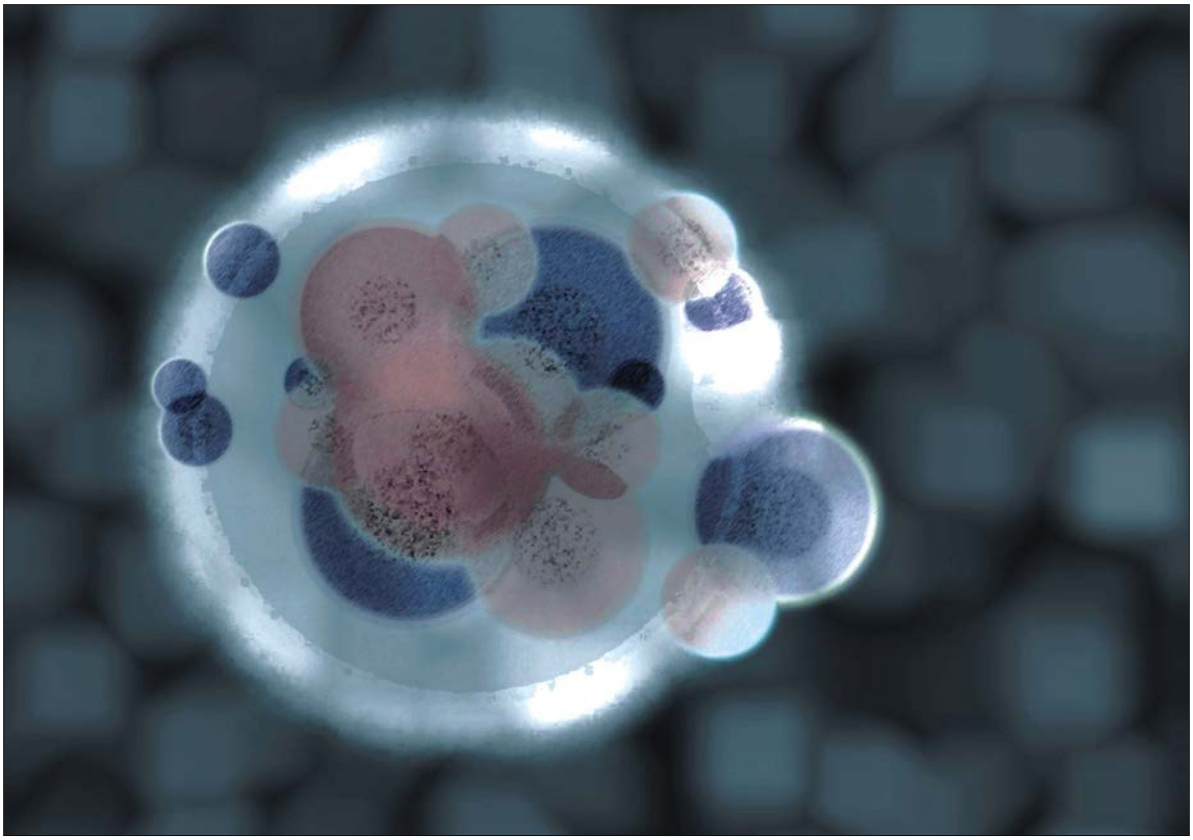

Journal of Biological Sciences

ISSN 1727-3048

Journal of Biological Science 6 (1): 202-208, 2006
ISSN 1727-3048

Therapeutic Properties of Probiotic Bacteria

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Abstract: In spite of its long history, public consciousness of probiotics has shifted dramatically in recent years. This is due to a number of factors, including an increased concern about the potential generation of antibiotic resistant bacterial strains due to widespread antibacterial use, and also to the spreading realization that one's health can be, not simply maintained, but actually improved with proper nutrition. Combined, these factors have stimulated a surge in probiotic research in the past decade, resulting in increasingly refined studies. Indeed, after Elie Metchnikov first printed his work suggesting a positive correlation between human longevity and the consumption of fermented milk, information on probiotics is leaving the realm of the anecdotal as recent, double-blind, placebo controlled randomized tests support beneficial probiotic activity. Concurrently, more is being learned about their activities *in vivo*. While much work remains to be done before a detailed understanding of probiotics can be achieved, there is mounting evidence that probiotics, when used in proper conditions, may indeed have prophylactic or preventative effects on a broad array of human and animal diseases. This article briefly surveys probiotic history and discusses recent research with a special emphasis on lactic acid bacteria probiotics. Finally, it discusses the inherent difficulties of their study and suggestions for standards for future work.

Key words: Probiotic bacteria, *Bifidobacterium*, *Lactobacillus*

INTRODUCTION

Probiotics: The definition of probiotic bacteria is constantly evolving. Attempts to provide a clear description of their biological details and effects on the health of their hosts are frustrated by the large number of species and genera existing in the category. While several authors have suggested amendments to it, a good starting point was offered[1] by Fuller: a live microbacterial feed supplement which beneficially affects the host animal improving the intestinal microbial balance.

Many different benefits have been attributed to the consumption of probiotics in both humans and in animals, most notably production animals such as cattle and chickens, in which they are often referred to as Direct Fed Microbials (DFM). Benefits include increased lactose tolerance and improved immunity in humans and increased feed efficiency and decreased pathogen shedding in animals, in addition the long-standing consensus that probiotics are able to encourage a healthy intestinal milieu in all hosts. In recent years, information on probiotics is finally leaving the realm of the anecdotal and earning its status based on double-bind, placebo-controlled, randomized testing.

History of probiotics: Humans have been consuming probiotic products since pre-biblical times, mostly in the form of fermented milk products. However an association between these products and human health was not suspected until the early 1900s. Metchnikoff[2] suggesting that the good health and long life of Bulgarians could be traced to their diets. This humble beginning has grown into the field of probiotics, an active avenue of scientific inquiry and an equally active commercial industry.

Metchnikoff[2] earlier research initiated study into the activity of probiotics. However, it was not until the end of World War 2 and the beginning of the "Antibiotics Era" that probiotic research began in earnest, catalyzed by indications that probiotics may be able to prevent or improve the diarrhoea associated with antibiotic use[3]. Probiotic research increased through the next several decades. By the 1980s researchers from around the world were coordinating their efforts in an attempt to elucidate the activity and mechanisms of probiotics in cattle and other production animals, as they were purported to increase the growth as well as improve the general health of these animals. Despite these efforts, laboratory results failed to provide consistent results and the 1990s saw a decrease in the work being done in this field. Commercial

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sales of probiotics for use in production industry, however, remained relatively high. With the discovery on BSE and the impending ban on antibiotics in animal feed in Europe, probiotic research for production animals is being pursued with renewed vigor in an effort to moderate the effects the ban is expected to have.

Meanwhile, researchers investigating the effects of probiotics on human health have obtained more consistent results. Despite the fact that a clear understanding of the mechanisms behind probiotic activity remains elusive, there exist a number of articles documenting well-controlled double blind tests reported clear and statistically significant results[4, 5].

PROBIOTIC BIOLOGY

Lactic acid bacteria: Probiotics commonly stem from the category of Lactic Acid Bacteria (LAB), especially of genera *Lactobacillus* and *Bifidobacterium*. These are gram-positive, catalase-negative, nonaerobic but aerotolerant, nonsporing, nonrespiring cocci or rods, and produce lactic acid as the major end product of the fermentation of carbohydrates[6]. The metabolites are considered to be quite important in their beneficial effects. These include organic acids (especially lactic acid), hydrogen peroxide deconjugated bile salts and bacteriocins[7].

Genus lactobacillus: Lactobacilli are gram-positive, non-spore forming and non-flagellated rods or coccobacilli. They have a low G+C content (below 50%)[8], usually between 32 and 51%. *Lactobacillus acidophilus* strains are the most common dietary components. Genetic studies have found that the original species consists of six DNA homology groups; *L. crispatus, L. gallinarium, L. gasseri, L. amylovarus and L. johnsonii.* Although these species are well defined, it is often difficult to allocate newly isolated strains to one of these groups.

L. acidophilus is a gram-positive rod with rounded ends and can occur as single cells, in pairs, or in short chains. Optimum growth occurs between 35-40°C and between pH of 5.5-6.0[7]. They are distributed throughout the gastrointestinal tracts of humans and other hosts, with the main factors affecting location being pH, oxygen availability, substrate levels, presence of gastrointestinal secretions, and interactions with other bacteria[7].

Genus bifidobacterium: Like Lactobacilli, Bifidobacteria are extremely important in the gastrointestinal and genito-urinary tracts of humans and other warm-blooded animals. While present throughout the life of the host, age and diet are important factors in the exact distribution of Bifidobacteria. They are the dominant species in the microflora of infants, but the number decreases with age until stabilizing at about 25% of the total adult intestinal flora, the third most abundant species after *Bacteroides* and *Eubacteria*[7]

Bifidobacteria were known as early as 1900, when Tissier described them as rod-shaped, non-gas-producing, and anaerobic microorganisms with bifid morphology[7]. They are currently characterized as gram-positive, non-spore forming, non-motile catalase-negative anaerobes. They are found in various shapes including Y-shaped rods, short and curved rods, and club-shaped rods. Unlike Lactobacilli, Bifidobacteria have a high G+C content (54-67%) and thus are grouped in the actinomycete branch of gram-positive bacteria. Those found in humans can use glucose, galactose, lactose and often fructose as carbon sources. Optimum growth conditions are found between pH of 6 and 7 and temperatures between 37-41°C.

Selection criteria: All probiotics are intended for consumption by humans-either directly through dietary supplements or in a food matrix, or indirectly as in the case of probiotics for use in production animals. Like anything intended for consumption by humans, the selection criteria for probiotics are necessarily strict. While probiotics must by definition possess activities beneficial for the consumer, they must also be able to survive in tact the often extremely harsh conditions involved in the manufacturing process as well as those found in the digestive tract of the host.

This involves an ability to survive the high acidity of the stomach, which can be as low as 1.0, as well as the concentrated bile found in the proximal small intestine.

Ability to adhere to the human intestinal mucosa is helpful for temporary Gastro-Intestinal Tract (GIT) colonization, although researchers disagree as to whether adhesion should be considered in selection criteria, as continuous administration can sometimes create an adequately constant probiotic population for positive results[7].

In addition to these biological criteria, probiotics intended for public use must also be commercially feasible. Thus, they must also be mass producible, remain viable while in storage and not affect the taste of the substrate. Finally, probiotics must, for obvious reasons, be non-pathogenic the intended host. For this reason, many researchers advocate the use of cultures that are indigenous to the GIT[9].

PROBIOTIC ACTIVITY

General: Colonization occurs in the first weeks after birth, and leads to crucial interactions with both the GIT and the Immune system. In healthy animals, the first colonizing microbes come mostly from the maternal flora[10]. As development progresses, the indigenous microflora is specific to both the species and the particular area of GIT inhabited. Through connection with mucus layer or adhesion to the surface or epithelial cells, this population serves as the first defence barrier against both pathogenic bacteria and other types of harmful elements ingested through the diet[10].

At birth, there is a fundamental shift as the gut switches from digesting amniotic fluid to digesting food. This stimulates the release of trophic hormones, which help to develop both digestive and protective roles of the GIT. As development progresses, mucosal proteins and digestive enzymes, along with the colonization of intestinal bacteria, are some of the first protective characters to develop. The highly acidic environment created by the secretion of hydrochloric acid is an effective antimicrobial characteristic, and develops within the first few months of life while goblet cells mucus covers GIT epithelial surfaces, preventing the attachment of luminal antigens.

Antigen attachment has been recognized as a fundamental step in foodborne virulence as soon as the early 1979s, when Jones and Rutter[11] showed that *Escherichia coli* was unable to establish pathogenesis without it. Normal bacterial populations aid in preventing overgrowth of pathogens and also help prevent the attachment of pathogens to the lumen through competitive exclusion, and this activity is thought to play a key role in probiotic protection[10].

Gut bacteria also perform other beneficial activities for the host. One example of this is found in adult ruminants in whom digestion of fibrous dietary components is mainly accomplished by the fermentative activities of bacteria located in the rumen. As mentioned, each part of the GIT is colonized by typical bacterial species, which have developed symbiotically with the host and are found in varying numbers throughout out the digestive tract. The mouth contains a large number of both facultative and strict anaerobes, while the upper bowel (stomach, duodenum, and jejunum) are only sparsely populated. Bacterial concentrations increase from the ileum through the remainder of the digestive tract, with the densest population found in the colon. Up to 35-50% of the contents of the human colon may be accounted for by the 500 species of bacteria commonly found in the human large intestine, mostly species representing five genera; *Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus* and *Fusobacterium*[10].

Probiotics have been accredited with a broad range of activity in the GIT. The most widely exhibited character is that of competitive exclusion, in which pathogenic and probiotic bacteria compete for limited nutrients and space on the gut mucosal layer[12]. In the case of successful probiotic administration, probiotics are more able to attach to the gut, thus preventing the pathogenic species from leaving the digestive system and entering the blood. However, it is widely accepted that this is not the sole active mechanism.

Since the 1950s, probiotics have been associated with an ability to prevent or treat intestinal ailments. Manifestation of this ability is seen in the treatment of problems as varied as Chrohn's disease, gastric enteritis, lactose intolerance, diarrhea resulting from numerous sources. An ability to work on such a broad range of discrete symptoms would suggest a large number of discrete activities, which indeed seems to be the case. The improved digestion of lactose seen when consumed in yogurt with live bacteria (as opposed to lactose in milk or yogurt in which the bacteria has been heat-killed) is thought to be due to luminal digestion of lactose by bacteria. The digested lactose would be released in the small bowel only when bile acids lyse these bacteria[4].

Probiotic mediated alleviation of diarrhea is thought to operate via multiple discrete or complementary pathways. Moreover, probiotics may successfully treat or prevent diarrhea associated with a number of different causes[13]. Antibiotic associated diarrhea occurs in up to 20% of medicated patients[4]. Statistically relevant results have been seen in multiple randomized double blind trials suggesting that certain probiotics may be able to ameliorate or prevent this[5]. Examples include the use of probiotics such as *Lactobacillus rhamnosus, Lactobacillus reuteri*[14] and *Saccharomyces boulardii* for treatment of acute diahrrea. Bifidobacteria [15] and *Clostridium butyricum*[16] have both been suggested for the prevention of acute diarrhea cause by rotavirus in infants.

Some probiotics are known to secrete antimicrobial molecules, referred to as bacteriocins, which are also able to prevent pathogenic bacteria from attaching to mucosal epithelial surfaces[17]. Many probiotics have also been shown to activate the immune system, possibly initiating activity in areas of the mucosa-associated immune system[18]. Researchers have reported that ingestion of probiotics has been shown to increase production of IL-10 or TGF-ß and/or regulatory cells. Additionally, preliminary findings indicate that probiotics may be able to bind with food contaminants in the GIT, thereby preventing them from being absorbed. Gratz *et al.*[19] have reported on the ability of *Lactobacillus rhamnosus L. rhamnosus* LC-705 and *Propionibacterium freudenreichii* subsp. *shermanii* JS (LC-705) to bind aflatoxin B1 *in vitro*. However, these results are as yet unconfirmed *in vivo,* where aflatoxin binding may be reduced by steric hindrance.

Probiotics and immunity: In addition to its fundamental role in digesting and absorbing nutrients, the gut must protect the host from the plethora of pathogens consumed with food. To this end, the intestinal mucosa works in conjunction with other components of the GIT such as saliva, gastric acid, peristalsis, mucus, intestinal proteolysis, intestinal flora, and epithelial cell membranes with intercellular junctional complexes.

Normal intestinal microflora is extremely important in gut immune function. The absence of an intestinal microflora can result in increased antigen transport across the gut mucosa. Gut microflora also elicit immune responses at local and systemic levels[10].

The high concentration of antigens in the gut requires highly evolved immune protection. Thus, the surface of mucosal membranes is protected by a local adaptive immune system. The gut-associated lymphoid tissue is the largest mass of lymphoid tissue in the human body and includes Peyer's patches and follicles distributed within the mucosa, secretory sites, and intestinal epithelium. What is commonly referred to as immune response is a complex and highly evolved combination of several different factors, including intraepithelial T lymphocytes, which are mainly involved in suppression of antigens through cytotoxicity, activated B cells, which secrete pathogen-specific antibodies, and numerous helper cells such as macrophages. The lamina propria cells are also active in the gut, working mostly as helpers and inducers, and it contains many B lymphocytes[18].

Probiotics have been shown to enhance humoral immune responses, promoting the intestine's immune potential. Studies indicate that probiotics are able to modulate both the specific and the non-specific immune system.

Nonspecific immunomodulation: Probiotics, including lactic acid bacteria have been shown to promote innate defence mechanisms. This often involves interactions with indigenous bacterial inhabitants of the gut. Such is the case in the stabilization of the gut microflora, thereby competitively limiting gut access to pathogens. Significantly, probiotics also stimulated nonspecific host resistance to microbial pathogens[20]. This aids in the elimination of a broad array of antigens.

In vitro experiments have indicated that several strains of Lactobacilli successfully induce the release of proinflammatory cytokines TNF-α and Interleukin-6, both of which are involved in nonspecific immunity[10]. *L. casei* and *L. bulgaricus* have both been reported to induce macrophages and activate phagocytosis in mice. *L. acidophillus* has also successfully induced phagocytosis, an early step in the immune response, in humans. This results in the release of toxic agents such as reactive oxygen intermediates and lytic enzymes and is also a step in the recruitment of specific immune response.

Many probiotics are also attributed with anti-inflammatory activity. Ma *et al.*[21] found that *L. reuteri* inhibits mRNA up-regulation, cellular accumulation, and secretion of IL-8 induced by TNF-α in human epithelial cells in vitro. *L. reuteri* was also shown to upregulate the anti-inflammatory NGF and inhibit NF-κB. This was suggested as a potential mechanism in the anti-inflammatory activities of this probiotic[21].

Studies on the lactic acid bacteria, *P. acidipropionici* and *P. freudenreichii*, have shown an ability to kill human colorectal carcinoma cell lines by apoptosis[22], preliminarily indicating that it may be useful in colon cancer prophylaxis or prevention.

Probiotic research in ruminants: While there has been a long standing interest in probiotics, increased concern that overexposure to antibiotics is resulting in the generation of treatment resistant strains of bacteria, compounded by the imminent ban on the use of antibiotics as feed additive in the E.U., has given extra impetus to research DFM in recent years. Thus, studies on the efficacy of different strains of DFM, alone or in conjunction with other bacteria, and also on the mode of action in production animals are becoming increasingly refined and important.

A healthy balance of microflora of the gastrointestinal tract is crucial to the health of an animal. Current production methods lead to heavy stress which can have negative effects on the performance of animals, especially on young ones whose gut microflora is not yet established. DFM have been reported to moderate the negative effects which such practices have on the health of the animals.

Beef cattle undergo severe stress in the process of weaning, transport, fasting, assembly, vaccination, castration, and dehorning. As previously mentioned, such stress can lead to an imbalance in intestinal microecology, leading to increased morbidity and even death. A number of studies have indicated that treatment with DFM can help to restabilize the gut biota, thereby improving overall health and performance. Examples include studies indicating that feed supplemented with probiotic bacteria has been shown to reduce the numbers of pathogenic bacteria in cattle rumen and feces. This, in conjunction with other mechanisms, are thought to result in the increased feed efficiency, live weight gain and disease resistance seen in research results[23].

DFM activity is limited to the GIT in most species. However, in ruminants the definition is broadened to include the rumen. In these animals, DFM use has been accredited with enhanced milk production in dairy cows, improved feed efficiency and daily gain in beef cattle. Additionally, there is mounting evidence that probiotic therapy can be used to decrease pathogen shedding in production animals. More than 76 million people are annually subject to foodborne illnesses in North America alone[12]. Traditionally, attempts to decrease this number have emphasized post-slaughter practices. However recent studies indicate further reduction in *E. coli* O157:H7 shedding feedlot cattle, and a decreased morbidity in neonatal and stressed calves can be achieved through pre-slaughter DFM treatment[24] .

Nocek *et al.*[25] reported that DFM (*Enterococcus faecium*, 2 separate strains) supplementation beginning several weeks prepartum and ending on day 70 of lactation results in increased dry matter intake, milk production, and milk-protein percentage. These results were especially visible through the third week of lactation, after which apparent effects tapered off. This study supported others which suggest that DFM use is most effective in times of environmental or physical stress on the host and that effects are less noticeable on healthy hosts.

DFMs have been credited with improving feed efficiency (approximately 2%) and the daily gain (most studies between 2.5-5% increase) of feedlot cattle. A strategy currently considered to have potential is phase feeding, in which specific species and strains could be targeted to specific phases of production. While there is much work to be done before this can be instituted, preliminary results are promising. Many researchers in the field suggest that a detailed understanding of proper targeting of probiotics would explain and reduce the number of inconsistent results, in which treatment was found to have no effect, or in a few cases, negative effects[26]. However, due to the complexity of the interactions, which are specific both to host-strain combinations and to the development phase of the host, a large amount of work remains to be done.

Probiotics in broilers: In poultry, the most common intestinal lactic acid bacteria are *Lactobacillus* and *Enterococcus*. Dalloul and coworkers[27] found that *Lactobacillus* treatment in broilers resulted in improved immunity in Vitamin A deficient birds, and were able to demonstrate a probiotic effect on local cell mediated immunity in chickens. This was evidenced by an apparent decrease in intestinal invasion by the pathogen *Eimeria acervulina* based on a higher serum level of IL-2 and a reduction in *E. acervulina* oocysts.

Many studies in poultry mirror the results found in cattle. Much like *E. coli* in cows, *Salmonella enteritidis* is a major food borne illness found in poultry and raw meat contamination has significant commercial implications. Tellez *et al.*[28] found that combining probiotics *L. acidophilus* and *Streptococcus faecium* with antibodies against *Sal. enteritidis, Sal. typhimurium* and *Sal. heidelberg* successfully reduce the colonization of *S. enteritidis* in market-aged broilers. Other studies have found that probiotics had a positive effect on chick weight gain[29-31].

Complications: As seen, the vast majority of properties discussed are both host and strain specific. These properties can be affected when the bacterial probiotics are used in conjunction with other products, such as other strains of bacteria, yeast probiotics, or antibiotics. Thus, the importance of clinical study and proper labelling during commercial packaging can not be stressed enough. Many additives are referred to as Probiotics despite a lack of quality assessment, standardized safety or proven clinical efficacy through reliable, controlled testing. The effects of this combination have taken a toll on the reputation of probiotics.

Manufacturing methods can also alter the activities of probiotics, both positively and negatively. Probiotics are most commonly administered in either fermented milk products or in capsule form. Shah[32] investigated the affects of different food matrices on the activity of *P. freudenreichii.* It was found that the delivery matrix could have profound effects on the bacteria, with some foods seeming to actively protect the bacteria from stress injury, while others had the opposite effect. Processes shown to increase viability of probiotics in yoghurt include microencapsulation, two-step fermentation, sonication to release β-galactosidase enzyme from the starter culture, and selection of containers impermeable to oxygen. The effects of processing and particular food matrices on the viability of probiotics must be further investigated in order to ensure consumers are purchasing active and effective cultures.

Suggestions for proper use: Lack of consistent results in laboratory studies, compounded by the large number of discrete strains with unique activities, prompted the meeting of a joint FAO/WHO Expert Consultation, resulting in the publication of standards to assure quality and reliability in using probiotics in humans. The list of suggestions include genus and strain identification by internationally accepted methods such as DNA-DNA hybridization or sequencing of DNA encoding 16srDNA and identification with pulsed field gel electrophoresis or randomly amplified polymorphic DNA. Also, proper product labeling stipulating the exact genus, species and stain, quantity (viable numbers of each stain), formulation (liquid, dry, micro-encapsulation, bacterial coating), storage condition, indication of the minimum daily amount required to confer specific health benefit, and level of evidence for the health claim. The committee further advocated standard safety tests including assessment of antibiotics resistance, toxin production, haemolytic activity, infectivity in immuno-compromised animal models, as well as recording of post-marketing side effects in consumers. Finally evidence based measurement of health benefits from well-designed randomized controlled trials of sufficient power should be conducted[9].

CONCLUSIONS

While many questions remain unanswered in the field of probiotics, an growing body of research indicates that they may be effective in treating or preventing a wide range of diseases in both humans and animals. The potential benefits of consuming probiotic bacteria include wide scale immuno-modulation as in auto-immune diseases and small scale suppression of specific intestinal pathogens as seen in the prevention of rotavirus infection. The list of targets is likely to grow as our understanding of the mechanisms behind probiotic activity continues to develop. Nevertheless, caution is necessary. The widespread use of live cultures can lead to undesirable side effects, such as a spread of antibiotic resistant strains of bacteria. To this end, it is imperative that individual strains be identified, carefully characterized and correctly labeled before being put on the market.

ACKNOWLEDGMENT

This is a part of a research project (Project Code MRG4680202) funded by the Thailand Research Fund which is gratefully acknowledged by the author.

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