REVIEW
Probiotics and their beneficial effects against various diseases

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Abstract: Joint FAO/WHO expert’s consultation report defines probiotics as: Live microorganisms which when administered in adequate amounts confer a health benefit on the host. Most commonly used probiotics are Lactic acid bacteria (LAB) and bifidobacteria. There are other examples of species used as probiotics (certain yeasts and bacilli). Probiotic supplements are popular now a days. From the beginning of 2000, research on probiotics has increased remarkably. Probiotics are now day’s widely studied for their beneficial effects in treatment of many prevailing diseases. Here we reviewed the beneficiary effects of probiotics in some diseases.

Keywords: Probiotics, Lactic acid Bacteria (LAB), Bifidobacteria, Probiotic supplements

INTRODUCTION
The emergence of resistance and tolerance to the existing drugs has created a decreased efficacy of these drugs in use. Along with the advancement in other fields of medicine (Qadir et al., 2006; Qadir et al., 2007; Qadir et al., 2008), the problem of resistance has been tried to be overcome by increasing the drug delivery to the target site by the use of polymers (Khalid et al., 2009; Hussain et al., 2011) or through nanotechnology (Naz et al., 2012; Ehsan et al., 2012), synthesis of new drugs, either by the use of proteomics (Qadir and Malik, 2010; Qadir, 2011; Qadir and Malik, 2011), or synthesis from lactic acid bacteria (Masood et al., 2011), or marine microorganisms (Javed et al., 2011). However, now a days, the trend is being changed from synthetic drugs to the natural drugs either from plants or microbes to control the diseases. The natural products are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ahmad et al., 2012; Ali et al., 2013), hypoglycaemic (Qadir et al., 2009; Nisa et al., 2010), amoebicidal (Asif and Qadir, 2011), anti-diarrheal (Janbaz et al., 2013a), anti-fertility, cytotoxic, antibiotic (Amin et al., 2012), spasmyolytic, bronchodilator (Janbaz et al., 2013b), antioxidant (Janbaz et al., 2012) and anti-Parkinsonism properties. As a natural product, probiotics have been emerged as new management tools for the control of different diseases.

The gastrointestinal tract of human, at different site is habited by beneficial bacteria. These bacteria have a relationship of symbiosis with the host. There are sites where the potentially beneficial micro-organisms are more in number than potentially harmful bacteria. This type of ecosystem composition is called Normobiosis. The environment where the potentially harmful bacteria dominate over health beneficial bacteria is called dysbiosis (Roberfroid et al., 2010). The potentially beneficial bacteria are called probiotics.

Probiotics (derived from Latin and Greek) means “for life” is defined in many ways. It was defined first time 50 years ago. The most recent and accepted definition of probiotics is “live micro-organisms administered in adequate amounts which confer a beneficial physiological effect on the host”. Joint FAO/WHO experts consultation report defines probiotics as: Live microorganisms which when administered in adequate amounts confer a health benefit on the host.

There are some substances normally oligosaccharides which serve as substrate for probiotics and thus important for their growth. These are called prebiotics. Prebiotics are those substances that are not used or metabolized by non-probiotic bacteria like Bacteroides spp and Escherichia coli. Prebiotics are found in breast milk and some vegetables (Hamilton and Miller, 2004).

Many of the publications define probiotics with reference to more or less same source. Most publications say that term probiotics is attributed to Lilly and Stillwell who had coined the term first in 1965. They defined probiotic as: a substance produced by one microorganism stimulating the growth of another microorganism. They understood a probiotics as opposite to an antibiotic. Parker (1974) gives a totally different overview: Organisms and substances which contribute to intestinal microbial balance. Most commonly, Kollath may be credited for the term
Probiotics and their beneficial effects against various diseases

‘probiotics’. In 1953, he coined probiotics as ‘Probiotika’, active substances that are essential for a healthy development of life.

There are different types of bacteria which are used as probiotics (fig. 1). Commonly used bacteria include Lactobacilli and Bifidobacteria (Macfarlane et al., 2004). These bacteria show symbiotic relationship with human. They are present in the mucus membrane present on epithelial cells of the gut (Holzapfel and Schillinger, 2002) where they inhibit the growth and attachment of harmful bacteria by producing bactericidal chemicals against these bacteria. With the development of evidences regarding usefulness and safety of probiotics, these bacteria are replacing the traditional prophylactic and treatment regimes.

Sources of probiotics

Yogurt is the most common source of probiotics. Yogurt consists of milk (usually from the cow, goat or sheep) fermented by bacteria that modify lactose into lactic acid. Lactic acid is responsible for giving yogurt its characteristics (sharp taste usually changed into good taste by using sweeteners and flavouring) and also denatures and precipitates casein, resulting in a semisolid consistency. “Bioyoghurts” are produced in a similar way, but bacteria used for fermentation are of different strains, usually L acidophilus. Fermented milk and fortified fruit juice are common sources of probiotics. Probiotics are also available in supplements consisting of freeze dried bacteria in tablets, capsules and powders. Selection of probiotic product depends on type of bacteria and type of beneficial effect expected. There are thousands of strains of probiotics and all of them show different beneficial effects.

Regulation of probiotics

It was usually understood that probiotics are not regulated (Sanders, 2008). But bacteria used as probiotics require a careful safety assessment (Wassenaar et al, 2008). FDA has established a regulatory authority to regulate probiotics production, manufacturers, labeling and safety of products. Of note, on 24 August 2007, the FDA issued rules that require current GMP for dietary supplements. Although these regulations do not address verification of efficacy claims, hopefully they will improve the compositional quality (identity, purity, and strength) of probiotic supplements in the market. Normally, the FDA Does not challenge the labeling or safety of a probiotic product except where the product is mentioned as a drug (i.e., to treat, cure, prevent, mitigate, or diagnose disease) and lacks approval as a drug (Sanders, 2008).

FDA regulations on probiotics depend on intended use of the product (indicated on Label). In FDA there are 4 regulatory categories for probiotics and each of these has different regulatory requirements. These are (1) Drug or biological products, (2) Dietary supplements, (3) food or

Fig. 1: Different types of bacteria which are recognized as probiotics (Furrie, 2005).
If probiotics are intended for use as drug, these are also considered as biological products and FDA regulations of biological products will also be applied on probiotics. If these are to be used as “dietary supplements”, the manufactures may market it without any pre-approval. But manufacturer must notify FDA the claims of the product. And it will be treated as “new dietary ingredient”. If it is launched to be used as food or food ingredient, the FDA only regulates its post-market controls related to adulteration. If the product is launched as medical food, then no pre-market clearance will be required (Degnan, 2008).

In Europe, the probiotic mediated food is not governed by European regulations. But microbial feed additives are subjected to regulations, regarding safety assessment of these additives in animals and humans. Scientific Committee on Animal Nutrition (SCAN) in Europe has launched a “Qualified Perception of Safety” (QPS) concept. According to this concept, the species which have long safety data are allowed to be marketed without extensive safety testing (Wright and Atte, 2005).

Clinical uses of probiotics

Diabetes

World health organization states diabetes as “Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces” Diabetes mellitus is one of the most prevalent diseases in the world with the ranking of 9th with respect to number of deaths. Hyperglycemia, or increased glucose level, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body’s systems, especially the nerves and blood vessels.

Diabetes has proved a silent killer for most of the Patients. So the main focus should be to devise the best treatment with lesser adverse effects.

Diabetes mellitus is classified into 2 major classes. Type-1 diabetes mellitus is related with destruction of pancreatic beta cells and failure to produce insulin. Type-2 diabetes mellitus is associated with decrease insulin production or increased insulin resistance with is mostly linked with obesity.

Diabetes management includes a large number of medications but none of them could be helpful in complete cure of disorder. Many researches are being carried out at bimolecular and pharmacological level. One of the efforts to cure this disorder is to use symbiotic (probiotic and prebiotics).

At the Start of 20th century, it was thought that probiotics beneficially affect the host by improving balance of gut microbiota. Recent researches show that there is a connection between bacterial population in gut and metabolic disease in human (especially diabetes). Recent studies based on large-scale 16S rRNA gene sequencing, quantitative real time PCR (qPCR) and fluorescent in situ hybridization (FISH), have shown that there is a connection between the composition of the intestinal microbiota and metabolic diseases like obesity and diabetes (Larsen et al., 2010). Danish researches worked on 36 Danish people and came to know that there is a difference in gut microbial flora of diabetic and non diabetic peoples. These men were of different age and body weight and 18 of them are diabetic and 18 are non diabetic they also found 3 types of major phyla of bacteria that reside in gut of human. These are firmicutes (most important is lactobacillus which is beneficial bacteria), proteobacteria and bactereodetes (opportunistic pathogens which can cause gum disease and tooth decay). There was a low level bacteria from phy lum firmicutes and greater level of bacteria from phylum bactereodetes. Researchers also found that increased level of bactereodetes and decreased level of firmicutes will lead to decreased glucose tolerance which is key problem with diabetes mellitus (Larsen et al, 2010). Probiotics are supposed to treat the diabetic patients by balancing microbial gut flora.

Low-fat (2.5%) dahi containing probiotics *Lactobacillus acidophilus* and *Lactobacillus casei* was tested in rats against high fructose-induced type-2 diabetes. Both these bacteria proved beneficial effect in lowering blood glucose by decreasing insulin resistance (Yadav et al., 2006). It is also suggested that the use of probiotics can decrease the insulin resistance and can also lower the incident of hypertensive conditions that are closely related to diabetes. It has also been found that *Bifidobacterium spp* delivers pharmaco nutritional support in treating insulin resistance (Cani and Delzenne, 2011).

Hypertension

The incidence of increased blood cholesterol has been increased in adults, children and adolescence. Most important causes of hypertension are lipid abnormality, hypercholesterolemia and obesity (Yekeen et al., 2003). Mann and Spoerry were among the first to illustrate that lactobacillus-fermented milk has hypocholesterolemic effects (Mann and Spoerry, 1974). New researches have shown that not only the Lactobacilli exhibit hypocholesterolemic effects, but Bifidobacteria could also cause a significant reduction in serum cholesterol when cholesterol is elevated. As we know that most of the cholesterol is synthesized and absorbed in intestine, therefore intestinal micro flora has shown to effect cholesterol level in blood. Studies have shown that probiotics have been proved beneficial in lowering hypertension by decreasing blood cholesterol level and increasing resistance of LDL to oxidation (Goel et al., 2006).
lactase. Mostly peoples deficit in lactase generally tolerate lactose due to lack of lactose metabolizing enzyme Lactose intolerance means the inability of adults to digest Lactose intolerance estimated 1 billion women have bladder or vaginal diseases which women are facing today. Globally, an et al. Urinogenital infections (Rahman and Qadir, 2011; Yousuf Urogenital heath care

Urogenital infections (Rahman and Qadir, 2011; Yousuf et al., 2012)) proved to be one of the most common diseases which women are facing today. Globally, an estimated 1 billion women have bladder or vaginal infections each year (Reid et al., 2004). UTI such as bacterial vaginosis can lead to increased risk of preterm delivery (Flynn et al., 1999). Mostly oral and vaginal sex play an important role in causing vaginitis and UTI, but these can also caused by a change in person’s own microorganism. This change occurs due to change in vaginal environmental in which lactobacilli decrease in concentrations or are absent and are substituted by pathogenic Gram-positive cocci, Gram-negative rods, or yeast. Many researches are carried out to find the different between normal and abnormal micro flora (Bruce et al., 1973; Stamey and Sexton, 1975; Wilson, 2004; Schwebke et al., 1999). Research on the vaginal micro flora during the whole menstrual cycle has proved that only 22% women maintain a lactobacilli-rich flora (Daus and Hafez, 1975) and it is also clear that epithelial cell receptivity to lactobacilli attachment enhances at peak estrogen levels midcycle (Chan et al., 1984), it is still not found what factors cause such a dramatic alteration in the flora.

Lactose intolerance

Lactose intolerance means the inability of adults to digest lactose due to lack of lactose metabolizing enzyme lactase. Mostly peoples deficit in lactase generally tolerate lactose better from yogurt than from milk. Reason for this is that it has been assumed that bacterial micro flora in the small intestine enhances lactose digestion (Shah, 2000 and Farnworth, 2008) possibly by increasing contact between lactose and lactase.

Crohn’s disease and ulcerative colitis
Crohn’s disease (CD) and ulcerative colitis (UC) are the chronic diseases of GIT with more or less common symptoms. Both are collectively called inflammatory bowl disease (IBD). In ulcerative colitis only mucosa of colon are inflamed. In case of Crohn’s disease, the mucosa, submucosa and serosa are inflamed and the inflammation can spread to whole GIT. Crohn’s disease is associated with diarrhea, want loss and abdominal pain while Ulcerative colitis has the symptoms of diarrhea and bleeding (Jonkers and Stockbrügger 2003). Exact cause of Inflammatory bowl disease in not known. Probiotics are used in the treatment of inflammatory bowl disease considering that bacteria are involved in the etiology of the disease. Different studies show beneficial effect of probiotic in the treatment of inflammatory bowl disease in animal models (Gionchetti et al., 2002).

Clinical placebo controlled studies also shows that Probiotics cause improvement in the condition of IBD (Jonkers and Stockbrügger 2003).

Colon cancer
Management of cancer is one of the hot issues in these days (Ilayas and Qadir, 2010; Tabasum and Qadir, 2010; Bokhari et al., 2012; Farooqi et al., 2013; Saleem et al., 2013). In laboratory experimentation, some stains of LAB (Lactobacillus delbrueckii subsp. bulgaricus) have shown anti-mutagenic effects because they have ability to bind with heterocyclic amines which are carcinogenic (Wollobski et al., 2001). Animal studies proved beneficial effects of LAB against colon cancer of rodents. Human trials also suggest that some types of LAB are anti-carcinogenic due to ability to decrease the activity of enzyme called β glucuronidase (Brady et al., 2000) (which can generate cancer producing substances in the digestive system). The incidence of colon cancer in people consuming dairy product has been low compared to others during population studies. But there is still lot more to do to confirm this effect.

Inflammation
Some strains of LAB may decrease the inflammation by modulating inflammatory and hypersensitivity responses. This effect is thought to be caused by regulation of inflammatory mediator called cytokines (Reid et al., 2003).

Immune function and infections
Some strains of LAB may decrease pathogen growth by means of competitive inhibition (i.e., by competing for 408 Pak. J. Pharm. Sci., Vol.27, No.2, March 2014, pp.405-415
growth) and thus these may support normal immune functions. They also improve normal immune system by increasing the concentration IgA-producing Plasma cells, improving phagocytosis as well as increasing the concentration of T lymphocytes and Natural Killer cells (Reid et al., 2003), (Ouwehand et al., 2002). These probiotics are thought to decrease dental caries and respiratory tract infection. Lactobacillus salivarius is found to protect the mice from infections caused by Listeria monocytogenes by producing a chemical called bacteriocin (Corr et al., 2007). Some strains of probiotics are useful in treating retrovirus infection (Trois et al., 2007) and sexually transmitted infections (Bolton et al., 2008) but no products are approved by FDA for such indications.

**Peptic ulcer**

Some strains of LAB may control Helicobacter pylori infections (cause of peptic ulcers) in adults when given concomitantly with standard medical treatments. But here also there is no product approved by FDA for this purpose.

**Atopic diseases**

Atopic dermatitis is the first symptom of atopic disease and it is a chronic skin condition associated with inflammation and pruritis (Simpson, 2010), eczematous papules, itch and plaques. It is one of the most prevalent skin diseases. 20% children and 1-3% adults are affected by atopic dermatitis. Atopic dermatitis tends to run in family. There is a high risk of developing atopic disease in children whose mother is atopic. It is found that probiotics play a major role in reducing occurrence of atopic diseases. The risk of occurrence of eczema during first 2 years of infant life was reduced significantly in those whose mother received probiotics as compared to those whose mother takes placebo (Rautava et al., 2002).

**Liver diseases**

The liver and gut has an important relation in a sense that the blood is carried form gut to the portal system. Liver functions are stimulated by intestinal blood content. Similarly bile secretion produced by liver affects gut performance. It is found that any change in the normal composition of gut micro flora alter liver function and can lead to initiation and progression of liver diseases (Cesaro et al., 2011). Many complications (Hepatic encephalopathy, cirrhosis, spontaneous bacterial peritonitis) are associated with overgrowth of harmful bacteria, changed intestinal permeability and improper immune function. Probiotics are useful in the treatment of chronic liver diseases as they block entry of microorganisms to blood flow and ultimately to liver by increasing the strength of intestinal barrier (Cesaro et al., 2011). Another mechanism involves the regulation of gut micro flora (Sheth and Garcia, 2008) and regulation of immune functions (Jonkers and Stockbrügger, 2007). These also reduce the development of Hepatic encephalopathy and also support other medication for the treatment of Hepatic encephalopathy (Hopkins, 2003).

**Food allergy**

Food allergy is caused by the antigens present in food and is associated with inflammation of intestine. Probiotics are helpful in reducing the symptoms of food allergy as they enhance gut defence by two mechanisms (non-immunologic and immunologic). First is carried out by normalizing the gut microflora and decreasing membrane permeability. Second mechanism involves the enhancement of immunological defense system of host by boosting the IgA action. This leads to enhanced degradation of food antigens and food allergy is reduced (Kirjavainen et al., 2001).

**Upper respiratory tract infection**

Upper respiratory tract infection includes laryngitis, tracheal inflammation and common cold and these are associated with symptoms like fever, headache, and pain and cough (Hao et al., 2011). Most of the upper respiratory tract infections (URTIs) are caused by viruses and these resolve in 3 to 7 days. To treat the symptoms like headache, pain and fever, analgesics and antipyretics are used most frequently. Fermented food containing probiotics like Lactic acid bacteria and bifidobacteria are found to reduce the episode of URTIs. Probiotics are also found to decrease the risk and incidence of respiratory tract infection (RTIs) in the children having age of 3-5 years (Ouwehand et al., 2008). These probiotics are also found to decrease the symptoms of pain, cough, and runny nose.

**Oral health**

Probiotics reside in oral cavity in a very less numbers, comprising only 1% of total micro biota of oral cavity (Haukioja, 2010). Probiotic species present in sliva includes L. paracasei, L. plantarum, L. salivarius, and L. rhamnosus (Haukioja, 2010; Ahrne et al., 1998). Bifidobacterial species which are found to be residing in oral cavity are B. bifidum, B. dentium, and B. longum (Haukioja, 2010). Probiotics enhance dental care by 3 possible mechanisms which include modulation of immune response, Normalization of oral microflora and metabolic effects (Parvez et al., 2006). Probiotics improve oral health by inhibiting incidence of following diseases.

**Dental caries**

One of the major causes of dental caries is mutant streptococci. It is suggested that the products containing probiotics when used, can decrease the number of mutant strain of streptococci (Nase et al., 2001; Cildir et al., 2009; Haukioja, 2010). In these studies it is found that consumption of food containing probiotics also enhances the level of slivary probiotics (Ahol et al., 2002; Montalto et al., 2006).
Periodontal diseases
Periodontal disease is an inflammatory disease which effect one or more periodontal tissues like alveolar bone, periodontal ligament, cementum, and gingiva. It is discovered that culture supernatant of *L. acidophilus*, when used in persons suffering from periodontal diseases (gingivitis, periodontitis and pregnancy gingivitis), treats most of the periodontal diseases. The probiotic species treating gingivitis include *L. brevis*, *L. casei*, *L. salivarius*, reuteri strains, *Bacillus subtilis*, *L. reuteri* and *L. brevis* (Krasse *et al.*, 2006; Della *et al.*, 2007; Twetman *et al.*, 2009). *L. brevis* was found to contain anti inflammatory activity and it also inhibit MMP (collaginase) activity (Della *et al.*, 2007). *B. subtilis* showed encouraging result in periodontitis by decreasing the number of pathogens in periodontal tissues (Tsubura *et al.*, 2009).

Halitosis
Halitosis is the unpleasant odour exhaled in breathing. It itself is not a disease but is caused by some other diseases like periodontitis. Probiotics can be used in both gut and mouth mediated halitosis (Delanghe *et al.*, 1997).

Oral candidiasis
Some strains of probiotics (*L. rhamnosus* strain, and *Propionibacterium freudenreichii*) are effective in reducing the candida yeast count (Haukioja, 2010).

Viral infections
Probiotics are useful against many viral diseases. It is also obvious that probiotics do not show antiviral effect by direct action on viruses but do so by the mechanism of immunostimulation (De Vrese and Schrezenmeir, 2002). Those probiotics which show immunostimulation also exhibit potential antiviral effect. Thermophilus species have some antiinfluezal effect. These species also show anti herpetic effect when administered to guinea-pigs (Liaskovs *et al.*, 2007). Antiviral activity of different probiotics against different viral infection is demonstrated in table 1.

Harmful effects of probiotics
Normally probiotics are considered as non-harmful bacteria that exert health benefit to the host. This is true in most cases. *Lactobecillus* are used for a long times due to
Table 1: Shows different infections mediated by different virus and the effect of different species of probiotics against these infections.

<table>
<thead>
<tr>
<th>Study</th>
<th>Probiotic spp</th>
<th>Result</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antirotaviral effect in Mice</td>
<td>B. breve</td>
<td>B. breve showed antirrotaviral activity by increasing production of IgA. It showed anti-influenzal effect by increasing IgG</td>
<td>Yasui et al., 1999 Liaskovs et al., 2007</td>
</tr>
<tr>
<td>Oral polio vaccination</td>
<td>L. rhamnosus, L. paracasei</td>
<td>Both showed anti polio effect due to increased IgG, IgA, and neutralizing antibodies.</td>
<td>De Vrese et al., 2001</td>
</tr>
<tr>
<td>Hepatitis A, B vaccination</td>
<td>L. acidophilus, B. bifidum</td>
<td>Against Hepatitis A, B</td>
<td>De Vrese and Schrezenmeir, 2000</td>
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<td>Anti herpes effect in Guinea pig</td>
<td>Thermophilus spp</td>
<td>Thermophilus showed anti herpetic effect.</td>
<td>Liaskovs et al., 2007</td>
</tr>
<tr>
<td>Treatment of RTI in childrens</td>
<td>L. rhamnosus</td>
<td>Significant reduction in infection.</td>
<td>Hatakka et al., 2001</td>
</tr>
<tr>
<td>Listeria monocytogenes infection in mice</td>
<td>L. salivarius</td>
<td>Protection of mice from infection by producing a chemical called bacteriocin.</td>
<td>Corr et al., 2007</td>
</tr>
<tr>
<td>Effect of probiotics in Rotavirus mediated gastroenteritis in children</td>
<td>Bifidobacterium lactis Saccharomyces boulardii</td>
<td>Useful in antirrotaviral therapy.</td>
<td>Erdogan et al., 2012</td>
</tr>
<tr>
<td>Effect of probiotics in HIV/AIDS infection</td>
<td>Yogurt bacteria</td>
<td>Probiotics increase CD4 count in blood and also reduce diarrhea incidence in HIV infected patients</td>
<td>Irvine et al., 2010</td>
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Table 2: Shows the risks of different species of probiotics

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<td>L. rhamnosis</td>
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<td>Pneumonia and sepsis</td>
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In addition most species are resistant to most of the antibiotics. This resistance is normally non transmissible. There is no safety concern regarding the use of these bacteria having intrinsic resistance. But it may be possible that resistance may be transmissible as present in plasmid mediated resistance (Salminen et al., 1998). Probiotics are also grouped into two classes based on their risk to health. Risk group 1 (No risk) consists of Lactobacillus and Bfidobacteria. Risk group 2 (small risk) contains L. rhamnosus and Bfidobacterium dentium (De Vrese and Schrezenmeir, 2002). Harmful effects of different bacteria are shown in the table 2.

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