCM and image analysis software.

Chapter Three incorporates the development of a fluorescent immune cell model using porcine progenitor immune cell lines, L45 and L23, and the fluorescent proteins, humanized Monster Green® Fluorescent protein (phMGFP) and pCIneo-DsRed2.

Chapter Four elaborates these studies to determine the effects on the proliferation of the transfected porcine progenitor immune cell lines by free and microencapsulated probiotic bacterial-produced soluble factors.

Chapter Five determines the effects on the proliferation of the transfected porcine progenitor immune cell lines by free and microencapsulated probiotic bacterial-produced soluble factors that have been co-cultured with the porcine fibroblast intestinal cells (IPI-1).

Chapter Six investigates cytokine gene expression by the treated porcine progenitor immune cells in response to the free, microencapsulated, IPI-1/free and IPI-1/microencapsulated probiotic bacterial-produced soluble factors.

Chapter Seven includes studies to determine if the porcine progenitor immune intracellular fluorescent protein diffusion rates are influenced by the free, microencapsulated, IPI-1/free and IPI-1/microencapsulated probiotic bacterial-produced soluble factors.

Chapter Eight incorporates a general discussion and suggestions for future research.

CONTENTS

Chapter 1

INTRODUCTION

The health benefits associated with probiotics, including immunomodulation, drive the world wide multi-billion dollar consumer market for probiotic food and drink products (Sanders, 1998). Numerous studies have evaluated the immunomodulatory properties of probiotics to identify the type of immunological response including anti-inflammatory, regulatory and maturation of immune cells (Iwasaki and Kelsall, 1999; Matsuzaki and Chin, 2000; Paturi et al., 2007; Takeda and Okumura 2007). The interactions between probiotics and intestinal cells have also been investigated by researchers, primarily the alleviation of intestinal inflammation by probiotics to ameliorate the symptoms of disease states such as Crohn's Disease (Boudeau et al., 2003).

A better understanding of the immune cell molecular behaviour and responses to soluble factors produced by probiotics and intestinal/probiotic co-cultures will benefit future research. Such understanding will allow for the identification of new probiotic strains with enhanced immunomodulatory effects, ascertain minimal probiotic dose requirements, distinguish the type of immunological response to probiotics and identify potential probiotic soluble factors that induce immunological reactions.

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1.1. History and Development of Probiotic Bacteria

Approximately 2,500 years ago, Hippocrates (460 – 377 BC) suggested that food could be of medicinal use advocating "let food be thy medicine and medicine be thy food". For thousands of years, microorganisms have been incorporated into food and beverages. The concept of microorganisms conveying beneficial effects to hosts was proposed over a hundred years ago. Metchnikoff, in 1907, suggested that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes". He also noted the long life of Bulgarian peasants who consumed fermented milk products and suggested that the ingestion of yogurts containing *Lactobacillus* sp. can replace harmful intestinal bacteria. Metchnikoff hence introduced the concept of functional foods containing beneficial microbes. Currently, increasing consumer awareness of personal health has led to health-promoting/functional foods becoming more popular. Contemporary consumers are looking beyond

immediate nutritional requirements from food products and seeking those that promote a number of health-enhancing, as well as disease preventing characteristics.

In the past 50 years, considerable research has been conducted into the beneficial properties conveyed by probiotic bacteria to hosts; the health-promoting characteristics associated with probiotic bacteria include immunological enhancement, re-establishment of intestinal microbial balance following antibiotic treatments, and the potential to treat gastrointestinal (GI) disorders (Gomes and Malcata, 1999; Shah, 2007; Prado et al., 2008). However, the underlying mechanisms of probiotic health-conveying activities, in particular immune cell responses to probiotics, have not been addressed. The health-promoting characteristics of probiotic bacteria have led to the incorporation of these microorganisms into many foods and beverages, in particular, but not limited to dairy products. The most recognised probiotic bacteria are gram-positive lactic acid bacteria (LAB), in particular the strains of *Lactobacillus* and *Bifidobacterium*, with both genera found in the human intestinal microbiota populations. Other probiotic microorganisms include *Bacillus cereus* (animal probiotic), *Streptococcus salivarius* sp. *thermophilus* and *Saccharomyces boulardii* (Prado et al., 2008). To be classified as probiotic, a strain must possess a number of desirable characteristics and activities such as validation of health benefits and strain identification.

1.1.1. Definition of Probiotics and Probiotic-Active Substances

Many proposed definitions attempt to reflect the role and characteristics of probiotic bacteria. The investigation of the health benefiting effects conveyed by probiotic bacteria to consumers has resulted in a change to past definitions. Subsequent to Metchnikoff's proposal in 1907 (to replace harmful microorganisms with beneficial microorganisms in the intestinal tract), the term probiotic was first used by Kollath (1953) to describe organic and inorganic substances in food that alleviated malnutrition in patients (Hamilton-Miller et al., 2003). Later, the term

probiotic was used by Lilly & Stillwell (1965) and Sperti (1971) to describe substances produced by microorganisms that encouraged the growth of other microorganisms. The term probiotic was also used to identify substances that improved resistance to infections (Fujii and Cook, 1973). The definition for probiotics was further developed to encompass microorganisms as well as substances. In 1974, Parker defined probiotics as "organisms and substances which contribute to intestinal microbial balance" in reference to advancing animal health using feed supplements. Currently, the most referenced definition was stated by Fuller in 1989 describing probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". Fuller emphasised that such microorganisms should be viable. At present, accepted definitions for probiotic bacteria portray viable microorganisms with the ability to convey beneficial health effects to the host. The World Health Organisation (WHO) and Food and Agricultural Organisation (FAO) of the United Nations (2001) proposed probiotic bacteria as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'.

Current definitions of probiotics describe these microorganisms as viable, whole cells. Probiotic cellular structure fragments, such as nucleic acids and cell wall elements, have been reported to influence the intestinal ecosystem. Interactions between these cellular components and the intestinal environment were described including induced immunomodulatory activities (Naidu et al., 1999). The potential use of non-viable microorganisms or cellular components in nutritional pharmabiotics was outlined by Shanahan (2004) who also declared that the concept of current definitions limit probiotic microorganisms to being viable and of human origin. Such bacterial components and non-viable cells have been termed probiotic-active substances (Naidu et al., 1999). The concept of stimulation by probiotic-active substances has been supported by studies using nucleic acids, cell wall structures and non-viable bacterial cells, among other probiotic bacterial components, that reportedly enhance immunological activities in mice (Lin et al., 2007) and humans (Lammers et al., 2003), and attenuated experimental colitis in mice