

## Probiotics for type 2 diabetes mellitus: an anti-diabetic intervention to see beyond the gut

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### ABSTRACT

The increasing cases of type 2 diabetes mellitus (T2DM) have made it a very concerning metabolic disease worldwide. The increasing understanding of the role of gut microbiota in metabolism process has lead to a promising intervention of T2DM that can relieve not only the symptoms but also help improve the disrupted metabolic mechanisms. Probiotics are now widely studied for its potential in the management of T2DM. From 50 journals reviewed, 43 were found suitable as references for this paper. The keywords used are “probiotics,” “gut microbiota,” “obesity” and “type 2 diabetes mellitus” on selected search engines. In general, it has shown that probiotics do have systemic therapeutic effects as it can increase the secretion of gut hormones, maintain gut barrier function and improve inflammatory response, thereby providing a hopeful future for a comprehensive management of metabolic disorders, especially T2DM.

**Keywords:** Probiotics, Gut Microbiota, Obesity, Type 2 Diabetes Mellitus

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### INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a type of metabolic diseases that is marked by hyperglycemia due to insulin insensitivity in the body. According to WHO, 90% of diabetes cases around the world are T2DM.<sup>1</sup> This data shows that T2DM has been one great concern of health issue worldwide. T2DM is closely related to obesity, where about 600 million of obesity people worldwide are suffering from T2DM.<sup>2</sup>

Many interventions are aimed to prevent or treat obesity so that it does not progress to T2DM, obesity being one of the highest risk factors. The interventions usually aim at reducing weight gain by setting goals and lifestyle changes to cut down calories. Weight loss helps improve insulin resistance, reduce heart rate, improve body composition and psychological well-being.<sup>3</sup> However, this kind of intervention might not work for all, as it is very challenging for most people to alter their lifestyle.

Treatments of T2DM currently revolves around the maintenance of blood glucose at a normal level (110 mg/dl) as possible with oral anti-diabetic drugs such as metformin, sulfonylurea, and thiazolidinediones (TZDs), as well as insulin injection. However, these treatments only cure the symptoms and are not yet able to interfere with the core problem, which is the cause of insulin

resistance in T2DM.<sup>4</sup> This question has raised such a challenge for researchers in medical field since a long ago to find another possible target along the pathological mechanism of T2DM for a more effective and efficient treatment.

A lot of studies have shown that the insulin resistance, the first stage of T2DM is a complex metabolic condition due to lipid overload. This lipid overload might come from either a high-fat diet or inadequate burning of fat that will cause inflammation and eventually result in insulin resistance.<sup>4</sup> This condition is closely related to the existence of gut microbiota, which has been known in the last decade to play such significant roles in the pathogenesis of obesity to T2DM. Indeed, the human gut consists of 100 trillion microorganisms that enclose thousands of species at an average concentration of  $10^{14}$  per ml with an average weight of 1.5 kg.<sup>6</sup> This shows that gut microbiota might convey an enormous amount of functions related to the endocrine system.

The studies of gut microbiota have launched the so-called theory gut microbiota-short-chained fatty acids (SCFAs)-hormone axis, where the interaction of host epithelial cells with microbes and the metabolites released by microbes is the key mediator in the cross-talk between the host and enteroendocrine cells. In general, most studies that relate gut microbiota to obesity and other metabolic disorders show that dysbiosis or alteration of the gut microbiota does contribute significantly to the disruption of gut hormone

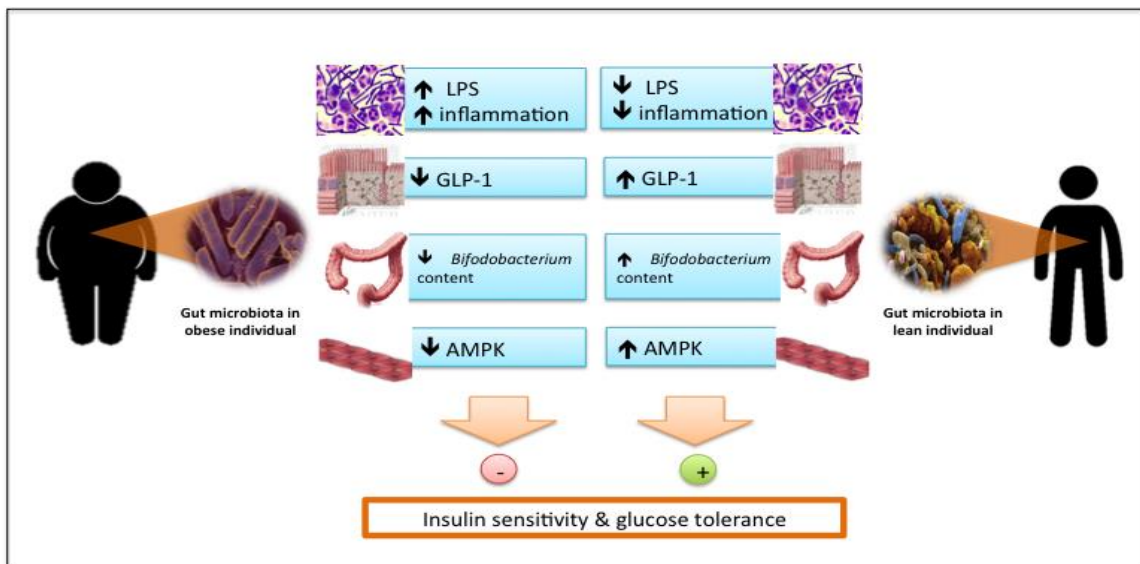
secretion essential for glucose and energy homeostasis, as well as inflammatory response. By that means, the induction of T2DM due to alteration of the gut microbiota occurs through both sectors; enteroendocrine and inflammation sectors. These findings of the relationship between gut microbiota, obesity and T2DM has raised a lot of expectation for a more comprehensive intervention that may treat not only the symptoms but also the core problem. Hence, some preventive and curative strategies targeting the gut microbiota have been proposed; one of the most widely talked about is probiotics.

The use of probiotics for their health benefits is widely known, but its supplementation as a preventive and curative strategy for T2DM has just widely discussed. Its potential in modulating the gut microbiota provides a promising result in the intervention of obesity and T2DM. Therefore, it would be such a valuable contribution to writing about probiotics as a potential intervention for T2DM. Given all the outstanding and up-to-date discoveries, the writer would like to discuss the role of gut microbiota in the pathogenesis of T2DM and

the mechanism of probiotics in alleviating the conditions of obesity and T2DM.

### The Role of Gut Microbiota in the Pathogenesis of Type 2 Diabetes Mellitus

The interaction between the gut microbiota and the host occurs through several metabolic pathways that involve enteroendocrine cells, which is the L cells. Enteroendocrine cells are just one of many types of cells that exist in the gut epithelium, which represent approximately 1% of all epithelial cells in the intestine and subdivided into more than ten different cell types depending on their major secretory products and their localization along the gastrointestinal tract.<sup>7</sup> The interaction of gut microbiota with these cells will lead to the secretion of gut peptides which are essential in controlling physiological processes of energy homeostasis, glucose metabolism, gut barrier function and even metabolic inflammation.<sup>7</sup> Any disruption to these interactions will impact the whole host physiology be it regarding the endocrine sector and inflammation sector (**Figure 1**).



**Figure 1.** Comparison of possible links of gut microbiota with the host in terms of glucose metabolism between obese and lean individuals.<sup>9-19</sup>

### Enteroendocrine Sector

For the gut microbiota to interact with enteroendocrine cells, there has to be some interaction between ligand and receptors working on the process. Firstly, gut microbiota ferment on non-digestible carbohydrates and fibers that cannot be digested by human gut. This fermentation will produce SCFAs that work as ligands for specific G-protein coupled receptors (GPCRs) expressed on L cells.<sup>8</sup> Despite the previous study that shows unfavourableness of SCFAs in

obesity,<sup>9</sup> the crosstalk between SCFAs and G-protein coupled receptors has been shown to lead the secretion of gut peptides such as glucagon-like peptides (GLP-1), PYY and GIP.<sup>10</sup> These gut peptides are known for their principal actions such as increasing insulin secretion and inhibiting food intake. Other ligands for GPCRs can also be produced by the human gut itself but still under the influence of gut microbes, these ligands involve endocannabinoids and bile acids.

SCFAs (acetate, propionate, and butyrate) control the glucose and energy homeostasis through activations of some essential GPCRs. A study with a GPR41-deficient mouse line shows attenuated GLP-1 secretion in response to butyrate, thus suggesting butyrate stimulation of GLP-1 secretion from L-cells partially mediated by the GPR41.<sup>11</sup> Research done by Tolhurst has also shown another important receptor GPR43, where GPR43-deficient mice were found to have reduced colonic GLP-1 protein content, reduced basal and glucose-stimulated levels of GLP-1 which result in impaired glucose tolerance.<sup>12</sup> Based on those results, both studies suggest that GPR41 and GPR43 might potentially have important roles in the secretion of gut peptides, specifically GLP-1.

Another substrate from gut microbiota and human gut, which is bile acids, was found to bind to both nuclear and cell surface receptors of enteroendocrine L cells. The activation of TGR5, a GPCR was found to improve liver function as well as glucose tolerance in obese mice through the regulation of GLP-1 production.<sup>13</sup> As for the nuclear receptor, the activation of farnesoid X receptor (FXR) was found to be essential for maintaining insulin sensitivity and glucose tolerance. It proved in a study by Ma et al., where the hyperinsulinemic euglycemic clamp confirmed insulin resistance in FXR-null mice as a sign of decreased inhibition of hepatic glucose production by insulin and reduced peripheral glucose uptake.<sup>14</sup>

The above metabolic pathways disrupted in the context of obesity and T2DM due to dysbiosis of the gut microbiota. Eighty to ninety percent of the bacterial phylotypes are members of two phyla: the Bacteroidetes (Bacteroides, Prevotella) and the Firmicutes (Clostridium, Enterococcus, Lactobacillus, Ruminococcus), followed by the Actinobacteria (Bifidobacterium) and the Proteobacteria (Helicobacter, Escherichia).<sup>6</sup> It was found that obese individuals have fewer Bacteroidetes and more Firmicutes compared to lean controls.<sup>15</sup> Another study shows that an increase in Actinobacteria follows the decrease in Bacteroidetes.<sup>16</sup> This result also supported by another study that shows obese mice with seventy-five percent of the obesity-enriched genes from Actinobacteria and twenty-five percent genes from Firmicutes, whereas forty-two percent of lean enriched genes came from Bacteroidetes.<sup>7</sup> This ratio of gut microbes in obese people indicates that Bacteroidetes species do have function in maintaining glucose tolerance, body weight as well as preventing up-regulation of serum LPS levels.

### Inflammation Sector

LPS (Lipopolysaccharides) play roles in the development of insulin resistance, and it correlates with an inflammatory reaction. LPS are endotoxins commonly found in the outer membrane of gram-negative bacteria that causes metabolic endotoxemia.<sup>17</sup> Metabolic endotoxemia leads to progression of insulin resistance in muscle, liver, and adipose tissue through the activation of TLR 2 and TLR 4 characterized by the increasing number of pro-inflammatory molecules. Excess in inflammatory factors, including IL-6, IL-1, and TNF- $\alpha$  can injure cellular insulin signals that contribute to insulin resistance.<sup>18</sup>

Gut microbiota themselves have been suggested to modulate inflammation. Gut microbiota during fermentation process produces SCFAs (Short Chain Fatty Acids) that has an important function in facilitating the bacterial translocation. If SCFA reduced, this will impair the bacterial translocation and increase plasma LPS.<sup>17</sup> SCFAs have significant roles in the immune system. Butyrate can modify the T-Helper cells and affect the integrity of intestinal epithelial barrier. Acetate also affects the immune response via G-protein coupled receptor, the Gpr43. Acetate has roles in maintaining the intestinal epithelial barrier which can prevent the translocation of the bacteria. It takes role in acetylation of lysine residues that regulated by SCFA and affect the proteins involved in signaling, metabolic process, and intimately involving in innate and adaptive immune system.<sup>19</sup>

Besides, obesity also causes an alteration in the gut microbiota composition, changes in activation of enzymes and pathways leading to increased inflammatory state. The obese individual has different proportion in phyla of gut microbiota, which is an increase of Firmicutes and decreasing the number of Bacteroides. Another possible mechanism is its correlation with AMP-activated protein kinase (AMPK) in the skeletal muscle and liver, increasing fatty acid oxidation and glucose uptake in the muscle. A study shows that there was a reduced level of AMPK in obesity and T2DM.<sup>17</sup> AMPK is essential for maintaining energy balance through activation of fatty acid oxidation in liver and muscle, thus reduced oxidation of fatty acids would result in a decrease of energy expenditure, excessive lipid storage as well as increasing total body fat. Weight increase is one of the initiating factor of low-grade inflammation.<sup>19</sup> Excess energy intake causes adipocyte hypertrophy and TNF- $\alpha$  production, thus stimulating the production of chemotactic factors. Adipocyte tissue will be ingested by the pro-inflammatory macrophages and resulted in production of IL-6 and IL-1.<sup>17</sup>

T2DM patients have higher production levels of IL-17 and IFN- $\gamma$  that produced by circulating T cells, leading to a pro-inflammatory state. Modulation of TNF- $\alpha$  by alteration of TACE expression, a disintegrin, and metalloproteinase or of tissue inhibitor of matrix metalloproteinase 3 (TIMP3) leads to glucose intolerance and vascular inflammation. Toll-Like Receptors (TLRs) play an important role in the activation of innate immunity. TLR which is activated by LPS recruits IL1RI (Interleukin-1 Receptor) associated protein kinase via adaptor MYD88 and activates nuclear factor- $\kappa$ B and mitogen-activated protein kinases, and the expression of inflammatory cytokines. The activation of TLR4 induces modulation of inflammatory pathways correlated to the induction of insulin resistance, c-Jun NH2-terminal kinase (JNK) and I $\kappa$ B kinase complex (IKK $\beta$ )/inhibitor of nuclear factor- $\kappa$ B (I $\kappa$ B $\alpha$ )/nuclear factor- $\kappa$ B, those can disrupt the regulation of glucose homeostasis in serine phosphorylation of IRS pathways.<sup>6</sup>

### **Probiotics to Interfere with Gut Microbiota in type 2 Diabetes Mellitus**

Having known for its health benefits widely, the widespread prescription of probiotics as therapies still limited due to inadequate knowledge of their mechanism of action.<sup>20</sup> Earlier known to improve and maintain immunity, probiotics now seen as a potential intervention for prevention and treatment of metabolic syndrome following T2DM. Not only does it alleviate inflammatory reactions, but it is also suggested to play important roles in the modulation of gut microbiota thus affecting the production of gut peptides essential in the glucose metabolism.

### **Probiotics Improve Insulin Sensitivity and Prevent Weight Gain**

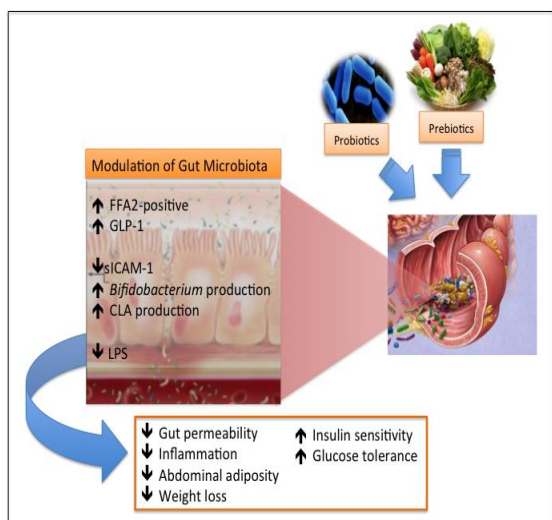
Probiotics have the potential to alleviate the disrupted glucose metabolism, thus working as preventive and therapeutic modalities for obesity and T2DM. A study by Yadav *et al.* shows that the administration of a probiotic VSL#3, which consists of 112.5 billion live lactic acid bacteria promotes the release of GLP-1 that results in reduced food intake and improved glucose tolerance.<sup>20</sup> This result also accompanied by a finding that demonstrates butyrate-stimulated release of GLP-1 from enteroendocrine L cells, thereby proving that modulation of glucose metabolism by VSL#3 is through the gut microbiota-SCFA-hormone axis. Due to its clinical effect, VSL#3 could suppress body weight gain and insulin resistance through modulation of the gut microbiota.

The probiotic strain was also found to prevent gain weight by significantly reducing the abdominal adiposity, thus preventing obesity and its progression to T2DM. A study that involves the administration of a type of *Lactobacillus gasseri* (LG2055) shows that the probiotic strain could significantly reduce the abdominal adiposity, body weight and other measures that suggest its beneficial effects on metabolic disorders.<sup>21</sup> In the following study, oral administration of another type of *L. gasseri* (BNR17) was also found to prevent increases in adipose tissue and body weight in diet-induced overweight rats, which proved by the inhibition of the enlargement of visceral adipocytes and prevention of inflammatory marker (sICAM-1) up regulation in obesity.<sup>22</sup> Another anti-diabetic effect of *Lactobacillus* strain also demonstrated in a study of *L.rhamnosus* which could reduce body weight without reducing energy intake through the production of conjugated linoleic acid (CLA).<sup>23</sup> Altogether those studies indicate that probiotics can help prevent and cure T2DM through the modulation of glucose and energy metabolism, as well as interfering with the adipocyte cells.

Another type of gut microbiome, *Akkermansia muciniphila* has been found to be important in the maintenance of gut barrier function. *A. muciniphila* is currently the only known species within its genus *Akkermansia* that occupy the human intestinal tract. A study shows that *A. muciniphila* administration can significantly increase the levels of 2-oleoylglycerol (2-OG), a bioactive lipid which triggers the secretion of GLP-1 through the activation of GPR119 located on enteroendocrine L cells.<sup>24</sup> This function supports the finding that increased gut permeability associated with a decrease of *A. muciniphila*.<sup>25</sup> However, its whole mechanism in the physiologic and pathologic conditions are still understudies, and its availability as probiotics is yet to discover as it is expected to enhance the wide range of probiotics.

The addition of prebiotics in the consumption of probiotics will also induce synergetic effects on body weight, food intake, glucose homeostasis and plasma lipid profile. Several mechanisms of probiotics have proposed, where through the modulation of gut microbiota, it could induce L cell proliferation thus modulating the secretion of the gut peptides such as GLP-1 and PYY. It supported by a study of supplementation of prebiotic dietary fibers oligofructose (OFS) in high-fat fed mice, where an increase in some *Bifidobacterium* spp. followed by increasing densities of free fatty acid receptor 2 (FFA2-positive) and increased production of SCFAs.<sup>14</sup>

These SCFAs stimulated intestinal proglucagon which is a precursor for GLP-1 as well as PYY secretion that is essential for inhibition of food intake.<sup>26</sup> By that means, regarding enteroendocrine sector, the administration of probiotics itself would have already been of a great favor to the patients of metabolic disorders. If added prebiotics, this will boost up the therapeutic effects since prebiotics work as maintenance of gut microbiota symbiosis (Figure 2).



**Figure 2.** Therapeutic effects of modulation of the gut microbiota by probiotics combined with prebiotics. Free fatty acids 2-positive (FFA2-positive), glucagon-like peptide-1 (GLP-1), serum soluble adhesion molecule (sICAM-1), conjugated linoleic acid (CLA),

### Probiotics Improve Inflammatory Response and Strengthen Intestinal Barrier

Probiotics give beneficial outcomes to insulin resistant individuals through intestinal microbiota modulation in inflammation or not inflammation condition. Consumption of probiotics can stimulate the immune system and reduction of the inflammatory response, those probiotics also secrete an antimicrobial substance, competing with other pathogens, intestinal barrier strengthening, and immune system stimulation. Most of the probiotics contain *bifidobacteria* and bacteria and lactobacilli and those who suffered DM T2 usually have to decrease in bifidobacteria.<sup>27</sup> Administration to *Bifidobacterium animalis* ssp. Lactis showed reduction in inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , Plasminogen Activator Inhibitor-1 (PAI-1), and IL-6 in mesenteric adipose tissue and to increase the sensitivity of insulin.<sup>3</sup>

*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *L. gasseri*, *L. fermentum* decrease the

production of Protein Kinase C-  $\delta$  (PKC-  $\delta$ ) that has the role to loosen the adherens junctions and increase the intestinal permeability. Those probiotics will modulate gene encoding junction and adhesion protein E-cadherin and  $\beta$ -catenin, these will support the adhesion junction to the cytoskeleton. *Lactobacillus rhamnosus* appears to reduce translocation of NF $\kappa$ B to the nucleus, decrease the degradation of I $\kappa$ B, and prevents the activation of TLR-4 by LPS.<sup>27</sup>

Probiotics are live microorganism when administered in an adequate amount give a health benefit to their host. Research explain and compares three different probiotics in gut microbiota, *L.paracasei* CNCM I-4270 (LC), *L. rhamnosus* CNCM I-3690 (LR) and *Bifidobacterium animalis* subsp. *lactis* CNCM I-2494 (BA) on mice with HFD-induced MS (Metabolic Syndrome) could decrease weight gain, increasing of glucose homeostasis, and reduce hepatic steatosis.<sup>28</sup>

Probiotics also can influence the circulating endotoxin levels, an endotoxin that comes from the gut bacteria act as a potent inflammatory stimulant.<sup>29</sup> Some studies suggest that probiotics may inhibit the progression of inflammatory cytokines production, but in muhammadshahi Majid research found no differences in the effect of probiotic yogurt consumption in comparison with conventional yogurt consumption in the T2DM patient. T2DM patients have an imbalance of microflora in their Gastrointestinal tract; this probiotic may improve the imbalance microflora by increasing a number of gram-positive bacteria. Probiotics bacteria such as *Bifidobacterium longum* act to suppress the NF $\kappa$ B activation, directly suppress the production of TNF- $\alpha$ , neutrophils, and macrophage. *Lactobacillus HY* could inhibit Nitrobenzene Sulfonic acid results in inhibition of TNF-  $\alpha$  gene expression.<sup>30</sup>

### The Beneficial Analysis of Probiotics as an Intervention for Type 2 Diabetes Mellitus

In general, probiotics does provide comprehensive therapeutic effects as it can improve not only the inflammatory response but as well as the production of gut hormones, thus recovering the disrupted metabolism of glucose and energy, as well as improving the gut permeability.<sup>12,14</sup> The known mechanisms of probiotics together with addition of other prebiotics show that these interventions offer a great preventive and curative strategy for people with metabolic disorders especially obesity and T2DM.

Compared to other forms of microbial manipulation such as fecal microbiota transplant

(FMT) and bariatric surgery, probiotics would be the easiest and friendliest way of modulating the gut microbiota. FMT is known to be a drastic intervention of the gut microbiota since it aims for total replacement of one microbiota by another. Even though it might provide a more approachable modulation of microbiota with a wider fold of the required organisms, in its application in the society, considering it's a fecal transplant surely requires procedural protocols and a very careful assessment of donor candidates.<sup>31</sup> This administration certainly needs to be introduced and educated carefully to the society, so that a well-managed pre-procedural screening can take place.<sup>32</sup> A lot different from FMT, probiotics have been widely known by the public for its health maintenance benefits,<sup>33</sup> therefore, its application for obesity and T2DM can be easily accepted and understood by the society. It needs no pre-procedural screening, no donors and can be consumed by all.

As for bariatric surgery or gastric bypass, despite its weight loss effect, several adverse effects have been linked to the post-surgery state. Diarrhea, constipation, dysphagia and the so-called dumping syndrome are just a few examples of the adverse effects of gastric bypass.<sup>34</sup> Besides that, bariatric surgery only applied if the candidate's BMI  $\geq 40$  or BMI  $\geq 35$  with at least two obesity-related comorbidities such as T2DM, hypertension, sleep apnea and other respiratory disorders.<sup>35</sup> It shows that this intervention cannot work as a preventive strategy, but rather serves as a very last choice if one is unable to attain a healthy weight loss. Based on the comparisons above, it has shown that probiotics stand as the easiest, yet a comprehensive intervention of gut microbiota, thus its application as a preventive and curative strategy for obesity and T2DM is highly recommended.

#### **Limitation of Probiotics as an Intervention for Type 2 Diabetes Mellitus**

One of the most challenging things about probiotics is that probiotics do not have a long shelf life. Probiotics are commercially available in the form of daily products such as yogurt or freeze-dried supplements.<sup>37</sup> Its viability is significantly influenced by some factors during storage such as moisture, temperature, light, and air. Probiotics cannot stand extreme temperatures, high pressure, shear forces and other forms of stresses especially during their production at the industry level or in the GI tract.<sup>38</sup> These characteristics cause probiotics to hardly survive the conditions during their production and may as well die during their transport from the upper intestinal tract to the colon due to gastric acidity and bile salts.<sup>39</sup>

However, this challenge can be overcome by several approaches that could increase probiotic viability such as genetic modification, immobilization, use of oxygen-impermeable containers as well as microencapsulation that has become of interesting research. The encapsulation might help reduce the loss of probiotics viability by inhibiting reactive components such as oxygen, high temperature, pressure, bacteriophage attack and cryo effects.<sup>39</sup> Nonetheless, despite the limitations of probiotics, these helpful bacteria can still serve a helpful result with some modification to protect and retain the viability.

#### **CONCLUSION**

The current studies about the role of gut microbiota in the pathogenesis of T2DM have led to a promising strategy for a more comprehensive therapeutic strategy. Probiotics indeed offer several helpful mechanisms be it regarding the enteroendocrine sector or inflammation sector. Probiotics reduce weight gain, thereby preventing inflammatory response due to lipid overload, improve insulin sensitivity, as well as glucose tolerance. It shows an extension mechanism that can protect the host body from the progression of T2DM. Despite its limitations, probiotics still considered as one of the easiest ways to gain a great range of health benefits, and the addition of prebiotics for a symbiotic is highly recommended for a better result.

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