Animal Models Used for Studying the Benefits of Probiotics in Metabolic Disorders

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Abstract

The GIT flora is disturbed due to various reasons such as metabolic disorders, immunosuppressive therapy, administration of antibiotics, radiations, etc. The introduction of beneficial bacterial species can therefore be an attractive choice for restoring microbial equilibrium in GIT and preventing disease. Probiotics are live microorganisms which appear to provide medical benefits when ingested usually by enhancing the intestinal microbiota. In literature, various studies have indicated the benefits of probiotics in metabolic disorders. Thus, in the first part of chapter, we will summarize available animal models to study benefits of probiotics in metabolic disorders. The advantages and limitations of individual animal model will be discussed in second part. Finally, this chapter will highlight current challenges and future perspectives.

Keywords

Animal models · Probiotics · Metabolic disorders · Intestinal microbiota · Microbial equilibrium

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cell
BBDP rat	BioBreeding diabetes-prone rat
BW	Body weight
FAO	Food and Agriculture Organization
GM	Genetically modified
HBA1c	Hemoglobin A1c
HFD	High-fat diet
HFSD	High-fat-sugar diet
HIV	Human immunodeficiency virus
HSD	High sucrose diet
IDDM	Insulin-dependent diabetes mellitus
IL-6	Interleukin-6
IM	Intramuscular
IP	Intraperitoneal
IV	Intravenous
MSG	monosodium glutamate
NIDDM	non-insulin-dependent diabetes mellitus
NOD mice	Non-obese mice for type 1 diabetes
ob gene	Obese gene
SC	Subcutaneous
STZ	Streptozotocin
TNFα	Tumor necrosis factor
WHO	World Health Organization

13.1 Introduction

According to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), probiotics are "live microorganisms which, when administered in adequate amount, confer a health benefit on the host" (Kumar et al. 2015). Probiotics help to maintain the body health in combination with the gut microflora. It has been reported that gut microflora is an important determinant of metabolic diseases like diabetes and obesity. The obesogenic diet can bring about a change in the bacterial population in intestine which may further lead to metabolic disorders. Thus, to prevent such deleterious effects, probiotics can be used to reshape the microflora in intestine and improve gut health. However, before administration of probiotics in human beings, there is need to evaluate their efficacy in metabolic disorders in animal models to understand their stability, safety, and mechanism.

Animal models are living, non-human organisms which are used scientifically to investigate the biological phenomenon and physiological function between the animal model and target species. Animal models are most widely used method to determine the pharmacological and toxicological profile of a drug before administration in human. Mice and rats are the most commonly used animals used to check the efficacy of probiotics in metabolic disorders. The animal model should mimic the anatomy, physiology, and pathogenesis so as to extrapolate the data for human. Thus, a sound knowledge of genetics, anatomy, and physiology will be of great help in choosing an animal model. Phylogenetic closeness, however, cannot guarantee the similar results, for example, chimpanzee does not acquire human immunodeficiency virus (HIV), thus cannot be used to study acquired immunodeficiency syndrome (AIDS) (King 1986). New animal model are constantly being developed to understand the mechanism of action, pharmacokinetics, metabolic diseases, diagnosis, therapeutic procedures, safety, and efficacy of chemical substances for human use. Different types of animal models can be used, the efficacy of probiotics is as follows:

1. In induced models, the disease condition to be investigated in induced using biological, chemical, and physical methods in healthy animals. However, there is difference between the etiology and categorization of disease in induced animal models (Hau 2008).
2. Spontaneous models are the animals that develop the disease under natural conditions. The manifestations of the disease in such models are similar to those of target species. It is a common practice to compare the disease and response between the animal model and the target species. For example, athymic nude mice (Pantelouris 1968).
3. Genetically modified (GM) animal models are developed by alteration in the genome to produce a desired disease. GM are further of two types, namely transgenic animals in which DNA is inserted into genome and knock-outs in which a specific gene is removed from the genome to produce a particular genotype. However, the development of GM animal models may lead to unpredictable and undesired results.
4. Negative models designed in such a manner that a specific disease does not develop but the animals exhibit absence of response to certain stimuli. These models are commonly used to understand the physiology and mechanism of resistance to a disease.
5. In orphan animal model, the disease occurs naturally which is not yet described in human, e.g., bovine spongiform encephalopathy in cows. These are useful to investigate similar diseases found in target species.

Various factors such as breed, species, strain, genotypes, etc. are known to affect the suitability and selection of particular model. During the investigation of the efficacy of probiotics in metabolic disorders, the microbial factor plays an important role as they may alter the outcome and inferences from the animal model. Infections in experimental animals may be attributed to various microbes such as bacteria,

virus, fungi, etc. Thus, it should be taken in account that the selected model is devoid of undesired microbial species in order to get accurate, valid, and reproducible results. However, there may be variations due to chemical substance, genotype, environmental conditions, or microbial flora, thus the efficacy of probiotics should be investigated carefully in animal models.

In the current book chapter, various animal models used to check the efficacy of probiotics in metabolic disorders such as diabetes and obesity are summarized. Further, the advantages and limitations of each model are also discussed. The chapter concludes with the current challenges and future perspectives.

13.1.1 Diabetes

Diabetes is a metabolic disorder characterized by high blood sugar level. Diabetes mainly develops due to inability of beta cell to produce sufficient insulin and insulin resistance, which leads to decreased consumption of glucose by tissues, thus raising blood glucose level. Thus, animal models used for diabetes tend to have beta cell failure and/or insulin resistance. Some of the frequently employed animal models to test the efficacy of probiotics for diabetes are outlined below:

13.1.1.1 High-Fat Diet Induced Diabetes

High-fat diet (HFD) fed model is the commonly used animal model to induce type 2 diabetes. This model was first introduced by Surwit et al. (1988). As compared to other strains, HFD fed model is found most effective model in C57BL/6J mice. HFD consists of 35.8% fat, 20.5% protein, 3.6% ash, 0.4% fiber, 36.8% carbohydrate, and 3.1% moisture administered for 1 week. HFD leads to increase in weight, stable hyperglycemia, followed by hyperinsulinemia (Winzell and Ahren 2004), indicating the continuous worsening of insulin resistance (Fig. 13.1). Moreover, after 1 week of HFD administration, there is elevation of baseline glucose and insulin, impaired insulin secretion, and reduced glucose elimination. Thus, the characteristic feature of type 2 diabetes, i.e., beta cell dysfunction and insulin resistance are induced in this mode.

The diabetes and obesity in human are induced majorly due to environmental manipulations, thus this model is of advantage as it mimics the human situation more

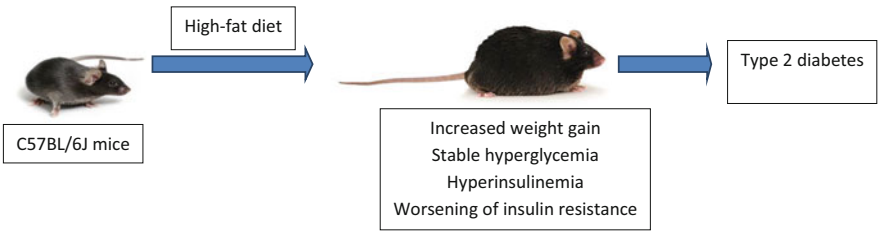

Fig. 13.1 Representation of type 2 diabetes induced by high-fat diet

accurately. However, the percentage of diet in the given diet exceeds the common dietary intake among the developed nations (Harika et al. 2013). Moreover, there may be some differences in the studies on HFD-induced diabetes model due to difference in age, strain and gender of mice, diet composition, fat content, and duration of feeding (King and Bowe 2016).

Yadav et al. (2013) used the HFD-induced type 2 diabetes model to show the antidiabetic activity of probiotic, De Simone Formulation. Also, it was shown that Lactobacillus rhamnosus enhances adiponectin and improved sensitivity to insulin in HFD fed mice (Kim et al. 2013).

13.1.1.2 High-Fat Diet and Streptozotocin Induced Type 2 Diabetes

Among the available models for type 2 diabetes, the streptozotocin (STZ) combined with HFD has been widely used by researchers to investigate the antidiabetic activity of chemical molecules. Also, this model has been employed to check the efficacy of probiotics against type 2 diabetes by researchers (Chen et al. 2018; Yan et al. 2019). In this model, rats are fed with HFD consisting of 48% carbohydrate, 22% fat, and 20% protein with a calorific value of 44.3 kJ/kg for a period of 4 weeks followed by IP administration of STZ (25–30 mg/kg) (Zhang et al. 2008) (Fig. 13.2). HFD helps to initiate the insulin resistance, an important feature of type 2 diabetes. Following insulin resistance by HFD, STZ causes alkylation of DNA, causing β cell death, thus inducing insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). In this model, a lower dose of STZ is used to induce mild insulin secretion impairment as its high dose impairs insulin secretion, thus mimicking type 1 diabetes.

HFD in combination with ST induced type 2 diabetes animal model is cheap, practical, and easily accessible for the investigation of antidiabetic property of probiotics. Moreover, stable hyperglycemia is achieved using this model. Further, this model can be customized as per need to resemble the slow pathogenesis of type 2 diabetes which cannot be found in most humans (Fang et al. 2019).

The major disadvantage of HFD in combination with STZ induced type 2 diabetes is that it is time-consuming, thus increases the cost of overall investigational protocol (Srinivasan et al. 2005). Moreover, the difference in fatty acid compositions in HFD may lead to considerable difference in the results and outcomes, affecting the

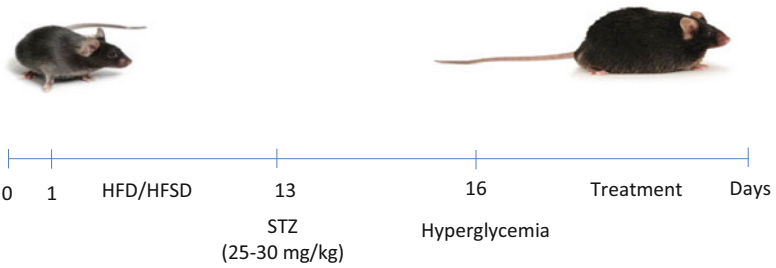

Fig. 13.2 HFD/HFSD and STZ induced diabetes

reproducibility of the result (Buettner et al. 2007). Further, the effects of HFD are difficult to prevent or reverse, thus the effect of antidiabetic drugs on obesity cannot be studied using this model, hence, model where low percentage of fat is administered might be useful to design such studies (Gheibi et al. 2017). Despite the mentioned limitations, HFD/STZ is a practical and reasonable animal model for type 2 diabetes to represent the later stage of ailment.

Chen et al. (2018) described the beneficial effects of *Lactobacillus* in HFD/STZ induced diabetic mice.

13.1.1.3 High-Fat-Sugar Diet and Low Dose of Streptozotocin Induced Type 2 Diabetes

In this model, high-fat-sugar diet (HFSD) consisting of 20% sucrose, 5% milk powder, 12% lard oil, 2% egg, and 61% normal fodder is administered orally for 6 weeks followed by once intramuscular (IM) or intraperitoneal (IP) injection of STZ (30–35 mg/kg) (Zhuo et al. 2018) (Fig. 13.2). The excess accumulation lipids due to HFSD trigger the mitogen-activated protein kinase (MAPK) pathway (Savary et al. 2012), which in turn increases the secretion of inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNFα). These further attack islets cells of pancreas and interfere with the insulin signaling mechanism and hence lessen glucose uptake efficiency. Also, as discussed earlier, the low dose of STZ helps to induce destruction of small part of beta cell, reducing the production of insulin rather than the complete destruction.

The main advantage of HFSD/STZ induced type 2 diabetes is that it helps in achieving the characteristic pathogenesis of type 2 diabetes. It brings out dysfunction of and insulin resistance without the genetic manipulation or multiple congenic breeding techniques which may lead to undesired results like β cell driven metabolic disorders or massive obesity. Thus, the development of this model is cheap, easy to breed, and widely available (Barrière et al. 2018).

Manaer et al. (2015) and Dang et al. (2018) reported the antidiabetic effect of shubat, a probiotic and *L. paracasei*, respectively, in high-glucose-fat induced type 2 diabetes.

13.1.1.4 Alloxan Induced Diabetes

Use of alloxan to induce insulin-dependent diabetes mellitus is very well documented (Dunn and McLetchie 1943; Gomori and Goldner 1945) for a variety of species like mice, rabbits, monkeys, dogs, and cats (Goldner and Gomori 1944; Cruz Jr et al. 1961). Alloxan can be administered through different routes such as intravenous (IV), subcutaneous (SC), and IP either in single dose or multiple doses. The species of animals, their status of nutrition, and route of administration also play a significant role in the determination of the dose of alloxan to induce diabetes (Federiuk et al. 2004). However, single IP dose of 170–200 mg/kg of body weight (BW) of alloxan is most preferably used and is effective to induce diabetes. The administration of alloxan causes blockage of secretion of insulin stimulated by glucose and leads to reactive oxygen species formation, thus promoting selective necrosis of pancreatic β cells (Fig. 13.3).

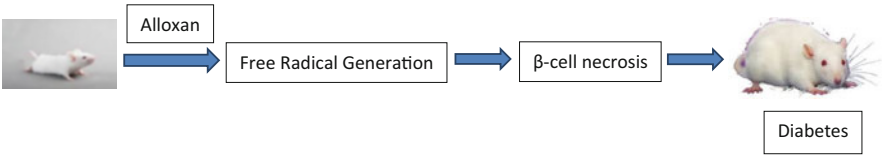

Fig. 13.3 Alloxan induced diabetes

There are some disadvantages of alloxan-induced diabetes like auto-reversal of hyperglycemia induced by alloxan and poor diabetogenicity after IP administration of 150 mg/kg and below. Moreover, very young animals are less susceptible and offer high resistance to the diabetogenic effects of alloxan, thus other animals should be used to induce diabetes using alloxan (Ighodaro et al. 2017).

Al-Salami et al. (2008) investigated the antidiabetic activity of probiotics in alloxan-induced diabetic animal model. They reported that probiotics have no effect on blood glucose level in healthy animals, however, it significantly decreases the blood glucose level in diabetic animals.

13.1.1.5 Db/db Mouse

Db/db mouse are genetically modified experimental animals for diabetes. They express autosomal recessive mutations in leptin receptor leading to obesity (Bogdanov et al. 2014), decreased insulin receptor sensitivity, decreased β cell function, and elevated Hemoglobin A1c (HBA1c) levels. This leads to the progressive development of hyperglycemia with age which provides clinical relevance. At 6 weeks of age, db/db mouse have near normal or slightly increased fasting plasma glucose level and comparatively normal β cell function. The level of fasting plasma glucose elevates gradually over several weeks. After 16 weeks of age, there is entire degeneration of β cell function and fasting plasma glucose becomes very high, i.e., >400 mg/dL. (Fajardo et al. 2014).

However, genetically modified db/db mouse are homogenous and development of hyperglycemia is genetically determined which differs from heterogeneity observed in humans. Moreover, db/db mouse have limited availability, are expensive, and require high maintenance (Srinivasan and Ramarao 2007).

Yun et al. (2009) demonstrated that *Lact. gasseri* BNR17 (derived from human breast milk) decreased blood glucose level and ameliorated the symptoms associated with diabetes after oral feeding in db/db mice. Wang et al. (2020) explored the antidiabetic mechanism of 14 probiotics in db/db mouse. They found that probiotics remarkably enhanced blood lipid and blood glucose levels. Furthermore, they improved morphological changes in liver, kidney, and pancreas and protected pancreas from apoptosis.

13.1.1.6 Non-obese Mice for Type 1 Diabetes (NOD Mice)

Type 1 diabetes is an autoimmune disease caused by infiltration of pancreatic islets by immune cells which attack β cells, leading to β cell destruction and ultimately insulin deficiency. The NOD mouse is being widely used as an experimental model

for Type 1 diabetes as it shares similarities with Type 1 diabetic patients (Li et al. 2019). The pathogenic events in NOD mouse begins after 3 weeks of birth with the presentation of islets antigens in lymph nodes of pancreas (Hoglund et al. 1999). At this stage there is infiltration of islets with antigen presenting cell (APCs), i.e., dendritic cells and macrophages, and then with lymphocytes, resulting in insulitis which gradually progresses over 15 weeks (Rosmalen et al. 1997). After 18–20 weeks, there is development of Frank diabetes, i.e., blood glucose level above 250 mg/dL (Kachapati et al. 2012).

The advantage of NOD mice is that the type 1 diabetes pathogenesis is similar to that in human, i.e., due to destruction of β cell resulting I insulin deficiency and ultimately hyperglycemia. Furthermore, chronic hyperglycemia can lead to serious complications like neuropathy, cardiovascular diseases, retinopathy, nephropathy, etc. Many of these complications are depicted by NOD mice and hence it provides a good animal model to study type 1 diabetes and its complications (Aldrich et al. 2020).

However, these mice require high maintenance (Caquard et al. 2010) and the physiological variations between mice and human, for example, islet architecture, immune system components, metabolism, etc. should be kept in mind while employing them to study type 1 diabetes therapy (Roep et al. 2004). It has been shown that it is relatively easy to treat diabetes in young NOD mice, therefore, the point of intervention should also be considered while using this experimental model (Roep 2007). Dose conversion of drug from NOD mice to human also poses a limitation in using it as an experimental model (von Herrath and Nepom 2005).

Despite the above-mentioned limitations of the NOD mouse, it is still used widely as it represents various aspects of human disease and is helpful to identify genetic as well as signaling pathway leading to type 1 diabetes. Kim et al. (2020) showed that the incidence of diabetes reduced significantly after the administration of a probiotic combination consisting of *Lactobacillus acidophilus*, *Lactobacillus reuteri, Lactobacillus casei, Streptococcus thermophiles*, and Bifidobacterium bifidum 6 times a week for 36 weeks to 4 weeks NOD mice. This combination also ameliorated insulitis and β cell mass in NOD mice. In a study, it was shown that probiotics belonging to families *Lactobacillaceae* and *Bifidobacteriaceae* and genus *Streptococcus thermophilus* ameliorated type 1 diabetes in NOD mice (Dolpady et al. 2016). In another experiment it was observed that oral administration of *L. lactis* in NOD mice prevents type 1 diabetes progression (Takiishi 2012). Calcinaro et al. (2005) showed that feeding De Simone Formulation prevents type 1 diabetes and reduces insulitis in NOD mice.

13.1.1.7 BioBreeding Diabetes–Prone (BBDP) Rat

The BioBreeding diabetes–prone (BBDP) rat is an important model to understand the pathogenesis as well as investigate the therapeutic intervention of type 1 diabetes. In BBDP rat, type 1 diabetes spontaneously through T cell mediated autoimmune destruction of β cells presents in pancreatic islets. In BBDP rats, diabetes develops after puberty with similar prevalence in females and males (Mordes et al. 2004). About 90% of rats develop diabetes within 8–16 weeks of age. The diabetes is quite

severe and is characterized by hyperglycemia, weight loss, hyperinsulinemia, insulitis, and ketonuria which requires insulin administration for survival. Metabolic and clinical symptoms are followed by histological abnormalities in islets of pancreas. The advantage of this model is that it is genetically similar to that of human diabetes and also insulitis is morphologically similar to that of human insulitis. Moreover, the hyperglycemia occurs in BBDP rat under well controlled circumstances and within short duration, thus facilitating its utility for understanding the stages of type 1 diabetes development (Bortell and Yang 2012).

Valladares et al. (2010) demonstrated that the administration of *L. johnsonii* isolated from BioBreeding diabetes resistant rat delayed or inhibited the onset of type 1 diabetes in BioBreeding diabetes-prone rats.

13.1.2 Obesity

The disparity between the intake of energy and its expenditure is the most common cause for the appearance of obesity. Microflora of the gut also plays a significant role in obesity as it influences the metabolism of whole body by influencing the energy balance, gut barrier function, and integrating peripheral as well as central intake regulatory signals. Thus, probiotics can be of use in obesity as they contribute to enhance the gut microflora, affect appetite, food intake, metabolic functions, and body weight through modulation of bacterial species in intestine and gastrointestinal pathways. Following are the most prevalent animal models to investigate the anti-obesity activity of probiotics:

13.1.2.1 Diet Induced Obesity

Diet induced obesity is the frequently used animal model to test the anti-obesity activity of probiotics. HFD and/or high caloric diet can be used to induce obesity in animals. HFD leads to lipid assimilation in the body and thus is detrimental to health, leading to quick gain of weight (Roseno et al. 2015; Sampey et al. 2011; Kumar et al. 2014).

In HFD-induced obesity, mice are fed with HFD consisting of 45% fat, 16.4% protein, and 25.6% carbohydrate (5.252 Cal/g) for 8 weeks. The physiological mechanisms involved in this model are the overconsumption of HFD leading to low satiety which in turn cause storage of dietary fat in the body and alteration in hormones required for the energy balance (Fig. 13.4) (For example, suppression of

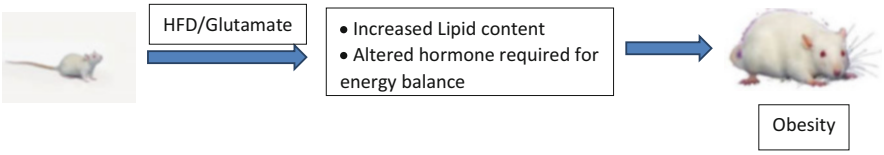

Fig. 13.4 High-fat diet/Glutamate induced obesity

ghrelin secretion after consumption of HFD; leptin and insulin resistance caused due to HFD-induced hyperinsulinemia and hyperleptinemia) (Hariri and Thibault 2010).

This model is simple to induce and closely mimic the time taken for the gradual development of obesity in human. However, there are some drawback of this models as the standardized, single, and defined diet is still lacking (Barrett 2016).

Another model used to induce obesity is high calorie diet induced obesity. In this model, the mice are ingested with high sucrose diet (HSD) consisting of 50.0% sucrose, 5.0% fat, 20.0% protein, and 15.0% cornstarch by weight (Kang et al. 2013). This model is commonly used by researchers to investigate the anti-obesity activity of testing compound as it takes longer time to induce obesity after hypercaloric diet as observed in humans (Rashmi et al. 2019).

However, HFD-induced obesity is more effective to induce obesity in animal as compared to HSD because of the low satiating effect of HFD and large storage capacity of adipose tissues as compared to low capacity of glycogen stores.

Various researchers have used diet induced obesity animal models to determine the anti-obesity activity of probiotics such as *L. plantarum* (Lee et al. 2007; Takemura and Sonoyama 2010; Park et al. 2013) and *L. curvatus* (Yoo et al. 2013; Kang and Cai 2018; Park et al. 2013); *Lactobacillus rhamnosus* (Lee et al. 2006; Liao et al. 2017; Kang and Cai 2018); *Bifidobacterium* (Stenman et al. 2014; Yin et al. 2010; An et al. 2011); *Lactobacillus gasseri* SBT2055 (Miyoshi et al. 2014); *Lactobacillus paracasei* ST11 (NCC2461) (Tanida et al. 2008); *L. casei* IMV B-7280 (Bubnov et al. 2017); *Pediococcus pentosaceus* LP28 (Zhao et al. 2012). Soundharrajan et al. (2020) recently checked the metabolic effect of selected probiotics in HFD fed mice. Their study showed that 29 potential probiotic strain may alleviate the obesity development and its associated metabolic disorders.

13.1.2.2 Glutamate Induced Obesity

Administration of glutamate leads to obesity by causing imbalance between the absorption and energy expenditure. In this model, 2–4 mg/g of BW of monosodium glutamate (MSG) can be administered SC or IP during the neonatal period for 4–10 doses, causing obesity (Von Diemen and Trindade 2006).

There is great increase in body lipid content and decrease in hormone-stimulated lipolysis in MSG-induced obese mice (Fig. 13.4), thus resembles the genetically induced mice. Moreover, it is found that the mortality rate in MSG-induced obese mice is low (Bunyan et al. 1976).

Savcheniuk et al. (2014) opined that multi-probiotic when administered from childhood stage prevents adiposity in glutamate-induced obesity in rats.

13.1.2.3 Other Models

Db/db Mouse

As discussed above can also be used as a genetically modified experimental animal for obesity. Everard et al. (2014) showed that the administration of *Saccharomyces boulardii* exhibits reduced body weight in db/db mouse.

Ob/ob Mouse

In these mice, there is a spontaneous mutation in obese (ob) gene leading to markedly obese phenotype. The obesity in ob/ob mouse is observed due to lack of leptin which further leads to hyperphagia, hypothermia, and decreased expenditure of energy. Further defects include hypercorticosteronemia, insulin resistance associated with hyperglycemia and hyperinsulinemia, growth hormone deficiency, and hypothyroidism leading to linear growth inhibition (Lutz and Woods 2012). The advantage of selecting spontaneous animal model is that there is no need to use labor-intensive feeding schemes to induce obesity. However, in humans, in most of the obese individuals, the obesity does not develop due to decrease in leptin production, therefore the physiology of ob/ob mouse does not entirely reflect to that of human. Moreover, ob/ob mice include high maintenance, expensive, and moreover they are infertile; therefore, limiting their use in routine study.

Yadav et al. (2013) demonstrated that administration of De Simone Formulation, a probiotic reversed obesity in ob/ob mice.

13.2 Current Challenges and Future Perspective

Mortality and morbidity are the risk factors often attached to disorders of metabolism like obesity and diabetes. Various studies have reported the beneficial activity of probiotics against obesity and diabetes by alteration in gut microflora, lowering of cholesterol, and regulation of insulin secretion. However, elucidation of the interaction between ingested probiotics and intestinal microflora possesses a great challenge to consider probiotics as a therapy against metabolic diseases. Thus, thorough and more specific in vivo studies can be of great help to understand the pharmacology and toxicology of probiotics when administered in the metabolic disorders. The in vivo studies will help researchers to gain insight regarding the basic mechanism and will enable researchers to conduct more optimal safety studies before such probiotics are approved for human consumption.

Moreover, there does not exist any standardized safety guidelines related to ingestion of probiotics in human. Thus, there is a need for the careful evaluation of individual probiotics in order to check the potential side effects. There is a need for advanced investigation to further improve the understanding of the relationships that exist between microflora of the intestine and the ingested probiotics.

13.3 Conclusion

Probiotics have shown to be beneficial in metabolic disorders as they help in improving lipid profile, insulin resistance, and glucose tolerance. A further exploration of the efficacy of probiotics in metabolic diseases may be beneficial in the management of metabolic diseases. Thus, further studies need to be designed in animal models to understand the exact mechanism, efficacy, and safety of probiotics in metabolic diseases.

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