Biology of Eukaryotic Probiotics

Sukanta Kumar Nayak

Contents

1 Introduction ............................................................................................................. 30
  1.1 Eukaryotic Probiotics: An Overview .......................................................... 31
2 Eukaryotic Probiotics Versus Prokaryotic Probiotics ....................................... 32
3 Sources of Eukaryotic Probiotics ................................................................. 34
4 Taxonomic Characterization of Eukaryotic Probiotics ..................................... 34
5 Properties of Eukaryotic Probiotics ............................................................... 35
  5.1 Ability to Tolerate Stress ............................................................................. 36
  5.2 Ability to Survive in the Gastrointestinal Tract ........................................ 37
  5.3 Ability to Adhere to the Gastrointestinal Tract ........................................ 38
6 Beneficial Effects of Eukaryotic Probiotics ..................................................... 38
  6.1 Nutritional Benefits .................................................................................... 39
  6.2 Disease Protection ...................................................................................... 40
7 Modes of Action of Eukaryotic Probiotics ....................................................... 41
  7.1 Trophic Effects on the Gastrointestinal Tract ............................................ 41
  7.2 Stimulation of Immunity ............................................................................ 41
  7.3 Synergistic Activity .................................................................................... 42
  7.4 Antagonistic Activity ................................................................................ 43
  7.5 Toxin Neutralization Activity ................................................................... 43
8 Safety Issues Related to Eukaryotic Probiotics ................................................. 44
9 Genetic Manipulation of Eukaryotic Probiotics ................................................ 44
10 Conclusion ........................................................................................................ 45
References ............................................................................................................ 45

Abstract Probiotics are viable microorganisms which upon ingestion confer health benefits to the host. Any microorganism irrespective of its origin, capable of surviving in the digestive tract of host and exerting such effects can be a candidate.

S.K. Nayak (*) Laboratory of Fish Pathology, Department of Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-8510, Japan e-mail: sukantanayak@rediffmail.com

Most of the currently used probiotics belong to prokaryotic origin. Unlike prokaryotes, several eukaryotic microorganisms can also be very useful to animal’s health. Since a long time, eukaryotes are used as single cell protein and/or as components of food starters for human and animal consumption throughout the world. Apart from these uses, certain eukaryotic microorganisms are also used as probiotics since they can withstand the harsh milieu of gut and execute beneficial effects in host. While bacterial probiotics are common, only limited eukaryotic probiotics belonging to fungi/moulds/yeasts are used in human and animal practices. Nowadays interest in eukaryotic probiotics is on the rise and in most of the cases, their efficacy and usefulness has been confirmed by firm scientific evidences. Among the eukaryotic probiotics, yeasts especially *Saccharomyces* species are dominant and routinely used in a broad range of hosts. This chapter deals with the occurrence, distribution, taxonomic characterization, and detail modes of action of eukaryotic probiotics with special reference to yeasts in human and other animals.

1 Introduction

The concept of using microorganisms as promoter of life perhaps dates back to almost a century when the Russian scientist Elie Metchnikoff proposed the, then revolutionary, concept of consuming live microorganisms for better health. As the knowledge on such types of microorganisms increases and their beneficial effects are scientifically established, the area of what is now known as “probiotics” has made tremendous progress. The term “probiotic” was originated from the Greek words “pro” and “bios” which mean “for life” (Gismondo et al. 1999) and are often called as promoter of life that help in a natural way to improve the overall health status of host. Probiotics are living microorganisms which upon ingestion in adequate amounts confer health benefits to host (FAO/WHO 2002). Therefore, any nonpathogenic microorganism, irrespective of its origin, capable of surviving inside the gut of host and exerting beneficial effects can be a candidate for probiotic use (Ouwehand et al. 2002). Over the years, probiotics are gaining scientific and commercial interest and are now quite commonplace in our daily life starting from health promoting functional foods to therapeutic, prophylactic, and growth supplements (O’Sullivan 2001; Ouwehand et al. 2002; Boyle et al. 2006). Apart from human beings, probiotics have also profound effect on a broad range of animals ranging from terrestrial to aquatic species. Probiotics, which encompass numerous prokaryotic and eukaryotic microorganisms, have become increasingly popular in the past few decades. Most of the currently used probiotics belong to prokaryotes and lactic acid bacteria, bifidobacteria, enterococci, and several other bacteria are the common examples of prokaryotic probiotics (Tuohy et al. 2003). On the contrary, the use of eukaryotic as probiotics is limited and only a few probiotics of eukaryotic origin are commercially available for human and animal practices (Czerucka et al. 2007; Martins et al. 2007, 2009).
1.1 Eukaryotic Probiotics: An Overview

Eukaryotic microorganisms can be very useful to animal’s health as probiotics. There are several food/feed grade eukaryotes, such as algae (e.g., *Chlorella, Spirulina* species), fungi (e.g., *Aspergillus, Penicillium* species)/yeasts (e.g., *Saccharomyces, Candida, Kluyveromyces, Pichia, Torulopsis* species), which are being consumed by human and animals throughout the world since a very long time. These organisms are mostly used as single cell protein and/or as components of food starters. However, there are certain eukaryotes when supplemented in live conditions through diet are found to execute probiotics like beneficial effects in host. Therefore, it is believed to be a very crucial event in the field of probiotics for the developing new candidate species beyond prokaryotic origin. Nowadays significant interest in eukaryotic probiotics is on the rise and in most of the cases, their efficacy and usefulness has been confirmed by firm scientific evidences. Most of the eukaryotic probiotics used in human and animal practices belong to fungi/yeasts/moulds with yeasts to be the dominant group. The common examples of eukaryotic microorganisms with probiotic properties include the genus *Saccharomyces, Pichia, Metschnikowia, Yarrowia, Candida, Debaryomyces, Isaatchenka, Kluyveromyces*, and *Aspergillus* (Table 1).

Historically, yeast has been used for fermentation purposes since 1,550 BC. However, nowadays yeasts are a part of nutritional supplements and health food realms due to their established beneficial probiotic effects. Yeast especially the genus *Saccharomyces* is widely used probiotics in human and animals throughout the world (Jakobsen and Narvhus 1996; Lourens and Viljoen 2001;

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basidiomycota</td>
<td>Urediniomycetes</td>
<td>Sporidiales</td>
<td>Sporidiobolaceae</td>
<td>Cryptococcus</td>
<td>mujuenisi cuniculi</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Saccharomycetes</td>
<td>Saccharomycetales</td>
<td>Dipodascaceae</td>
<td>Yarrowia</td>
<td>lipolytica</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metschnikowiae</td>
<td>Metschnikowia</td>
<td>lochheadii</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saccharomycetaceae</td>
<td>Candida</td>
<td>humilis pintolesii</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>saitoana</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>utilis pararugosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hansenii cereviae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Debaryomyces</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kluyveromyces</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pichia</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Saccharomyces</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Isaatchenka</td>
<td></td>
</tr>
<tr>
<td>Euromycetes</td>
<td>Euromycetes</td>
<td>Trichocomaceae</td>
<td></td>
<td>Aspergillus</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Systemic position of some of the eukaryotic microorganisms showing probiotic activities in different animals including human beings
Saccharomyces cerevisiae and Saccharomyces cerevisiae var boulardii are the only yeast strains commercialized for human uses (Sargent and Wickens 2004; Czerucka et al. 2007; McFarland 2010). S. cerevisiae possess the generally recognized as safe (GRAS) status from the Food and Drug Administration (FDA, USA). Similarly, the European Union has also approved five probiotic strains of S. cerevisiae (NCYC SC 47, NCYC 1026, CNCM I-1077, CNCM I-1079, and MUCL 39885) for application in animal feed (Buchl et al. 2010). Besides, several other yeasts such as Candida pintolopesii, Candida utilis, and Candida saitoana (Bovill et al. 2001; Leuschner et al. 2004) and many filamentous fungi belonging to Aspergillus species (A. niger, A. oryzae) are also used as probiotics in animal feeds (Lee et al. 2006b). Like S. cerevisiae, A. oryzae has the GRAS status from FDA and its safety is also supported by the World Health Organization (FAO/WHO 1987).

While various strains belonging to these species are already been used as probiotics, several other yeasts belonging to Kluyveromyces, Isaatchenki a, and Debaryomyces species are emerging as new generation of eukaryotic probiotics. Strains of these species possess all the desired features of an ideal probiotic including antioxidative, antifungal, antibacterial, antiinflammatory, and antitumoral properties (Oh et al. 2002; Diniz et al. 2003; Lopitz-Otsoa et al. 2006; Lee et al. 2008; Chen et al. 2010).

2 Eukaryotic Probiotics Versus Prokaryotic Probiotics

Bacteria are the natural and predominant colonizers (∼99%) in the gastrointestinal tract of an organism and hence most of the currently used probiotics belong to prokaryotes. On contrary to prokaryotes, eukaryotes are not dominant and/or even part of the naturally occurring microbiota of gastrointestinal tract of many animals and till date only limited strains are available for human and animal uses. So far, tremendous efforts have been given on prokaryotic probiotics, whereas limited emphasis has been placed on eukaryotic probiotics. Both the groups differ greatly in their properties and modes of action and a comparative account of eukaryotic probiotic (yeast) and prokaryotic probiotics (bacteria) is given in Table 2.

It is already established that probiotics can exert beneficial metabolic and immunological effects in host. However, there are also chances of adverse metabolic and immunological responses like deconjugation and dehydroxylation of bile salts, transformation of conjugated primary bile salts into free secondary bile salts, and excessive degradation of host intestinal mucus layer due to manipulation of gut microbiota with the use of probiotics. Transformation of conjugated primary bile salts into toxic free secondary bile salts in the small bowel due to ingestion of fermented dairy products containing Lactobacillus acidophilus and Bifidobacterium species has been reported in healthy humans with a terminal ileostomy (Marteau et al. 1995). However, no such effects are observed for any of the eukaryotic probiotics.
Likewise, the transfer of antibiotic resistance gene which is now believed to be a major concern for bacterial probiotics is not reported for any of the eukaryotic probiotics (Czerucka et al. 2007). There exists the possibility of antibiotic resistant gene transfer from bacterial probiotics to pathogens in the gut of host. However, most of the probiotic yeasts lack such plasmid encoded genes (Kourelis et al. 2010a) and till date no such transfer of genetic material from yeast to bacteria is reported. Finally, the hallmark of probiotic yeasts is that they can be used during antibiotic treatment.

Table 2: Comparison between prokaryotic and eukaryotic probiotics with reference to yeast

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>Prokaryotic (bacteria)</th>
<th>Eukaryotic (yeast)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Size</td>
<td>Small (≈0.5 x 5 μm)</td>
<td>Large (≈10 x 5 μm)</td>
</tr>
<tr>
<td></td>
<td>Cell wall composition</td>
<td>Peptidoglycan, lipoteichoic acid, lipopolysaccharide</td>
<td>Chitin, glucan, mannose, phosphoheptidomannan, phospholipomannan</td>
</tr>
<tr>
<td></td>
<td>Optimum growth conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6.5–7.5</td>
<td>4.5–6.5</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>10–80°C</td>
<td>20–30°C</td>
</tr>
<tr>
<td></td>
<td>Tolerance to gastric acid</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Tolerance to bile salts</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Resistant to antibiotics</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Ability to transfer genetic materials</td>
<td>Yes Predominant (up to 99% of gut microbes)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Natural occurrence in gut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ability to colonize in the gut</td>
<td>High</td>
<td>Low to moderate</td>
</tr>
<tr>
<td></td>
<td>Synergistic effects on other microbes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Application as probiotics</td>
<td>Wide range of animals</td>
<td>Limited application</td>
</tr>
<tr>
<td>3</td>
<td>Effect on host</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Growth and nutrition</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Immunostimulation</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Protection</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Ability to produce antagonistic compounds</td>
<td>High</td>
<td>Low (occasionally)</td>
</tr>
<tr>
<td>5</td>
<td>Ability to neutralize enterotoxin</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Likewise, the transfer of antibiotic resistance gene which is now believed to be a major concern for bacterial probiotics is not reported for any of the eukaryotic probiotics (Czerucka et al. 2007). There exists the possibility of antibiotic resistant gene transfer from bacterial probiotics to pathogens in the gut of host. However, most of the probiotic yeasts lack such plasmid encoded genes (Kourelis et al. 2010a) and till date no such transfer of genetic material from yeast to bacteria is reported. Finally, the hallmark of probiotic yeasts is that they can be used during antibiotic treatment.
Further, probiotic strains of *S. cerevisiae* and *S. cerevisiae* var *boulardii* are sensitive to nonabsorbable antifungal agents such as nystatine but can be used with reabsorbable antimycotics such as fluconazole (Dixit and Gandhi 2010). On the other hand, bacterial probiotics cannot be used during antibiotics treatment.

### 3 Sources of Eukaryotic Probiotics

The isolation and characterization of suitable eukaryotic probiotics from natural sources warrants special considerations. Over the years, there is a great interest in developing suitable eukaryotic probiotics in general and probiotic yeasts in particular for human and animal practices. Yeasts may not be as ubiquitous as bacteria but they can thrive in diverse niches such as plants, animals, soil, water, and atmosphere. They are also associated with the skin and gastrointestinal tract of human and animals including aquatic animals (Suh et al. 2005; Gatesoupe 2007; Scanlan and Marchesi 2008; Urubschurov et al. 2008). Yeasts are predominantly associated with many food items especially dairy products (Jakobsen and Narvhus 1996; Fleet 2006; Chen et al. 2010). A scan of the literature suggests that dairy and dairy-related products could be a good source of many potential probiotics such as *Candida* (*C. humilis*), *Debaryomyces* (*D. hansenii, D. occidentalis*), *Kluyveromyces* (*K. lactis, K. lodderae, K. marxianus*), *Yarrowia* (*Y. lipolytica*), and several other species (Fleet 1990; Lopez-Daz et al. 1995; Kumura et al. 2004). Many times the gastrointestinal tract of human and animals is a good source of probiotic yeasts. Recently, Kourelis et al. (2010a, b) have succeeded in developing suitable probiotic strains belonging to *Saccharomyces* and *Kluyveromyces* species isolated from feta cheese and human gastrointestinal tract. Apart from these sources, several yeasts from marine sources such as *Yarrowia lipolytica* and *Candida tropicalis* are not only found to colonize in the gut of animals but also exert nutritional and other beneficial probiotic effects in host (Hirimuthugoda et al. 2007; Chi et al. 2010).

### 4 Taxonomic Characterization of Eukaryotic Probiotics

One of the most important aspects of assessing the efficacy of probiotics requires the understanding of individual strains, each of which is unique and different. Characterization of a strain is very important which in turn provides exact information on the nomenclature of the strain, its origin, and even presumed safety (Salminen et al. 2001). Detailed information on the taxonomic position of an organism is therefore very important in the selection process of probiotics. As per joint FAO/WHO guidelines (http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf), the presumptive probiotics should be identified up to genus and strain level by internationally accepted methods such as DNA–DNA
hybridization or sequencing of DNA encoding 16SrRNA and strain identification with pulsed field gel electrophoresis or randomly amplified polymorphic DNA.

Over the years, researchers have succeeded in identifying and characterizing several probiotic yeast strains from different sources by using molecular techniques (Posteraro et al. 2005; MacKenzie et al. 2008; Buchl et al. 2010; Chen et al. 2010). The ITS regions situated between the small and large subunits of rDNA, and NTS that separate the ribosomal gene clusters present high degrees of inter- and intraspecies variability (Baleiras Couto et al. 1996), in contrast to the rRNA genes, which are subject to greater evolutionary constraints and are thus more highly conserved (Molina et al. 1993). Biolog YT microplate and chromosomal PFGE karyotyping are often used for identification of yeasts (Baleiras Couto et al. 1996; Valente et al. 1996). As an alternative to sequencing, restriction fragment length polymorphism analysis of amplified rDNA regions has also been used. However, the sequencing of the D1/D2 fragment of the 26 S rRNA gene is suitable for identification of yeasts from different sources since strains of one yeast species differ in less than 1% of D2 nucleotide sequence (Peterson and Kurtzman 1991; Kurtzman and Robnett 1997, 1998). Similarly, the taxonomic characterization of Aspergillus species is very important due to their economic importance as both pathogenic and beneficial activities. Nowadays, several useful strains of Aspergillus species have been characterized by various molecular techniques (Klich and Mullaney 1987; Klich et al. 1995; Kumeda and Asao 2001; Montiel et al. 2003; Lee et al. 2006a).

5 Properties of Eukaryotic Probiotics

Eukaryotic microorganisms often exhibit potentially exploitable physiological and metabolic characteristics of an ideal probiotic. Several features which contribute to the success of eukaryotic microorganisms like yeasts as probiotics include their robust size, morphological diversity (budding, pseudomycelial), nutritional flexibility (ability to utilize a broad range of nitrogen, carbon, and phosphorous sources), stress tolerance ability (to low pH/oxygen/water activity, high osmotic pressure), enzyme secreting potency (secrete a broad range of enzymes such as lipase, peptidase, amylase, invertase, phytase, etc.), antioxidative/antitumor/antimicrobial activity (effective against a wide range of pathogens), and ability to produce several other useful metabolites. Furthermore, most of the presumptive probiotic yeasts are nonmycotoxic, nonallergenic, and nonpathogenic in nature (Fredlund et al. 2002).

All these characteristics not only contribute to their ability to resist the harsh milieu of the digestive tract of host but also are indispensable for their beneficial probiotic effects in host. However, the biological properties of various yeasts show considerable intraspecies variability and their beneficial properties are considered to be strain specific (Posteraro et al. 2005). S. cerevisiae var boulardii is genetically identical to S. cereviceae but often exhibits very distinct behavior particularly in relation to growth pattern, resistance to stress and temperature variations (Fietto et al. 2004; Nicoli and Castro 2004; Graff et al. 2008). S. cerevisiae var boulardii strains
are better tolerant to acidic stress and grow faster at 37°C than *S. cereviceae* (Fietto et al. 2004). Edwards-Ingram et al. (2007) also recorded similar type of viability of *S. boulardii* to that of the control at pH 2 while laboratory *S. cerevisiae* strains was less than 4% of the control at same pH.

Yeast can tolerate a wide range of temperature, salt concentration, and pH depending upon the strain. Temperature can affect the growth and metabolic activities of yeast. Generally, yeasts grow best at a temperature range from 20 to 30°C. The lower temperature limit is around 20°C while the higher limit for certain probiotic yeasts can be as high as 50°C. Koedrith et al. (2008) isolated a thermostolerant strain of *S. cereviceae* capable of growing rapidly at both high (40/41°C) and low temperatures from banana leaves. With regard to pH, most of the yeasts can grow very well in between the pH 4.5–6.5 but nearly all species can survive at low pH (up to 2.5). Similarly, the salt tolerance potency varies greatly among different probiotic yeasts. Probiotic strains of *S. cerevisiae* can resist up to 1.5 M NaCl while several other natural probiotic strains like *D. hansenii* which can resist NaCl up to 2.5 M with optimum growth at 0.5 M NaCl.

### 5.1 Ability to Tolerate Stress

Probiotics irrespective of origin are expected to undergo various stress conditions during industrial processing such as drying, heating, and freezing. Therefore, the stress tolerance nature of any probiotics is of paramount importance. Yeasts can survive the adverse environmental fluctuations by rapidly adopting their internal systems to meet challenges of new environment. However, it depends upon the strains, cell age, and type. The ability of any probiotics to tolerate heat stress is very crucial since they have to undergo the industrial processing. *S. cerevisiae* usually respond to heat stress in two phases. The initial phase of the response is the gaining of thermostolerance with accumulation of trehalose followed by the induction of heat shock proteins when it begins to grow at the increased temperature. Besides, antioxidants enzymes and plasma membrane ATPase are also involved in this process (Piper 1993). Trehalose protects the membranes and proteins from stress (Hounsa et al. 1998) and its accumulation/depletion is closely correlated with changes during the induction of thermostolerance in yeast (Hottiger et al. 1987, 1992; de Virgillio et al. 1994; Martins et al. 2008). Further, Hsp104 is believed to play a role not only in thermostolerance but also in other kinds of stress in *S. cerevisiae* (Sanchez et al. 1992; Holubarova et al. 2000). Li et al. (2006) observed that heat stress (39°C) rapidly lead to a notable increase in the binding of HSF to ScSSA1 and HSP104 genes with a peak after 10 min of heat stress followed by decline with continuous exposure to heat stress for up to 1 h.

Yeast also exhibit broad range of tolerance towards osmotic stress and studies indicate that KCl and sorbitol are less inhibitory to yeast growth than NaCl (Gaxiola et al. 1992). While KCl and sorbitol only cause osmotic stress, NaCl in addition to osmotic stress also contributes to Na⁺ toxicity (Serrano 1996).
Usually yeasts adopt several mechanisms to avoid depletion of water in the cell during osmotic stress that could lead to the cell shrinkage and eventually death. Glycerol plays a critical role in overcoming the stress to balance the osmotic pressure across the cell membrane and adjusting the external water pressure (Blomberg and Adler 1989, 1992; Blomberg 2000). Studies indicate that among the two genes that are responsible for the production of glycerol, namely glycerol-3-phosphate dehydrogenase (GPD1 and GPD2), only GPD1 is induced by stress in yeast (Eriksson et al. 1995). There is also an indication of accumulation of intracellular trehalose during osmotic stress (MacKenzie et al. 1988; Sharma 1997; Kofli et al. 2006). Further, increase of intracellular $K^+$ and decrease of intracellular $Na^+$ by overexpression of either HAL1 or HAL3 is crucial for NaCl tolerance in $S. \text{cerevisiae}$ (Gaxiola et al. 1992; Rios et al. 1997).

While thermo- and osmotolerance nature of probiotics is necessary to withstand the processing stress, their ability to tolerate low temperature is equally important for maintenance. The ability of probiotics to resist low temperature is also very crucial since decreased efficiency of several probiotics is often reported when maintained at low temperature for long duration. Yeasts cells show distinct resistance to freezing injury and the growth conditions of yeasts significantly modify the cellular responses to freezing injury (Morris et al. 1986, 1988). The cells in their early exponential phase or stationary phase of culture show more resistant to freezing injury (Morris et al. 1988; Pardo et al. 2009). Morris et al. (1988) observed a clear relationship between the morphology during freezing of $S. \text{cerevisiae}$ cells from the late exponential phase of culture and viability upon thawing. Recently, Pardo et al. (2009) also reported that $-20^\circ \text{C}$ maintained $S. \text{cerevisiae} \ var \ boulardii$ cells to show similar types of specific growth rate and biomass as that of normal cells with survival percentage of 0.31 and 11.5 when freezed the cells in their exponential phase and stationary phase of growth, respectively. Furthermore, they have found pretreatment of $S. \text{cerevisiae} \ var \ boulardii$ cells in media with water activity 0.98 can lead to a tenfold enhancement in survival at $-20^\circ \text{C}$ for 2 months.

However, the entire process of tolerance of yeasts to various stresses is very complex which need detail information from genome, proteome, and metabolome studies.

### 5.2 Ability to Survive in the Gastrointestinal Tract

Probiotics need to survive the inevitable biological barriers of gut (Saarela et al. 2009). It is apparent from the previous in vitro studies that eukaryotic probiotics can tolerate the simulated environmental conditions of the digestive tract. This fact is also supported by several in vivo studies where eukaryotic probiotics are not only found in viable conditions but also execute positive effects. In vitro studies indicate that yeasts belonging to $\text{Saccharomyces}$, $\text{Debaryomyces}$, and $\text{Kluyveromyces}$ species are extremely tolerant to bile salt (Kumura et al. 2004; van der Aa kuhle et al. 2005; Pardo et al. 2009; Chen et al. 2010; Kourelis et al. 2010a). van der Aa kuhle
et al. (2005) recorded that probiotic *S. cerevisiae* can withstand 0.3% Oxgall at a pH of 2.5 while certain strains of *S. cereviceae* and *K. lactis* can tolerate 0.15% bile salt at low pH of 3.0 (Kourelis et al. 2010a). The high tolerance potency of *Torulaspora delbrueckii*, *D. hansenii*, *K. lactis*, *K. marxianus*, and *K. lodderae* towards simulated gastrointestinal conditions, further accentuates their possible use as probiotics in near future (Kumura et al. 2004; Psani and Kotzekidou 2006).

In vivo studies also support this fact and probiotic strains of *S. cerevisiae*/*S. cerevisiae* var *boulardii* are not only able to survive in the digestive tract of host but also are found to persist at high populations in the gut of host. The pharmacokinetics study indicates that probiotic *S. cerevisiae* var *boulardii* when ingested orally achieved a steady state in the gut within 3 days but eliminated within 2–5 days after it is discontinued (Blehaut et al. 1989). Such elimination from the digestive tract is believed to be due to the barrier effect of the complex established resident microbiota of the gut (Fiems et al. 1993; Chaucheyras et al. 1998).

### 5.3 Ability to Adhere to the Gastrointestinal Tract

The necessity of probiotics adherence and subsequent colonization to the gut of host for exerting beneficial effects remains unsolved (Fedorak and Madsen 2004). However, adhering potency of probiotics to the intestinal tract of host is believed to be crucial in order to ensure their maintenance in the intestinal tract for a longer period of time (Ouwehand et al. 1999a). Probiotic strains of *Saccharomyces, Debaryomyces, Candida, Isaatchenkia*, and *Kluyveromyces* species possess noteworthy adhesive potency as evidenced from their in vitro adhesive capabilities to various cells (Kumura et al. 2004; Kourelis et al. 2010a). However, these yeasts differ greatly in the attachment with respect to sources, species, and strains. Ouwehand et al. (2000) reported that *S. cerevisiae* var *boulardii* from Precosa R exhibits good adhesive tendency which is congruent with their earlier study (Ouwehand et al. 1999b). Similar type of high adhesive potency of *S. cerevisiae* isolates from blue veined cheeses is also reported by van der Aakuhle et al. (2005). On contrary to these findings, several other strains of *S. cerevisiae* var *boulardii* are found to possess poor to low in vitro adherence potency to various cells such as Vero cells (monkey kidney cells), Caco-2 (Tasteyre et al. 2002; Kumura et al. 2004). In a comparative study, Kumura et al. (2004) recorded best adhesive potency of *K. lactis* to human enterocyte-like Caco-2 cells followed by *K. marxianus*, *K. lodderae*, and *D. hansenii* while *C. humilis*, *D. occidentalis*, *S. cerevisiae*, and *Y. lipolytica* strains exhibited least adhesive potency.

### 6 Beneficial Effects of Eukaryotic Probiotics

Probiotics are now becoming a popular and important tool in the health management strategy of human and animals. Eukaryotic probiotics, when ingested orally as food/feed supplements exert several types of nutritional benefits in hosts. Some of
the nutritional and growth benefits which are mainly documented in animals are as follows.

### 6.1 Nutritional Benefits

Probiotics have profound effect on nutrition of host and can influence on various digestive processes, especially cellulolysis and synthesis of microbial protein and increase in the absorption of nutrients. *S. cerevisiae* is considered as one of the probiotics that, when administered through the digestive tract, have a positive impact on the hosts health (Patterson and Burkholder 2003). In various animals, *S. cerevisiae* supplementation often lead to enhanced body weight gain with increase in feed intake (Churchil and Mohan 2000; Sharma et al. 2001; Kim et al. 2002; Nilson et al. 2004; Kabir 2009; Shareef and Al-Dabbagh 2009). Apart from *S. cerevisiae*, *A. oryzae* is also very useful to animals from nutritional point of view (Lee et al. 2006a, b, c). *A. oryzae* often secretes various digestive (amylolytic and proteolytic) enzymes which are indispensable to the host nutrition. Dietary supplementation of *A. oryzae* can significantly enhance the weight gain (Wallentine et al. 1986) and the dry matter digestibility in cows (Wiedmeier et al. 1987; Gomez-Alarcon et al. 1990). In broilers, *A. oryzae* is not only reported to enhance the feed intake and weight gain but also reduce the ammonia and serum cholesterol level (Kim et al. 2003). Similarly, another probiotic belonging to *Aspergillus* species, *A. niger* is also reported to improve the blood quality with low cholesterol and glucose level in broilers (Al-Kassie et al. 2008).

Probiotics can influence the production as well as the quality of milk. Yeasts either live or fermented have been used in dairy cattle for more than 60 years (Schingoethe et al. 2004). Eukaryotic probiotics help in improving the dry matter intake (Williams et al. 1991; Wohlt et al. 1991, 1998; Yu et al. 1997; Dann et al. 2000), the percentage of milk fat, Solids-Not-Fat, protein (Putman et al. 1997; Wang et al. 2001a), and production level (Harris and Lobo 1988; Arambel and Kent 1990; Piva et al. 1993; Robinson and Garrett 1999; Masek et al. 2008). In dairy cows, supplementation of *S. cerevisiae* can increase the production of acetate, propionate, and total volatile fatty acids (Nisbet and Martin 1991; Piva et al. 1993; Miller-Webster et al. 2002). Likewise, other eukaryotic probiotic like *A. oryzae* is also found to enhance the milk yields (Marcus et al. 1986; Wallentine et al. 1986; Kellems et al. 1987).

Probiotics can influence the egg quality and production percentage and inclusion of live yeasts into laying hen diets significantly improve the egg production percentage (Kim et al. 2002; Shivani et al. 2003), egg weight/mass (Han et al. 1999; Park et al. 2001, 2002; Omar 2006), egg shell breaking strength (Park et al. 2002), as well as reduce the percentage of soft or broken eggs (Park et al. 2001). On the other hand, Dizaji and Pirmohammadi (2009) could not observe any substantial influence of dietary supplementation of eukaryotic-based probiotics “Biosaf SC 47” (*S. cerevisiae*, strain NCYC SC 47) on the overall egg performance of laying hens. Earlier, Yousefi and Karkoodi (2007) also failed to record any significant effect on
egg mass, weight, and production, except improvement in yolk weight and Haugh unit of egg by supplementing *S. cerevisiae* diets of laying hens.

The involvement of probiotics especially prokaryotic in improving the overall meat quality has been questioned due to several contradictory findings. However, not much work has been done on these aspects of eukaryotic probiotics. Preliminary reports indicate that whole *S. cerevisiae* as well as its extract can improve the meat tenderness and oxidative stability of broiler meat. It is assumed that certain antioxidant factors present in *S. cerevisiae* are involved in shifting the oxidative factor of fatty acid profile in the meat (Zhang et al. 2005).

### 6.2 Disease Protection

Over the years, several studies have been conducted to establish the disease protecting ability of yeasts. The therapeutic potency of yeasts especially *Saccharomyces* species and their mechanisms, pharmacokinetics, and pharmacodynamics is well documented in animal models as well as in clinical trials (Rodrigues et al. 1996; Periti and Tonelli 2001; Girard et al. 2003; Dalmasso et al. 2006; Dixit and Gandhi 2010). Out of 16 species of *Saccharomyces*, specific strains of *S. cerevisiae / S. cerevisiae* var *boulardii* are only used as biotherapeutic agents. Their biotherapeutic effects especially in the treatment of general digestive problems, diarrhea, amebiasis, irritable bowel syndrome, inflammatory bowel syndrome, bacterial overgrowth in short bowel syndrome, Crohn’s disease, ulcerative colitis, and lyme disease are noteworthy (McFarland et al. 1994, 1995; WHO 1995; Bleichner et al. 1997; Guslandi et al. 2000). Probiotic strains of *S. cerevisiae* and *S. cerevisiae* var *boulardii* are extremely effective in treatment and prevention of various types of diarrhea, including infectious types such as acute diarrhea in children, diarrhea caused by pathogens in adults, traveler’s diarrhea, AIDS-associated diarrhea, antibiotic-associated diarrhea, diarrhea in ill tube-fed patients, and *Clostridium difficile*-associated diarrhea (Surawicz et al. 1989a, b; Cetina and Sierra 1994; Bleichner et al. 1997; Aloysins et al. 2005; Martins et al. 2005; Czerucka et al. 2007).

Furthermore, *S. cerevisiae* var *boulardii* is also effective for lactose intolerance, urinary tract infections, vaginal yeast infections, high cholesterol levels, hives, fever blisters, canker sores, and teen-age acne. Besides, their usefulness against food allergies, yeast (candida) infection (Murzyn et al. 2010), and parasitic infestation and in reestablishing the normal gut functions after long-term antibiotic therapy in hosts (McFarland et al. 1994) are also recorded. In a recent study, Murzyn et al. (2010) found that *S. cerevisiae* var *boulardii* as well as its extract can inhibit the expression of various genes associated with the virulence of *C. albicans*. They have established the involvement of capric acid as an active compound responsible for preventing the growth, hyphae formation, partly adhesion, and biofilm formation of *C. albicans*. Nonetheless, strains of *S. cerevisiae* are also effective in preventing the adhesion of *Entamoeba histolytica* trophozoites (Elliot et al. 1991; Rigothier et al. 1994).
Further, *S. cerevisiae* can reduce the *C. albicans*, *Candida krusei*, and *Candida pseudotropicalis* in the digestive tract of normal and antibiotic treated rats/mice (Seguela et al. 1978; Ducluzeau and Bensaada 1982) as well as other pathogens such as *Salmonella typhimurium* and *Shigella flexneri* (Rodrigues et al. 1996). *S. cerevisiae* var *boulardii* is also reported to decrease the inflammatory reaction and colonization of mouse intestine by the *C. albicans* infection (Jawhara and Poulain 2007).

### 7 Modes of Action of Eukaryotic Probiotics

Probiotics often exert beneficial effects through a variety of disparate and overlapping mechanisms. Eukaryotic probiotics adopt several mechanisms like trophic effect on gut for maintaining the intestinal homeostasis, stimulating effect on both local and systematic immunity, antimicrobial and toxin neutralizing activities.

#### 7.1 Trophic Effects on the Gastrointestinal Tract

Eukaryotic probiotics especially yeasts exert trophic effects on gut to restore its homeostasis. Although the precise mechanisms by which yeasts exert various trophic effects are yet to be established, Buts (2009) summarized the possible mechanisms of trophic effects of *S. boulardii* in human and animals. It is well known that probiotics often improve enzymatic activity of the gut by producing several enzymes which are not produced by host to break down complex macromolecules. In human beings, *S. cerevisiae*/*S. cerevisiae* var *boulardii* can increase the lactase, glycosidase, and alkaline phosphatase activities both at the basal and apical parts of villi (Jahn et al. 1996). Oral ingestion of *S. cerevisiae* can also lead to marked increase in the specific and total activities of brush border membrane disaccharidases including sucrase, lactase, isomaltase, and maltase in human and animals (Buts et al. 1986). Therefore, *S. cerevisiae* can help to improve malabsorption in patients with sucrase-isomaltase deficiency that intentionally or unintentionally consumes sucrose and also improves some diarrheas that are associated with a decrease of the intestinal disaccharidase activities. Further, the involvement of polyamines (spermine and spermidine), present in *S. cerevisiae*/*S. cerevisiae* var *boulardii* in trophic effect on the intestinal mucosa could not be ruled out (Balasundram et al. 1994; Buts et al. 1994).

#### 7.2 Stimulation of Immunity

Eukaryotic probiotics have also been shown to stimulate both innate and adaptive immunity which in turn may contribute to good health and disease resistance of
host. Eukaryotic probiotics unlike bacterial probiotics can stimulate the immune system of diversified hosts ranging from mammalian to piscine. Yeasts are rich source of many biologically active substances which can potentially trigger various biological systems including the immune system of host (Buts et al. 1990; Rodrigues et al. 2000; Martins et al. 2007, 2009). The cell wall components of yeast are predominantly composed of complex polymers of $\beta$-glucans, $\alpha$-mannans, mannoproteins, and a minor component of chitin (Smits et al. 1999). These macromolecules can eventually stimulate the immune system of host especially inflammatory response and reticuloendothelial system. Recently, Pothoulakis (2009) has elaborately discussed the anti-inflammatory mechanism of $S. \textit{cerevisiae}$ var $boulardii$.

$S. \textit{cerevisiae}$ strains are found to be better stimulator of immune system. In a comparative study, Martins et al. (2009) reported better immunostimulating activity (in terms of sIg A and IL10 production) of $S. \textit{cerevisiae}$ var $boulardii$ as compared to bacterial probiotics such as $\textit{Bifidobacterium animalis}$, $\textit{E. coli}$, and $\textit{Lactobacillus casei}$. Probiotic strains of $S. \textit{cerevisiae}$ var $boulardii$ have been shown to enhance the phagocytic activity as well as production of different cytokines (Cuaron 1999; Rodrigues et al. 2000; Czerucka et al. 2007). Kourelis et al. (2010b) found the ability of presumptive probiotic yeast strains belonging to $S. \textit{cerevisiae}$ and $K. \textit{lactis}$ species to increase polymorphonuclear cell influx, phagocytic activity, and various cytokines in an air pouch model. However, many times, elevation of immune responses is often strain specific. Recently, Kourelis et al. (2010b) have reported significant variation in the ability of various probiotics strains in inducing the increased production of TNF-$\alpha$, IFN-$\gamma$, and IL-10. However, strains like $K. \textit{lactis}$ and $K. \textit{loddrae}$ showed no significant effect on the secretion of proinflammatory cytokine, IL 8 by Caco-2 cells (Kumura et al. 2004).

$\textit{Saccharomyces}$ species are found to stimulate sIgA level in different animal models. In gnotobiotic mice, it triggered the phagocytic system along with enhancement of sIg A (Rodrigues et al. 2000) while Buts et al. (1990) found significant increase in sIgA and secretory component of immunoglobulins in rats by oral administration of $S. \textit{cerevisiae}$.

### 7.3 Synergistic Activity

Eukaryotic probiotics can exert synergistic effects on the indigenous gut microbiota of host. Supplementation of probiotic yeasts often favors the growth of certain bacteria (Newbold 1996; Chaucheyras et al. 1996, 1997), fungi, and even protozoa (Chaucheyras et al. 1995; Miranda et al. 1996). $\textit{Saccharomyces}$ species have been found to stimulate the growth of other microorganisms by providing essential metabolites such as pyruvate, amino acids, and vitamins (Jespersen 2003). The redox potential in the rumen which is mainly increased due to the infusion of oxygen during intake of feed and water can have detrimental effect on the anaerobes in the rumen. Yeasts can remove oxygen from the rumen and help to increase microbial population. This is further confirmed from the fact that respiration-deficient mutants of $S. \textit{cerevisiae}$ lack the ability to enhance
microbes (Newbold et al. 1996). Recently, Hassanein and Soliman (2010) also recorded such increase in the lactobacilli population along with decrease of many pathogens in the gut of layers fed with probiotic *S. cerevisiae*.

### 7.4 Antagonistic Activity

Bacterial probiotics inhibit pathogens by competing for nutrients, adhesion sites, and/or by producing several antimicrobial compounds/metabolites. However, the situation may be little different for eukaryotic probiotics. There is a general consensus that eukaryotic probiotics lack the ability to produce any antimicrobial metabolites and/or inhibit pathogens (Martins et al. 2009; Kourelis et al. 2010a) but several reports indicate the successful inhibition/killing of various pathogens such as *Clostridium albicans*, *E. coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae*, *Salmonella enteritidis*, and *Clostridium difficile* by eukaryotic probiotics (Izadnia et al. 1998; Filho-Lima et al. 2000; Czerucka and Rampal 2002).

Pathogens usually adhere to the epithelium for establishing lethal infection. But eukaryotic probiotics can prevent the pathogens to bind to intestinal cells by direct antagonistic effect and/or secreting several metabolites/enzymes. Further, *S. cerevisiae* var *boulardii* strains can compete with pathogenic microorganisms for food and mucosal receptors in the gut and thereby prevent the pathogens to colonize in the gut of host (Filho-Lima et al. 2000). Similarly, certain pathogens like *E. coli* or *Salmonella* which posses such mannose-specific type-1 fimbriae bind to mannose residues on epithelial cell membranes (Ofek et al. 1977). Yeasts like *S. cerevisiae* contain mannan in its outer layer of their cell wall and therefore can induce a protective effect against these pathogens. Such pathogens readily bind to the mannans present on the surface of yeast instead of attaching to intestinal epithelial cells (Gedek 1999; Spring et al. 2000) and then eliminated from the digestive tract of host after agglutinated by yeast.

### 7.5 Toxin Neutralization Activity

Lethal toxins produced by pathogens bind to specific receptors on intestinal epithelial cells and then cause mucosal damage and inflammation. Eukaryotic probiotics often destroy bacterial toxins and their receptor sides by releasing various enzymes like proteases (Castagliulo et al. 1999). In vitro studies indicate the possible involvement of protease present in both whole yeast and cell wall fraction of *S. cerevisiae* var *boulardii* in inhibiting the adhesion of *C. difficile* to Vero cells despite lacking the adhesion ability to bacteria or Vero cells (Tasteyre et al. 2002).

Yeasts not only can inhibit the production of toxins of several pathogens such as *C. difficile*, *V. cholerae*, and *E. coli* but also prevent their adverse effects (Massot et al. 1982; Corthier et al. 1986; Vidon et al. 1986). A serine protease from
*S. cerevisiae* effectively hydrolyzes the toxin A, one of the two potent toxins (A and B) produced by *C. difficile* (Pothoulakis and Lamont 2001). Similarly, *S. cerevisiae/S. cerevisiae var boulardii* are reported to reduce the liquid secretion and mannitol permeability caused by *C. difficile* toxin A in the rat ileum (Pothoulakis et al. 1993). A 54-kDa serine protease of *S. cerevisiae* can reduce the toxin A-induced rat ileal secretion and prevent the toxin A-mediated inflammation and villus damage by inhibiting toxin A binding to its brush border glycoprotein receptor (Pothoulakis et al. 1993; Castagliuolo et al. 1996). Nevertheless, another 120-kDa protein of *S. cerevisiae var boulardii* is responsible for preventing the cholera toxin action and is found to reduce the cholera toxin-mediated stimulation of cyclic adenosine monophosphate in mouse intestinal loops (Brandao et al. 1998; Czerucka et al. 2000; Czerucka and Rampal 1999; Neves et al. 2002).

### 8 Safety Issues Related to Eukaryotic Probiotics

Probiotics are usually safe beyond any doubt but sometimes can cause complications and side effects in susceptible individuals. Most of yeasts used as probiotic are safe and also possess Qualified Presumption of Safety Status, assigned by the European Food Safety Authority (http://www.efsa.europa.eu) and are rarely associated with outbreaks or cases of food-borne illness. Although, *S. cerevisiae* and *S. cerevisiae var boulardii* are routinely used in human and animal practices for several decades, few cases of infection are reported in patients. *S. cerevisiae/S. cerevisiae var boulardii* induced fungemia has been reported in humans using biotherapeutic products containing *S. cerevisiae var boulardii* (Zunic et al. 1991; Pletincx et al. 1995; Fredenucci et al. 1998; Niault et al. 1999; Cesaro et al. 2000; Hennquin et al. 2000; Rijnders et al. 2000; Cassone et al. 2003; Riquelme et al. 2003). However, such cases are only restricted to immunocompromised patients and/or patients being contaminated through a central venous catheter, and therefore raise some concerns over the use of live yeasts especially in the debilitated and immunosuppressed patients.

Animal model studies also indicate the low to moderate virulence nature of *S. cerevisiae/S. cerevisiae var boulardii* (McCullough et al. 1998). They can penetrate the intestinal mucosa of animals to reach other organs (Cartwright-Shamoon et al. 1996) as evident from the translocation of *S. cerevisiae* to mesenteric lymph nodes in immunosuppressed mice.

### 9 Genetic Manipulation of Eukaryotic Probiotics

Nowadays, much emphasis has been given on the genetic improvement of probiotics for developing suitable candidate with desirable characteristics (Steidler 2003). The genetic improvement of *Saccharomyces* and other yeasts has traditionally been relied
on random mutagenesis (Wang et al. 2001b) but now protoplast fusion technique is adopted to generate fusants with desired characteristics (Martins et al. 2004). Several researchers have also succeeded in developing highly efficient biotherapeutic strains of *S. boulardii* which can resist low pH degree, high bile salts, possess high vitamin content, and exhibit antagonistic effect on several pathogens through genetic improvement (using mutation/interspecific protoplast fusion) (Nivien et al. 2006; Abosereh et al. 2007; Pasha et al. 2007; Sharaf et al. 2009). However, there are several issues associated with such manipulations and detail information on various aspects of the genetic improvement of yeast/fungi needs to be addressed before its practical application in the field of probiotics. Furthermore, issues like safety, effectiveness, impact on environment associated with such manipulations warrants further elaboration but it is beyond the scope of this review.

## 10 Conclusion

Although the epicenter of probiotic research has been prokaryotes, scientific and commercial interest in eukaryotic probiotics has significantly increased in recent years. There is no doubt that eukaryotic probiotics can execute a myriad of beneficial effects in a broad range of hosts. However, there are identifiable limitations to the use of eukaryotic probiotics and more in-depth studies are needed to establish scientific rationale of eukaryotic probiotics. There is a dearth of information on the diversity and function of the eukaryotic microbiota in the gut of human and other animals. Additionally, adequate knowledge on the individual strain, its source, safety, and host ranges is required for the firm establishment of eukaryotes as probiotics. More comprehensive ecological surveys are needed to determine the diversity of probiotic fungi/yeasts in nature other than *Saccharomyces* species. Successful application of modern technologies will certainly allow in developing tools for analyzing the functionality of these strains as probiotics. Integrating metagenomic and metaproteomic approaches will certainly help to develop the new generation of eukaryotic probiotics.

## References


Cetina SG, Sierra BG (1994) Evaluation thérapeutique de Saccharomyces boulardii chez des enfants souffrants de diarrhée aigue. Annales de Pediatrie (Paris) 41:397–400


Gedek BR (1999) Adherence of *Escherichia coli* serogroup O 157 and the *Salmonella typhimurium* mutant DT 104 to the surface of *Saccharomyces boulardii*. Mycoses 42:261–264


Klich MA, Mullaney EJ (1987) DNA restriction enzyme fragment polymorphism as a tool for rapid differentiation of *Aspergillus flavus* from *Aspergillus oryzae*. Exp Mycol 1:170–175


Martins FS, Nardi RMD, Arantes RME, Rosa CA, Neves M, Nicoli JR (2005) Screening of yeasts as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice. J Gen Appl Microbiol 51:83–92

Biology of Eukaryotic Probiotics 51
Nilson A, Peralta JMF, Miazzo RD (2004) Use of brewer’s yeast (S. cerevisiae) to replace part of the vitamin mineral premix in finisher broiler diets. XXII Worlds Poultry Congress, Istanbul, Turkey
Omar AHM (2006) Nutritional studies on using some feed additives in poultry ration. M. Sc Thesis, Department of Animal Production, Faculty of Agriculture, Minia University, Minia Egypt


Probiotics
Biology, Genetics and Health Aspects
Liong, M.-T. (Ed.)
2011, X, 330 p., Hardcover
ISBN: 978-3-642-20837-9