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Biological attributes and enzyme profiling of B*ifidobacterium sp.*: A State-of-art review

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Abstract- Bifidobacterium represents a genus within the phylum Actinobacteria. It representing ubiquitous inhabitants of the human orogastrointestinal tract and vagina. Some of its species and typical inhabitants in the human gut.The genus consists of more than 50 species, with only 10 species being found in humans. The *Bifidobacterium* species were classified into four classes viz. oxygen hypersensitive, oxygen sensitive, oxygen tolerant and microaerophilic. *B.adolescentis* and *B.longum* subspecies are the major bifidobacterium species in the adult intestinal flora and *B.longum* species. All Bifidobacterium strains possessed leucine aminopeptidase, α- and β – galactosidase and α-glucosidase activities. β – glucosidase was not detected in *B.bifidum* or *B.longum*, but it was present in all other species. N-acetyl - D - glucasaminidase activity was present in *B.bifidum* (ATCC 11863) had higher N-acetyl - D – glucasaminidase activity than the other bifidobacteria.
The present research investigation is focused on the state-of-art review on the various biological and enzymatic attributes of *Bifidobacterium sp.*
It is well established that Bifidobacteria confer positive health benefits to their host via their metabolic activities. The availability of complete bifidobacterial genomes and corresponding comparative analysis allow for the identification of mechanisms underlying bifidobacterial metabolic activity. Carbohydrate utilization studies and identification of metabolic pathways also provides fundamental information allowing for the identification of novel & effective probiotic compounds.
Keywords: B.longum, B.bifidum, β – glucosidase, α-glucosidase, aminopeptidase

Introduction- There are trillions of Bacteria on and in our body and they are extremely important for our health. And one of the most important type is called Bifidobacterium [1]. These are also called Baby bacteria. Breast Milk contains Bifidobacterium and they can be transmitted to the infant gut through breastfeeding . Bifidobacteria were first identified in 1899 by Tissier from the feces of breast-fed infants. Bifidobacterium is the genus of gram positive anaerobic nonsporuling bacteria.
Bifidobacterium represents a genus within the phylum Actinobacteria.It representing ubiquitous inhabitants of the human orogastrointestinal tract and vagina. Some of its species and typical inhabitants in the human gut.The genus consists of more than 50 species, with only 10 species being found in humans [2]. The Bifidobacterium species were classified into four classes :- oxygen hypersensitive, oxygen sensitive, oxygen tolerant and

microaerophilic. *B.adolescentis* and *B.longum* subspecies are the major bifidobacterium species in the adult intestinal flora and *B.longum* species.

Metabolism- The genus Bifidbacterium possesses a unique fructose-6-phosphate phosphoketolase pathway employed to ferment carbohydrate.

Response to oxygen - Recent research has reported that some Bifidobacterium strains exhibit various types of oxic growth. A H_2O_2 – forming NADH oxidase was purified from O_2 sensitive Bifidobacterium bifidum and was identified as a b-type dihydroorotate dehydrogenase [3].

Genomes - The Bifidobacterium have genomes sizes ranging from 1.73-3.25Mb. Three or more complete genome sequences are available for certain bifidobacterial species such as B.adolescentis, B.animalis, B.breve, B.bifidum, B.longum. Bifidobacterial genomes typically encode 52-58 tRNA genes for genome, although there are exceptions [4].

Probiotic aspect

Literally means "for life", It firstly, observed by Lilly & Stillwell in 1965. Probiotics are live bacteria and yeast that are good for our health, especially for our digestive system. Probiotics, are often called 'good' or 'helpful' bacteria because they help keep our gut healthy.Probiiotics may contain a variety of micro-organisms. The most common are bacteria that belongs to groups called Bifidobacterium [5], Lactobacillus, and so many yeast such as Saccharomyces boulardii.

Synthesis of antimicrobial compounds - Produce bacteriocin and other antimicrobial compounds such as hydrogen peroxide, diacetyl and short - chain fatty acids.

Isolation of *Bifidobacterium sp.* from Breast Milk - For many decades, breast milk has been regarded as a sterile body fluid which exerts its influence on the infant's microbiota environment via presenting only some growth factors and optimum conditions for helping the growth of these important groups of bacteria.However, in recent years, breast milk has been hypothesized to be a continous source of commensal bacteria for the infant gut [6].

Clinical implications of *Bifidobactrium sp.* Bifidobacteria and colorectal cancer

Several studies have investigated that potential of bifodbacteria to prevent or treat colorectal cancer. It was shown that B.animalis displays anti-mutagenic activity during growth in MRS broth thereby antagonizing the action of carcinogen 2- amino -3- methylimidazo quinolone.

Bifidobacteria and diarrhea

The use of bifidobacteria to treat various gastrointestinal disorders has also been reported. Successful treatment diarrhea afollowing administrations of B.longum subspecies infants CECT 7210 and B.breve K-110 was found to be due to inhibition of rotavirus, Another example involves a double-blind study investigating whether oral treatment with a commercial probiotic formula containing B.bifidum and Streptococcus thermophiles would reduce antibiotic – associated diarrhea in infants.

Bifidobacteria and inflammatory bowel disease

Although, the exact mechanism of action is not understood, reduction is the symptoms of inflammatory bowel disease following treatment by probiotic strains has been reported. However, it does not seem to increase bowel movements. Taking a specific product containing species of Bifidobacterium,Lactobacillus and Streptococcus seems to decrese bloating in people with IBS [7].

Lung infection

Some research suggests that taking a specific combination product containing Lactobacillus acidophilus and Bifidobacterium with milk help reduce symptoms of fever, cough, runny nose and decrease the amount of antibiotics needed in children [8].

Scaly, itchy, skin (eczema)

Some research show that giving Bifidobacterium lactis by mouth reduce eczema severity in infants.

High cholesterol

Early research suggest that taking milk containing Lactobacillus acidophilus 145 and Bifidobacterium longum BB536 reduces ''bad low density lipoprotein (LDL) cholesterol in people with high cholesterol. However, it also seem to reduce ''good'' high-density lipoprotein (HDL) cholesterol.

Bifidobacteria and functional foods - The inclusion of micro-organisms in the human diet has been on going thousand of years. Throughout history the most common form of administration of microorganisms was through fermented diary products and this is still the case today. Certain lactic acid bacteria, in particular certain members of the genus Lactobacillus, and members of the Bifidobacterium genus make up the vast majority of the functional ingredients present in currently commercialized prebiotic food products. Probiotics have been defined as "selectively fermented ingredients that allow for specific changes, both in the composition and activity of the gastrointestinal microflora that confere benefits upon host well –being and health.

Bifidobacterial carbohydrate metabolism

The human genome is predicted to encode just eight glycosyl hydrolases that are directly linked to carbohydrate digestion. Therefore, many complex dietery carbohydrate remains un-digested and end up in the colon where they may be degraded by members of the microbiota. The human GIT is home to complex microbial community that encompasses approximately 100 fold more genes than the number of genes present in the host genome. Colonization of the human GIT which is believed to occur immediately after birth, is influenced by various factors such as the method of delivery (i.e vaginal or cesarean), type of feeding (breast fed or formula fed), exposure to antibiotics,frequency, and nature of diseases and hygiene condition. Bifidobacteria dominate the total gut bacterial population in health breast-fed infants,although this dominance decreases following weaning. During adult life the bifidobacterial population stabilizes to represent 3-6% of the total gut microbial population whereas in elderly the bifidobacteria numbers usually decline with age. Digestable and simple sugars such as lactose and sucrose are metabolized in the upper gut by the host & bacteria such as lactobacilli, a prevalent inhabitant of the upper GIT. A diverse set of non-digestable carbohydrate are metabolized in the lower gut, including complex plant derived polysaccharides (eg :- pectin, hemicellulose & xylans), host-derived

carbohydrates (such as mucin & glycosphingolipids), and extracellular polysaccharides that are produced by members of the gut microbiota [9]. In fact a recent study performed on the genome sequences from the type strains of each of the 47 Bifidobacteriumm (sub) species found that 5.5% of the core bifidobacterial genomic coding sequences is associated with carbohydrate metabolism. Bifidobacteria present in the infant gut are presumed to metabolize human milk oligosaccharides (HMOS), and the genomes of *B.bifidum* & *B.longum* subsp infantis are indeed tailored toward HMO metabolism. However, other bifidobacterial species such as *B.breve* & *B.longum* subsp. Longum are also commonly present in the infant gut. Although, they do not encode the same HMO catabolic arsenal found in *B.bifidum* & *B.longum* subsp infantis, they can degrade certain HMOs and may also scavenge on carbohydrates that are released by other bifidobacteria. The species *B.biavatti* specifies the largest number of pathways (14 complete pathways), whereas themembers of the species *B.bombi,B.longum , B. infantis, B.minimum* etc. specifying just nine complete pathways. Bifidobacteria lack a number of key enzymes involved in the Emden-Meyerhoff Parnas (EMP) pathway instead, bifidobacteria metabolize hexose sugars through a metabolic pathway named the "bifido shunt" which is centered around the key enzyme, fructose-6-phosphate [10].

Enzyme profiling of Bifidobacteria

All Bifidobacterium strains possessed leucine aminopeptidase, α- and β – galactosidase and α-glucosidase activities. β – glucosidase was not detected in *B.bifidum* or *B.longum*, but it was present in all other species. N-acetyl - D - glucasaminidase activity was present in B.bifidum, var pensylvanicus (ATCC 11863) had higher N-acetyl - D – glucasaminidase activity than the other bifidobacteria. Only B.longum enzyme profile was differenciate from that of other species, as it lacked both β – glucosidase and N – acetyl - D – glucosaminidase. Aside from providing a simple and rapid way of differenciating B.longum from all other bifidobacteria.

Production of fermented milks containing bifidobacteria is increasing as the beneficial effects of bifidobacteria become better known. Fermented milks and yogurt are the most useful media for administering bifidobacteria. However, bifidobacteria often grow poorly on milk, and growth – promoting substances must be added. Bifidobacteria are nutritionally fastidious micro organisms, an donly a limited number of bifidobacteria can grow in minimal culture conditions. Rapid growth rate and acidification of milk are desirable characteristics in a strain of Bifidobacteria to be used in making fermented milks. These characteristics would reduce costs by requiring only a short incubation time and thereby decrease the possibility of contamination. Moreover, in the intestinal tract of humans, bifidobacteria capable of growing on diverse sources of carbohydrates would be favoured for their ability to use sugars that are not digested or absorbed by the host strains of bifidobacteria with high glycosidase activities should be preferred over strains with lower activities.

Potential enzyme of Bifidobacterium

Enzyme β – galactosidase

Three β - galactosidase genes from Bifidobacterium bifidum DSM20215 and one β – galactosidase gene from bifidobacterium infantis DSM20088 were isolated & characterized. The three B.bifidum β – galactosidases exhibited a low degree of amino acid sequence similarity to each other and to previously published β – galactosidases classified as family 2 glycosyl hydrolases.The other two B.bifidum β – galactosidases an dthe enzyme from B.infantis were multimeric intracellular enzymes with molecular masses similar to typical family 2 and family 42 glycosyl hydrolases, respectively. It played a central role in Jacob's & Monad's development of the operon model's development of the operon model for the regulation of gene expression.β - galactosidase has

three enzymatic activities. First, it can cleave dissacharide lactose to form glucose & galactose, which can the enter glycolysis . Second, the enzyme can catalyze the transgalactosylation of lactose to allolactose, and third, the allolactose can be cleaved to the monosaccharides. It is allolactose that binds to lacZ repressor and creates the positive feedback loop the regulates the amount of β – galactosidase in the cell. β - galactose has high specificity for the galactose part of its substrates but low specificity for the remainder [11]. β – Galactosidase, as proteins in general, forms crystals that include about 50% proteins and 50% solvent by volume. β – Galactosidase is a tetramer of four identical polypeptide chain, each of 1023 amino acids. The crystal structure was initially determined in a monoclinic crystal form with four tetramers in the asymmetric unit. As active site are also contributed by amino acids from elsewhere, in the same polypeptide chain as well as from other chains within the tetramer. It has been suggested that β – galactosidase arose from a much simpler, single domain TIM barrel enzyme that had an extended active site cleft and could have cleaved extended oligosaccharides.

β – Galactosidase catalyzes reaction with β – D – galactopyranosides with an oxygen glycosidic bond. The enzyme also reacts with substrates having other glycosidic linkages, including nitrogen, sulphur and fluorine, but with much reduced catalytic efficiency. Reversion (reverse) β – galactosidase reactions done at very high concentrations of sugars having orientation changes an dthe absence of individual hydroxyls at different positions showed that only D – galactopyranose, I – arabinopyranose, D – fucopyranose, and D – galactal reacted in the reverse direction when D – glucose was the other reactant.This results in the formation of covalently attached 2 – deoxy galactose that is released on hydrolysis.

Aminopeptidase

Aminopeptidases are enhance fermentation because bifidobacteria have a high demand for essential growth factors, such as various amino acids and peptides to the growth of bifidobacteria. Aminopeptidases activities were determined in a cell extract of bifidobacterium breve, bifidobacterium infantis, Bifidobacterium longum & B.adolescentis show amino, di, tri and carboxypeptidase activities. Bifidobacterium strains like B.longum contains more than 20 predicted peptidases that could provide amino acids from proteinaceous substrates in the gastrointestinal tract (GIT) and vagina. Aminopeptidases relates to its role as cys-gly dipeptidase or cysteinyl – glycinase and that this peptidase activity present in apparently purified RNA preparations contributed to polypeptide biosynthesis [12]. The enzyme has also been referred to in its earlier days as aminopeptidase M (for microsomal or membrane aminopeptidase). In 1980 it was renamed amino peptidase N, reflecting its preference for action on neutral amino acids and that is the commonly recognized terminology today. The enzyme is widespread but is particularly abundant in brush border membranes of kidney, small intestine and placenta and is also rich in liver.

α - Glucosidase

Bifidobacterium is an important genus of prebiotic bacteria colonizing the human gut. These bacteria can uptake oligosaccharides for the fermentative metabolism of hexoses & pentoses producing lactate, acetate as well as short chain fatty acids & propionate. These end – products are known to have important effects on human health. β – glucosidases (EC 3.2.1.21) are the pivotal enzymes for the metabolism & homeostasis of Bifidobacterium, since they hydrolyze small and soluble saccharides, typically producing glucose.

Two α – glucosidase – encoding genes (agl 1 & agl 2 from Bifidobacterium breve UCC 2003 were identified & characterized. β – Glucosidases (EC.3.21.21) are the enzymes which hydrolyze the glycosidic bond of a

carbohydrate moiety to release non reducing terminal glycosyl residues, glycoside & oligosaccharides. These enzymes are present in all kinds of organisms including :- bacterial,archaea and eukaryotes and play several important roles such as biomass conversion in micro organisms, breakdown of glycolipids and process of lignifications involve in defence against pests, phytohormones activation, catabolism in cell walls in plants and both plant microbes and plant insect interaction. β – Glucosidase also play an important role in the treatment of Gaucher's disease (resulting form a deficiency of β – glucosidase) in which accumulation of glycoceramides takes place in the lysosomal tissues. β – Glucosidases are the essential part of cellulose system (cellulose metabolizing enzymes) & catalyze the last and final step in cellulose hydrolysis. All the enzymes, which are involved in cellulose hydrolysis are normally grouped as cellulose system. It consists of the following systems :- endoglucanase, exoglucanase and β – glucosidase. β – Glucosidases are widely used in the various biotechnological processes, including the production of biofuel & ethanol from cellulosic agricultural wastes and synthesis of useful β – glucosides [13]. These enzymes are employed in industry for hydrolysis of bitter compounds during juice extraction & liberation of aroma from wine groups. In flavour industry β – glucosidases are the key enzymes in the enzymatic release of aromatic compounds from glucosidic precursors present in fruits & fermenting products.

Conclusion

It is well established that bifidobacteria confer positive health benefits to their host via their metabolic activities. The availability of complete bifidobacterial genomes and corresponding comparative analysis allow for the identification of mechanisms underlying bifidobacterial metabolic activity. Carbohydrate utilization studies and identification of metabolic pathways also provides fundamental information allowing for the identification of novel & effective probiotic compounds.

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