Beneficial Properties of Probiotics

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Abstract: Probiotics are live microorganisms that can be found in fermented foods, cultured milk and widely used for preparation of infant foods. They are well-known as “health friendly bacteria” which exhibited various health beneficial properties such as prevention of bowel diseases, improving immune system, lactose intolerance and intestinal microbial balance, exhibiting antihypercholesterolemic and antihypertensive effects, alleviation of postmenopausal disorders and reducing traveler’s diarrhea. Recent studies also have been focused on their uses in treating skin and oral diseases. In addition, modulation of the gut-brain by probiotics has been suggested as novel therapeutic solutions for anxiety and depression. Thus, this review discusses on the current probiotics-based products in Malaysia, criteria for selection of probiotics and evidences obtained from past studies on how probiotics have been used in preventing intestinal disorders via improving immune system, acting as antihypercholesterolemic factor, improving oral and dermal health and performing as anti-anxiety and anti-depression agents.
**INTRODUCTION**

Probiotics are live microorganisms which upon ingestion in sufficient concentrations can exert health benefits to the host. The definition of probiotics was established in 2001 by United Nations Food and Agriculture Organization and the World Health Organization and has been the term of reference for science and regulation thereafter (FAO/WHO 2002). Demands on food containing probiotics are expanding globally due to continuous generation of research evidence indicating their potential health benefits on consumers. This growing market has pulled in probiotics research into Malaysian mainstream in line with government policies promoting health living styles and related products are being marketed as functional food products. Functional foods products resemble conventional foods in terms of appearance but compose of bioactive compounds that may offer physiological health benefits beyond nutritive function (Arora et al. 2013; Emms & Sia 2011). Food processing companies that are widely involved in the manufacturing of probiotics or cultured drinks in Malaysia are Yakult (M) Sdn. Bhd., Nestlé Malaysia, Malaysia Milk Sdn. Bhd. and Mamee-Double Decker (M) Berhad.

Hundreds of different bacterial species are the natural and predominant constituents of intestinal microbiota. Among the numerous intestinal microbes, those anticipated to exhibit potential health benefits to the host through modulation of the intestinal microbiota are commonly selected as probiotics. Species belonging to the genera *Lactobacillus* (*L.*), and *Bifidobacterium* (*B.*) have been reported to be the beneficial probiotic bacterial strains. The representative species include *L. acidophilus*, *L. casei*, *L. plantarum*, *B. lactis*, *B. longum* and *B. bifidum* (Kailasapathy & Chin 2000; Ishibashi & Yamazaki 2001). Some of the major health benefits attributed to probiotics, including improvement of gastrointestinal microflora, enhancement of immune system, reduction of serum cholesterol, cancer prevention, treatment of irritable bowel-associated diarrheas, antihypertensive effects as well as improvement of lactose metabolism (Saarela et al. 2000; Nagpal et al. 2012). This article reviews on the past studies involving the use of probiotics in strengthening the immune system, prevention of bowel diseases, modulation of
hypocholesterolemic effect as well as promoting dermal and oral health. Besides, potential uses of probiotics for the management of anxiety and depression as well as boosting dermal and oral health are also discussed.

**Current Probiotics-based Products in Malaysian Market**

Mostly probiotics products in Malaysia are marketed in the form of fermented milks and yoghurts (Ting & DeCosta 2009). However, in recent years, the probiotics products from non-dairy based sources are gaining attention due to ongoing trends of vegetarianism and also to meet demands of those who are lactose intolerant (Vasudha & Mishra 2013). The following examples represent some of the dairy based-products with types of probiotics that can be found currently in the Malaysian market. Nestlé Bliss is marketed by Nestlé Malaysia which is made up from real fruit juices added with live cultures of *Streptococcus thermophilus*, *B. lactis* and *L. acidophilus* (as on Feb 22, 2015, http://www.nestle.com.my/brands/chilled_dairy/nestle_bliss). Vitagen is a cultured milk drink manufactured by Malaysia Milk Sdn. Bhd. composed of live probiotics such as *L. acidophilus* and *L. casei* that are imported from Chr. Hansen Laboratory, Denmark (as on Feb 22, 2015, http://www.vitagen.com.my/benefits.html). Yakult (M) Sdn. Bhd. is producing Yakult Ace cultured milk drink that contains a strain named *L. casei* Shirota strain (as on Feb 22, 2015, http://www.yakult.com.my/html/about_yakult.html). HOWARU Protect™ is a non-dairy product containing patented probiotic formulation in the form of powder which contains *B. lactis* Bi-07™ and *L. acidophilus* NCFM® and marketed by Cambert (M) Sdn Bhd (as on Feb 22, 2015, http://www.danisco.com/product-range/probiotics/howarur-premium-probiotics/howarur-protect-probiotics/). Nutriforte Lactoghurt is a product by Cell Biotech, a Danish-Korean bio-venture enterprise that introduces the dual coating technology, Duolac® in order to increase the viability of the probiotics during manufacturing, shelf life and when passage through the gastrointestinal tracts (GIT). The product is based on synergistic combination of 5 dual-coated live bacteria strains of *L. acidophilus*, *L. rhamnosus*, *B. longum*, *B. lactis* and *Streptococcus (S.) thermophilus* (as on 16 Oct, 2015, http://www.nutriforte.com.my/Lactoghurt+Probiotics_20_1.htm). Hexbio© is a probiotic formulation consisting of *L. acidophilus* BCMC™ 12130, *L. lactis* BCMC™ 12451, *L. casei* BCMC™ 12313, *B. longum*
Selection of Probiotics

There are number of criteria that must be met during selection of a probiotic bacterial strain with utmost importance is placed on safety issues. Strains of the genera Lactobacillus and Bifidobacterium are usually regarded as safe from the basis of long-term human experience. Members of other genera such as Bacillus licheniformis also have been investigated as probiotics. However, it should not be concluded that all members belonging to genera of Bacillus can be used as probiotics. This is because there are some strains from Bacillus genus that are associated with diseases such as Bacillus cereus, which can cause food-borne illness. It is critical to perform safety assessment when the probiotics are not from the genera of Lactobacillus or Bifidobacterium, (EFSA 2007; Leuschner et al. 2010).

Pathogenicity and infectivity, intrinsic properties as well as virulence factors related to toxicity and metabolic activity of the microorganisms are factors that need to be addressed during safety assessment process of probiotics (Ishibashi & Yamazaki 2001). Viability and activity of probiotics during storage and when passing through the gastrointestinal tract (GIT) is also essential. Stomach and the surroundings of GI have the highest acidity; therefore it is critical to establish the behaviour and the fate of the microorganism when passage through this condition. In vitro tests typically resembling the conditions in GI commonly used as a screening tool to identify potential probiotics. This is because colonization and potential health benefits only can be anticipated when these viable cells able to survive natural barriers that exist in GI such as low pH conditions and degradation by digestive enzymes as well as bile salts (Kailasapathy & Chin 2000; Ishibashi & Yamazaki 2001). The viable cell numbers of probiotic in a product should be at least $10^6$ CFU/ml at the expiry date for health and functional claiming as the recommended minimum effective dose per day is $10^6–10^9$ cells. Many factors such as pH, titrable acidity, molecular oxygen, redox potential, hydrogen peroxide, flavoring agents, packaging materials and packaging conditions are associated with viable cell count of a microorganism in a product throughout manufacturing and shelf-life period (Mortazavian et al. 2012). Another important selection criterion for a probiotic is the ability to adhere to host tissues especially to intestinal mucus and epithelial cells to promote efficient host-
microbial interactions. This interaction is particularly important to prolong the retention period of the specific strain in the gut. However, continuous intake of orally administered probiotics is necessary because permanent colonization of probiotics is uncommon. Many factors involved in the adhesion of probiotic microorganisms to the host tissues. Microbial cell density, buffer components, fermentation duration and growth medium are associated to the in vitro culture parameters while intestinal microflora, digestion and the food matrix are referred to in vivo conditions (Ouwehand & Salminen 2003; Forssten et al. 2011).

There are ongoing studies on the identification of new strains for potential exploitation as probiotics concurrently with existing strains are being explored for novel applications. These new strains need to be evaluated and assessed based on established selection criteria which include safety, functional and technological characteristics prior to the selection of a particular strain for probiotic application.

IN VITRO AND IN VIVO STUDIES ON BENEFICIAL EFFECTS OF PROBIOTICS

Bowel Diseases and Immune System
Ulcerative colitis and Chron’s disease are types of bowel diseases that have been linked to the gut's microbial, genetic predisposition and environment. Breaking the balance between intestinal immunity and microbiome may lead to these bowel diseases (Khor et al. 2011). Enteric bacteria may change the equilibrium of pro-inflammatory and anti-inflammatory cytokine level of intestine that becomes the predisposing factor for intestinal disorders. Pro-inflammatory cytokines that produced by Th1 cells and anti-inflammatory cytokines that secreted by Th2 cells are important in maintaining the homeostasis of the immune system in the intestinal barrier (Elgert 2009).

Probiotic organisms are increasingly known for its ability to prevent and/or treat intestinal disorders and improve the immune system in both in vitro and animal models. Peran et al. (2005) investigated the role of L. salivarius CECT 5713 in colitis induced rats. The research showed that orally administered probiotics were able to exert anti-inflammatory effect and reduced the necrosis in the
treated group which subsequently ameliorated the colonic condition. It was proved with histological findings where the affected intestine of the treated group showed a pronounced recovery and the markers of inflammation and necrosis such as MPO, TNF-α and iNOS expression have been greatly reduced. This result also matches with the research conducted on human peripheral blood mononuclear cells (PBMCs) that certain probiotic bacteria such as lactobacilli have anti-inflammatory effect where the highest level recorded with *L. salivarius* Ls 33. The inflammatory status was assessed by the ratio of IL10/IL12 where high ratio indicates anti-inflammatory effect whereas low ratio shows pro-inflammatory effect. In addition, the ranking of the tested strains’ ability to improve experimental colitis that obtained on the basis of an *in vivo* IL-10/IL-12 cytokine stimulation ratio closely resembles the order in an animal model such as mice (Foligne et al. 2007).

Additionally, a downregulation of TNF-α, COX-2 and upregulation of anti-inflammatory cytokines for instance IL-4, IL-6 and IL-10 were observed in colitis mouse fed with *L. plantarum* 91 (Duary 2011). A similar *in vivo* result was also obtained where an increased in anti-inflammatory cytokine IL-10 and a decreased in pro-inflammatory cytokine were found in dextran sulphate sodium (DSS) induced colitis mouse treated with *L. kefiranofaciens* M1. In the same study, anti-colitis effect was also examined using *in vitro* assays. Results showed that *L. kefiranofaciens* M1 was able to increase the amount of apical and basolateral chemokines, CCL-20 and strengthen the barrier function of epithelia via improving the transepithelial electrical resistance (TEER) (Chen et al. 2011). In addition, Ganguli et al. (2013) conducted a study to investigate effect of probiotics in necrotizing enterocolitis (NEC). NEC is considered a lethal condition in premature infants. The effect of probiotics was observed on developing human intestinal xenografts and the research proved that *L. acidophilus* ATCC 53103 and *B. infantis* ATCC 15697 were able to modulate intestinal inflammatory response. The secreted glycolipid or glycan could be the reason for the anti-inflammation effect.

On the other hand, a clinical trial was conducted involving 187 ulcerative colitis patients where *L. rhamnosus* GG (LGG) was given at the dosage of $18 \times 10^9$ viable bacteria/day with and without standard treatment of mesalazine at the dosage of 2400mg/day. Administration of LGG or a combination of LGG and mesalazine by the subjects increased the relapse-free time compared to the standard treatment
As shown in both *in vitro* and *in vivo* studies, probiotic treatment may alleviate the bowel diseases through modulating immune responses.

Although probiotic treatment improves the severity by decreasing the inflammation but they did not treating the actual root cause. Moreover, there is fear of opportunistic infection by probiotic strains as they modulate the inflammatory status of a subject. Thus, more clinical trials will be needed to disclose the controversies regarding the effectiveness and safety issues in order to provide better understanding on the control mechanisms of diseases. Longer duration of studies also required to prove the sustainability of the positive effects on human health.

**Hypocholesterolemic Effect**

Probiotics have been suggested to have hypocholesterolemic effect through numerous mechanisms such as assimilation of cholesterol, binding of cholesterol to cellular surface (Lye *et al*. 2010), co-precipitation of cholesterol (Zhang *et al*. 2008), interfere with the formation of micelle for intestinal absorption and bile acids deconjugation through the secretion of bile salt hydrolase (BSH) (Lambert *et al*. 2008).

Hypocholesterolemic effect exhibited by probiotics is mostly claimed due to BSH activity and it can be detected in all lactobacilli and bifidobacteria strains. The major role of BSH is deconjugation of bile acid which makes the bile salt less soluble and excreted out as free bile acid via feces. This will reduce the cholesterol in serum and increase the de novo synthesis to replace the lost bile acid (Nguyen *et al*. 2007). Besides, cholesterol could be removed in greater amount in the presence of bile as it acts as a surfactant and allows cholesterol to attach onto bacterial cell membrane. Additionally, Lye *et al* (2010) reported that the attachment of cholesterol on the bacterial cell membrane was growth dependent. However, the efficacies of treatment of each probiotic strain have not been explored in detail with respect to dose and duration. Table 1 shows the summary of findings for hypocholesterolemic effects of probiotics.

**Dermal Health**

Probiotics have been proven to have some new benefits for skin health. Recent studies showed that probiotic could improve atopic eczema, wound and scars healing and help skin-rejuvenating.
To date, effects of probiotics on skin diseases are extensively studied by both administration and topical application methods. However, research data are still inconclusive to support the concerned potential of probiotics. Results from the clinical trial of probiotic treatment are conflicting due to differences in dosage, probiotic strain, duration of application, length of follow-up and time slot of administration.

A recent study done by Yesilova et al. (2012) revealed that probiotics treatment containing *B. bifidum, L. acidophilus, L. casei* and *L. salivarius* was effective in reducing atopic dermatitis patients’ SCORing Atopic Dermatitis (SCORAD) index and stimulate cytokine production. The authors suggested that the impact of probiotic on SCORAD could be due to the modification of immunogenicity of potential allergens. On the other hand, *Escherichia coli* Nussle 1917 (EcN, serotype O6: K5: H1) has been evaluated to be beneficial for treatment of several chronic inflammatory diseases. Weise et al. (2011) demonstrated that oral administration of EcN induced the immune regulatory mechanisms in allergen-induced dermatitis mouse model (BALB/c mice) via stimulating the cytokine production.

Furthermore, a few evidences suggesting that nonviable bacteria as well as, extract component from bacteria could also exert health potentials. Research findings showed that bacterial compounds can evoke certain immune responses and improve skin barrier functions. Stability of the cell components and metabolites at room temperature when compared to viable cell make them more suitable for topical applications (Gueniche et al. 2010). Furthermore, Lew and Liong (2013) reported that some of the bacterial compounds such as hyaluronic acid, lipoteichoic acid, peptidoglycan and sphingomyelinase exhibiting beneficial dermal effects with some possible mechanism actions. However, the exact mechanisms remain unclear, and more research should be directed to explore the potential in fulfilling the demand for probiotic dermal formulations.

Lactic acid bacteria can produce bioactive peptides known as bacteriocins that possess antimicrobial activity against pathogenic bacteria. Lordache et al. (2008) revealed that in the presence of soluble molecules produced by lactic bacteria with probiotic potential could suppress the expression of opportunistic bacterial virulence factors. These findings could lead to a new alternative treatment for bacterial infections although the exact mechanism of action remains to be ascertained.
Based on studies that have been done on probiotics, probiotics pose a promising potential although its effects could be strain specific, dosage dependent and application reliance.

**Oral Health**

The emergence of antibiotic-resistance bacteria has recently attracted attention of researchers for potential application of probiotics in boosting oral health. To date, research findings suggested that probiotic is useful in preventing oral diseases such as dental caries, periodontal infection and halitosis.

**Dental caries**

Dental caries is a bacterially mediated process that is characterized by acid demineralization of the tooth enamel (Selwitz et al. 2007). In the event of preventing dental caries, probiotic needs to adhere to dental surfaces and antagonize the cariogenic species such as mutans streptococci and lactobacilli. Probiotics that incorporated into a dairy product such as cheese could neutralize the acidic condition in the mouth and prevent demineralization of the enamel (Jensen & Wefel 1990).

An *in vitro* study done by Ahola et al. (2002) has revealed that *L. rhamnosus GG* could potentially inhibit the colonization by streptococcal cariogenic pathogens, thus help to reduce tooth decay incidence in children. Nase et al. (2001) demonstrated a significant decrease in dental caries and lower salivary counts of *S. mutans* in patients after consumption of dairy products containing *L. rhamnosus* for seven months. An *in vitro* study done by Haukioja et al. (2008) also revealed that lactobacilli and bifidobacteria were able to modify the protein composition of salivary pellicle and thus specifically prevent the adherence of *S. mutans*.

In addition, Nikawa et al. (2004) revealed that the consumption of yogurt containing *L. reuteri* for two weeks reduced the concentration of *S. mutans* in saliva up to 80%.

**Periodontal disease**

Primary pathogenic agents such as *Porphyromonas (P.) gingivalis, Treponema (T.) denticola* and *Tannerella forsythia* possess variety virulent characteristic that can allow them to colonize the subgingival site, interfere with the host’s immune system and cause tissue damage. Hojo et al. (2007) reported that *L.*
salivarius, L. gasseri, L. fermentum and Bifidobacterium are among the common prevalent species residing the oral cavity and significant for the oral ecological balance.

Krasse et al. (2006) found that after two weeks of ingesting chewing gum containing L. reuteri, oral cavity of the patients with a moderate-to-serve gingivitis has been colonized by the strain and a significant reduction of plaque index was observed. In addition, Riccia et al. (2007) evaluated the anti-inflammatory effects of L. brevi in a group of patients with chronic periodontitis. The result demonstrated a positive improvement in plaque index, gingival index, bleeding and probing for all patients after four days of treatment with lozenges containing L. brevis. Moreover, substantial reduction of salivary level prostaglandin E$_2$ (PGE$_2$) and matrix metalloproteinases (MMPs) were also observed and these could be due to ability of L. brevis in preventing the production of nitric oxide, thus suppressed PGE$_2$ expression and MMPs activation.

Recent studies have reported the ability of lactobacilli flora to inhibit the growth of periodontopathogens such as P. gingivalis, Prevotella intermedia and Aggregatibacter (A.) actinomycetemcomitans. According to Koll-Klais et al. (2005), isolated oral lactobacilli suppressed the growth of S. mutans, A. actinomycetemcomitans, P. gingivalis, Prevotella intermedia up to 69%, 88%, 82% and 65%, respectively. In recent study, Chen et al. (2012) determined the antagonistic growth effects of L. salivarius and L. fermentum on growth inhibition of periodontal pathogens including S. mutans, S. sanguis and P. gingivalis. A similar finding was also reported by Ishikawa et al. (2003) on the in vitro inhibition of P. gingivalis, Prevotella intermedia and Prevotella nigrescens by daily oral administration of a tablet containing L. salivarius.

Comprehensive studies are required to clarify the correlation between regular consumption of the product containing probiotics and periodontal health. Further clinical investigation on the dosage of probiotic, mean of administration and safety aspect are required in order to establish the potential of probiotics in the treatment of periodontal diseases.

Halitosis
The unpleasant odour from the oral cavity in halitosis is due to the volatile sulphur compounds (VSC) which produced by anaerobic bacteria that degrade food proteins. Fusobacterium (F.) nucleatum, P.
gingivitis, *Prevotella intermedia* and *T. denticola* are the bacteria that responsible for VSC production. Kang *et al.* (2006) suggested that the production of hydrogen peroxide by *Weisella (W.) cibaria* caused the growth inhibition of the *F. nucleatum*. They also found that the gargle solution containing *W. cibaria* reduced the production of hydrogen sulphide and methanethiol by *F. nucleatum*. Moreover, another species, *S. salivarius* is known to produce bacteriocins that could colonize with and suppress the growth of volatile sulfide-producing species (Burton *et al.* 2005).

Preliminary data obtained by numerous studies have been encouraging, but apparently more clinical studies are necessary to establish its potential application in oral health. More studies are required to identify the most safe and functional probiotic strains, optimal target population, optimal dosage and mode of administration. The effects of probiotic on oral health and its maintenance are remaining unclear. The exact mechanisms of action for immuno modulation in host and its interaction with pathogenic species need further clarification. Long-term effects of probiotics consumptions are remain ambiguous. Thus, well-designed long-term clinical trials are needed to evaluate the potential of probiotics. Promising strains need to be tested in an extended clinical trial with various methods of applications in order to prove the oral disease treatment using probiotics conclusively.

**MODULATION OF GUT-BRAIN AXIS USING PROBIOTIC**

In a human body, GIT is the most heavily colonized organ by various species of bacteria such as *Bactroidetes, Firmicutes* and *Actinobacteria* (Vyas & Ranganathan 2012). The human GIT is inhabited with $10^{13}$ to $10^{14}$ microorganisms, which is tenfold greater than human cell number and carries 150 times more genes than that of the human genome (Cryan & Dinan 2012).

On the other hand, gut-brain axis is the bidirectional interactions between the GIT and the brain (Grenham *et al.* 2011). It is regulated at the hormonal, neural and immunological levels for maintaining homeostasis and dysfunction of the axis which causes pathophysiological consequences. The frequent co-occurrence of stress-related psychiatric disorders for instances gastrointestinal disorders and anxiety
has also further emphasized the importance of this gut-brain axis (Cryan & Dinan 2012; Matsumoto et al. 2013).

The scaffolding of the gut-brain axis consists of the central nervous system (CNS), the enteric nervous system (ENS), the sympathetic and parasympathetic arms of autonomic nervous system (ANS), the neuroendocrine and neuroimmune systems, and also the gut microbiota (Grenham et al. 2011). A complex reflex network is formed to facilitate signaling along the axis, with afferent fibers projection to integrative CNS structures and efferent that project to smooth muscle in intestinal wall (Cryan & Dinan 2012). Through this bidirectional communication network, brain signals can affect the motor, sensory and secretory functions of the GIT and contrarily, the GIT signals can affect the brain function (Grenham et al. 2011).

There have been increasing evidences showed that the alterations in gut microbiota can greatly influence the interaction between gut and the brain, affect brain function as well as modulates behaviour. The use of germ-free animals is one of the approaches used to study the gut-brain axis. Neufeld et al. (2011) carried out a comparison study on the basal behavior of female germ-free (GF) mice and conventionally reared specific pathogen-free (SPF) mice. A higher plasma corticosterone level was observed in GF mice which indicated a higher stress response compared to SPF mice. An altered gene expression level of brain-derived neurotrophic factor (BDNF), glutamate and serotonin receptors which imply anxiety also observed in GF mice. This was the first study demonstrated the effect of intestinal microbiota on the behavior development and neurochemical changes in the brain (Neufeld et al. 2011).

Gut-brain axis modulation has been considered as a potential therapeutic solution to treat disorders like anxiety and depression due the emergent concern on gut-brain interaction and its ability to affect the development of psychiatric disorders. Studies also supported that probiotics play a role in modulation and improvement of mood, stress response and anxiety signs in irritable bowel syndrome (IBS) and chronic fatigue patients (Lakhan & Kirchgessner 2010). A number of researches have been conducted to examine the impact of probiotics on gut-brain axis.

An in vivo study on the effect of psychotropic-like properties of probiotic in rats and human subjects was performed by Messaoudi et al. (2011). The authors found that the daily consumption of probiotics mixture of *L. helveticus* R0052 and *B. longum* R0175 (10⁹ cfu) significantly (P<0.05) decreased
anxiety-like behaviour in rats and showed a reduced psychological distress in human subject. The research findings indicated that probiotics are not only able to modulate gut microbiota but also involved in stress, anxiety and depression management which can be used as a novel therapy in psychiatric disorders (Messaoudi et al. 2011). In another study, a reduction in the post-myocardial infarction depressive behavior and an improvement in intestinal permeability in a rat were observed upon administration of similar probiotics mixture. The authors postulated that the probiotics mixture might exhibit therapeutic effect on depressive behavior via reduction of pro-inflammatory cytokines, which subsequently leads to depression induction and restores intestinal integrity by apoptosis inhibition (Arseneault-Breard et al. 2012).

In addition, Desbonnet et al. (2009) studied the effect of *B. infantis* on twenty Sprague-Dawley rats. The authors reported that an increase in serotonergic precursor (tryptophan) and decrease in pro-inflammatory immune responses, which both implicated in depression, were found in rats upon consumption of *B. infantis* for 14 days. Results showed that *B. infantis* might possess antidepressant properties and might be beneficial in depressive therapy. This was supported by Desbonnet et al. (2010) where *B. infantis* treatment enable the normalization of peripheral immune response, reverse behavioral deficits and restore concentrations of basal noradrenaline in the brain of maternal separation rats (Desbonnet et al. 2010).

Moreover, an experiment was performed by Bravo et al. (2011) to examine the antidepressant effect of *L. rhamnosus (JB-1)* in mice. The authors observed a decreased stress-induced corticosterone and reduced anxiety- and depression-related behavior in mice as well as induced region-dependent alterations in gamma-aminobutyric acid receptors (GABA<sub>A</sub> and GABA<sub>B</sub>) mRNA expression via vagus nerve. GABA is the main CNS inhibitory neurotransmitter. Pathogenesis of depression and anxiety was implicated by alteration in expression of GABA receptor. The results revealed that administration of *L. rhamnosus (JB-1)* was able to modulate the GABAergic system and alter anxiety- and depression-related behavior in mice.

Chronic fatigue syndrome (CFS) is a complex and debilitating disorder characterized by intense fatigue that may be worsened by physical or mental activity and will not be improved by bed rest. About 97% of CFS patients claimed neuropsychological disturbances such as headaches and symptoms in
emotional realm. The most prevalent emotion-related symptoms are anxiety and depression. In a pilot study, CFS patients receiving *L. casei* strain Shirota (LcS) \(24 \times 10^9\) cfu daily for two months showed a significant \(P<0.01\) decrease in anxiety symptoms (Rao *et al.* 2009). This study provides further support on the presence of the gut-brain communication which can be mediated by gut microbiota. In another study, human subjects were required to consume either a cultured drink containing *L. casei* Shirota \(10^8\) cfu/ml or a placebo control daily for three weeks. Measurements on cognition and mood using questionnaire-based profile of mood states (POMS) were conducted at baseline and after ten and twenty days of administration. Six basic mood dimensions were measured daily which includes confident/unsure, clearheaded/muddled, elated/depressed, agreeable/angry, energetic/tired and composed/anxious on 10 cm visual analogue scales. Every evening subjects were requested to rate their mood all through the day on the scales. Human subjects with poor mood at the beginning of the experiment exhibit a significant \(P<0.05\) improved in mood after probiotic treatment (Benton *et al.* 2007).

It has been reported that an alteration of normal gut microbiota in adult rodents with probiotics can modulate pain, behaviour and brain biochemistry (Bravo *et al.* 2011). Thus, another study proposed that the alteration of gut microbiota might possess a similar effect on human behavior and brain function. Tillisch *et al.* (2013) evaluated an effect of consuming fermented milk containing a mixture of probiotics (*B. animalis* subsp *Lactis*, *S. thermophiles*, *L. bulgaricus*, and *Lactococcus lactis* subsp *Lactis*) on gut-brain communication in humans. Results revealed that brain activity which plays a role in controlling emotion and sensation in healthy women was influenced after administration of aforementioned fermented milk. This study clearly demonstrates the relationship of consumption of probiotics on the modulation of brain activity and also provides evidence for the modulatory effect of probiotics in the gut-brain interactions.

An increasing of experimental data has supported the existence of gut-brain axis and the modulatory effect of probiotics on the axis to treat psychiatric disorders. However, the exact mechanisms involved in modulation of the gut-brain axis with probiotic remain ambiguous. In recent research by Bercik *et al.* (2011), administration of *B. longum* NCC3001 was determined to normalize anxiety-like behavior of the dextran sodium sulfate-induced colitis mice model. The authors hypothesized that it might be vagal
pathways that mediate the anxiolytic signals of *B. longum* which can be initiated either on vagal afferent terminals innervated with gut or at the enteric nervous system level.

Altogether, accumulating evidences prove the presence of the gut-brain communication and its importance in altering brain function and behavior. Capabilities of certain probiotics to regulate different aspects of gut-brain axis simultaneously provide potential benefits in the management of stress, anxiety and depression behaviour. However, the findings are still in preliminary stages and further studies are warranted to examine the exact mechanisms of action involved. In addition, investigation on the specific gut microbes, intestinal structure and function should be carried out to better understand the interactions that take place. Evaluation on the signaling pathways between gut microbiota and the brain in humans also critical to elucidate whether the gut-brain communication plays a homologous role in modulating stress, mood and anxiety as reported in rodent models. Advance understanding of the interaction that occurs during gut-brain communication can provide insight into the development of novel treatment strategies for patient with psychiatric disorders or other diseases.

**CONCLUSION**

This review has focused on several beneficial properties of probiotics. One of the most known health effects of probiotics is preventing and ameliorating bowel diseases by improving the immune system. Besides, probiotics have found to exhibit hypocholesterolemic effects via cholesterol assimilation, binding of cholesterol to cellular surface, co-precipitation of cholesterol, interfere the formation of micelle for intestinal absorption, deconjugation of bile acids by BSH and improving the lipid profiles. Apart from these conventional beneficial effects, probiotics have been reported to improve atopic eczema, wound and scars healing and possess skin-rejuvenating properties. It has been suggested that probiotics could exhibit beneficial dermal effects by producing bacterial compounds which evoke certain immune responses and improve skin barrier functions. Probiotics could also be used to prevent and treat oral diseases. They are found to improve/prevent dental caries and periodontal infection via growth inhibition of cariogenic bacteria and periodontopathogens. Additionally, they have been shown to reduce the
production of nitric oxide, which subsequently suppressed the prostaglandin and matrix metalloproteinases levels in saliva. Moreover, the unpleasant odour from the oral cavity in halitosis could also be ameliorated by inhibiting the growth of volatile sulfide-producing species. On the other hand, improvement of stress-related psychiatric disorders such as anxiety and depression via modulation of gut-brain axis by probiotics has also further emphasized the importance use of probiotics. However, more scientific developments are needed to establish the potential application of probiotics. There is no doubt that the application of probiotics in human health will expand to a greater degree with the current significant research progress.

ACKNOWLEDGEMENT

This work was supported by the UTAR research fund provided by Universiti Tunku Abdul Rahman, Perak, Malaysia.

REFERENCES


### Table 1: Summary of findings for hypocholesterolemic effects of probiotics.

<table>
<thead>
<tr>
<th>Probiotic organism</th>
<th>Experimental system</th>
<th>Major findings</th>
<th>Reference</th>
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<tr>
<td><em>Yogurt</em> (unknown)</td>
<td>human</td>
<td>Reduced total cholesterol and LDL</td>
<td>Agerholm-Larsen <em>et al.</em> 2000</td>
</tr>
<tr>
<td>Fortified buffalo milk-yogurts with <em>B. longum</em></td>
<td>rat</td>
<td>Reduced total cholesterol, LDL-cholesterol and triglyceride</td>
<td>Abd El-Gawad <em>et al.</em> 2005</td>
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<td><em>L. plantarum</em></td>
<td>mice</td>
<td>Reduced blood cholesterol</td>
<td>Nguyen <em>et al.</em> 2007</td>
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<td>rat</td>
<td>Decreased total cholesterol and LDL-cholesterol</td>
<td>Kumar <em>et al.</em> 2011</td>
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<td>Increased HDL-cholesterol</td>
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<td><em>L. plantarum</em></td>
<td>rat</td>
<td>Decreased LDL, VLDL and increased HDL with decrease in cholesterol</td>
<td>Mohania <em>et al.</em> 2013</td>
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in deposition of cholesterol and triglyceride in liver and aorta.

**In vitro**

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<tr>
<th>Strain</th>
<th>Culture media</th>
<th>Activity</th>
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<td>BSH activity</td>
<td>Pereira <em>et al.</em> 2003</td>
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<td>Attachment of cholesterol onto cell surface</td>
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