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Review of Literature
2.1 LACTASE

Lactase, a part of the β-galactosidase family of enzymes; also known as Lactase phlorizin hydrolase (LPH) is a bifunctional enzyme having lactase (β-gal, EC 3.2.1.23) and phlorizin hydrolase (EC 3.2.1.62) activities (Skovbjerg et al. 1981; Skovbjerg et al. 1982; Montgomery et al., 1991). The phlorizin hydrolase activity is present in all the vertebrates that is involved in the breakdown of substances such as phlorizin, flavonoid, glucosides and pyridoxine-5′-β-D-glucoside, whereas lactase activity is present only in mammals wherein hydrolysis of lactose into glucose and galactose, occurs normally in the brush border of small-intestinal mucosa and then body absorbs these simple sugars into the bloodstream (Leese and Semenza, 1973; Mackey et al., 2002; Nemeth et al., 2003; Kaur et al., 2006). But lactase usually disappears after the weaning stage of young mammals, except in some humans. Accordingly, enzymatic hydrolysis of milk sugar (lactose) can be accomplished either by free enzyme (β-gal) usually in a batch fermentation process, or by immobilized β-gal (Gikas and LopezLeiva, 1985; Ladero et al., 2003), which are the most popular techniques to produce lactose-free milk and related dairy products for consumption by lactose intolerant people (Ladero et al., 2000; Jurado et al., 2002; Sener et al., 2006).

2.2 MECHANISM OF ENZYME (LACTASE) ACTION ON LACTOSE HYDROLYSIS

Lactose (an abundant disaccharide) occurs in the milk of humans (7.5%), cows (4.5%) and other mammals. After ingestion of lactose in humans, it is essential to hydrolyzed into its constituent galactose and glucose monomers by the enzyme called lactase through the cleavage of a glycosidic bond (β-1, 4-d-galactosidic linkage of lactose) in the brush-border membrane of the small intestinal epithelium (Figure 1). This catalytic mechanism of lactose hydrolysis retains the substrate anomic
configuration in the products (Sinnott, 1990). Substrate modification studies have been confirmed that 3’-OH and 2’-OH moieties on the galactopyranose ring are important for enzymatic recognition and hydrolysis. This demonstration of a 2-deoxy analog is an effective competitive inhibitor (Ki = 10 mM). Elimination of specific hydroxyl groups on the glucopyranose moiety does not completely eliminate catalysis (Fernandez et al., 1995).

Figure 1: Schematic presentation of lactose hydrolysis by β galactosidase (lactase) enzyme (Courtesy: http://cbc.arizona.edu/lactose_hydrolysis.jpg)

2.3 PROPERTIES AND FUNCTIONS OF LACTASE

Lactase may also cleave fucosides and arabinosides but with much lower efficiency and even as it is able to catalyze transglycosylation reactions. In E. coli, the lacZ gene is the structural gene for β-galactosidase. It is present as part of the inducible system lac operon which is activated in the presence of lactose when glucose level is low and β-gal synthesis stops when glucose levels are sufficient (Juers, et al., 2003). The deficiency of lactase may cause lactose intolerance to most of the people that are unable to digest high levels of lactose properly in dairy products such as milk, ice cream
and yogurt. Hence, they may well be treated by the intake of supplemental enzyme (lactase) to break down any lactose before its consumption. In recent biotechnological era, a potential treatment for lactose intolerant have been researched through gene replacement therapy with β-gal could be placed into the human DNA so on their intestinal absorption of lactose break down easily by an individual’s (Salehi, 2009; Ishikawa, 2015). Conversely, Lactase has potential importance and accepted as a vital enzyme for dairy industry i.e., used to prevent crystallization of lactose, to improve sweetness, and to boost solubility of the milk products (Voget et al., 1994; Montanari et al., 2000; Domingues et al., 2005).

### 2.4 STRUCTURE AND BIOSYNTHESIS OF LACTASE

The 1024 amino acids of *Escherichia coli* β-galactosidase were first sequenced in 1970 (Fowler and Zabin, 1970) and later in 1994, β-gal protein structure of 464-kDa homotetramer with 222-point symmetry was determined (Jacobson, 1994). Thereafter, in 2005 reported that each unit of β-gal composed of five domains; domain 1 is a jelly-roll type barrel, domain 2 and 4 are fibronectin type III-like barrels, and the central domain 3 is a TIM-type barrel contains the active site and catalytic site while domain 5 is β-sandwich (Matthews, 2005). The 3D ribbon diagram of domain 3 has alpha/beta barrel structure and loops resided in first and fifth domains of the same unit to form active site (Figure 2). The active site is made up of elements from two subunits of the tetramer, and disassociation of the tetramer into dimers removes critical elements of the active site. To complete four functional active sites, domain 2 of different monomers extends into the neighboring active sites called activating interface. Monomer A donates its domain 2 loop to complete the active site of monomer D and in monomer D donates its domain 2 to complete the active site of monomer A. The small intestinal enzyme lactase or lactase-phlorizin hydrolase (LPH) is encoded by the gene LCT that comprises
17 exons with a genomic size of 50 kb located on second chromosome (2q21) in humans as reviewed by Boll et al., (1991) (Figure 3). LCT is transcribed into Lactase-messenger RNA (mRNA) with 6274 bases by RNA polymerase, and Lactase-mRNA is translated by a membrane-bound ribosome into a polypeptide called pre-pro-lactase; that is the primary translation product, has a single polypeptide primary structure consisting of 1927 amino acids (Harvey et al., 1993; Mantei et al., 1988).

Figure 2: 3D Structure of β-galactosidase (Lactase) from *E. coli* represents tetramer color indicates different domains, orange domain 1, blue domain 2, yellow domain 3, cyan domain 4 and red domain 5 (Matthews, 2005).

Figure 3: Location of LCT gene represent on the q (long) arm of chromosome 2 at position 21 indicates with yellow downward arrow (http://commons.m.wikimedia.org/wiki/File:LCT_location.png)
Pre-pro-lactase includes five domains: (i) a 19 amino acid cleaved signal sequence involved in translocation over the endoplasmatic reticulum (ER); (ii) a large prosequence domain that is not present in mature lactase; (iii) the mature lactase segment; (iv) a membrane spanning hydrophobic anchor; and (v) a short hydrophilic carboxyl terminus as depicted in Figure 4 a (Mantei et al., 1988; Neele et al. 1995; Arribas et al. 2000).

Figure 4: Outline Structure and Biosynthesis of Lactase Protein (Troelsen, 2005)

The prodomain has been shown to act as an intramolecular chaperone in the ER, preventing trypsin cleavage and allowing LPH to adopt the necessary 3-D structure to be transported to the Golgi complex (Naim et al., 1994). Mature human lactase consists of a single 160 kDa polypeptide chain that localizes to the brush border membrane of
intestinal epithelial cells. It is oriented with the N-terminus outside the cell and the C-terminus in the cytosol as depicted in Figure 4b (Mantei et al., 1988). At this point, the enzyme lactase will perform its function of breaking down dietary lactose which also contains two catalytic sites of glutamic acid: Glu-1749 associated with the lactase activity and another Glu-1273 is the site of phlorizin hydrolase function (Zecca et al., 1998).

2.5 TYPES OF LACTASE DEFICIENCY

Lactase deficiency is either a primary or a secondary event. Primary lactase deficiency mainly occurs as an effect of autosomal recessive inheritance (Savilathi et al., 1983). Secondary lactase deficiency is the major cause of multiple gastrointestinal diseases with histological evidence of mucosal damage and increased transit time in the jejunal mucosa (Srinivasan and Minocha, 1998).

2.5.1 Primary Lactase Deficiency

Primary lactase deficiency is the normal post-weaning loss of intestinal lactase in the majority and is the most common cause of lactose intolerance (Heyman, 2006). This type of deficiency is further sub-divided into three types:

2.5.1.1 Congenital Lactase Deficiency

Congenital lactase deficiency is the most severe form of lactase deficiency in babies born with little or rarely entire deficient of lactase activity in the intestinal mucosa; generally subjected to dehydration and malnutrition due to the inability to digest the mammalian milk (Torniainen et al., 2009). It manifests as a persistent watery diarrhea during the first days of life of an infant’s fed lactose-containing milk, which leads to poor weight gain (usually weigh less than their birth weight), also suffer from dehydration and acidosis (Jarvela, 2005; Bhatnagar and Aggarwal, 2007).
2.5.1.2 Developmental Lactase Deficiency

Developmental lactase deficiency results from low lactase levels in born babies and is a consequence of prematurity (Heyman, 2006). Initially increase in lactase activity among preterm infants of less than 34 weeks during late gestation periods. Reduction of lactase activity in premature infants born at 28 to 32 weeks of gestation causes severe cramps, abdominal pains, diarrhea, and continuous discomfort and crying; possibly these symptoms identical to babies suffering indigestion. However this condition is temporary for premature babies to be lactase deficient. As such they develop and improve as the intestinal mucosa matures, most of them able to resolve enough lactase production (Vesa et al., 2000; Westland and Crawley, 2013).

2.5.1.3 Adult Lactase Deficiency

Adult lactase deficiency is the primordial common deficiency due to the low lactase activity in healthy adults, independently discovered in 1963 by two group’s scholars (Auricchio et al., 1963; Dahlqvist et al., 1963). This phenotype mainly causes lactose malabsorption and it is also known as adult-type hypolactasia (lactase non-persistance) (Sahi, 1994a; Heyman, 2006). It is characterized by decline in lactase activity in the jejunal mucosa of healthy adults following weaning, between the ages of 3 and 5 years; resulting into symptoms of lactose intolerance (Sahi, 1994b; Moore, 2003).

2.5.2 Secondary Lactase Deficiency

Secondary lactase deficiency is the acquired hypolactasia and is the most commonly associated with gastrointestinal illness such as microbial infections, giardiasis, celiac disease, inflammatory bowel syndrome or malnutrition, and cow milk allergies or viral infections that damage the intestinal villi lead to lactose malabsorption
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(Kosnai et al. 1980; Nieminen et al. 2001; Crittendon & Bennett, 2005; Lomer et al., 2008; Campbell et al., 2009). Usually it is prevalent in the children with rotaviral diarrhea wherein association with celiac disease, Crohn’s disease and HIV (Heaney, 2002; Heyman, 2006; Nicklas et al., 2011). This type of lactase deficiency may also occur due to certain drugs like neomycin, colchicines, tetracycline and methotrexate can result in the severe injury of intestinal mucosa that causes villous atrophy or gastrointestinal mucositis; moreover intake of alcohol may initiate or worsen the lactose intolerance (Sonis, et al., 2004; http://www.allergyclinic.co.nz/guides/21.html).

2.6 LACTOSE INTOLERANCE: AN OVERVIEW OF A DEVELOPMENTAL DISORDER

The concept of prevalence to ‘systemic lactose intolerance’ is disputed and still mysterious, often it is important to distinguish that lactose malabsorption or maldigestion (lactase non-persistence) is not consistent or equivalent to lactose intolerance (Table 1).

<table>
<thead>
<tr>
<th>Lactase Persistence (Normolactasia)</th>
<th>There is persistent to high lactase activity in middle age of an individual can afford larger of amounts of lactose digestion (usually consume 12g of lactose content in &lt; 240 mL of milk).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactase non-persistence (Hypolactasia)</td>
<td>Low lactase activity in the jejunal mucosa of many individuals after the ingestion of lactose.</td>
</tr>
<tr>
<td>Lactose maldigestion or malabsorption</td>
<td>Incomplete digestion of lactose due to hypolactasia or less absorption of lactose capacity without symptoms.</td>
</tr>
<tr>
<td>Lactose intolerance</td>
<td>Gastrointestinal symptoms outcome with lactose malabsorption in an individual.</td>
</tr>
</tbody>
</table>

Source: definitions modified from Benjamin et al., (2013).
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Lactose maldigestion is a common occurrence in many infants as milk is the only fed for them during the first few months of life regardless of having high intestinal lactase activity. It is also prevalent in adults normally who have low intestinal lactase activity (Brown-Esters et al., 2012). Lactose malabsorption occurs when the passage of lactose is not absorbed through the gastrointestinal tract to the colon epithelial cells of an individual after the consumption of milk or dairy products. It is caused by primarily adult lactase deficiency (as discussed above) due to the reduction or loss of lactase activity in the intestinal brush border. Although the colonic bacterium in each individual is relatively stable but differs noticeably between individuals (Arola and Tamm 1994). As a result an individual’s abdomen develop a functional gastrointestinal disorder called as an irritable bowel syndrome (IBS) causes irregular bowel movement, flatulence and abdominal fullness, abdominal pain and discomfort; these symptoms are also associated with lactose malabsorption frequently among lactase deficient patients in India (Shaw et al., 1999; Bohmer et al., 2001; Gupta et al., 2007). Lactose intolerance is the most common disorder of intestinal carbohydrate digestion in humans, and is a widespread problem occurring in ~70% of the world's population (Horner et al., 2011). It is caused by the deficiency of lactase (β-galactosidase) in the digestive tract. The typical clinical symptoms consist of tissue dehydration due to short chain fatty acids (SCFA) stimulate absorption of salt water, and calcium (Ca) reduced, generation of gases [carbon dioxide (CO₂), hydrogen (H₂), and methane (CH₄)] leads to flatulence and cramps, abdominal pain, bloating, nausea, blanching, and sometimes diarrhea may also occur from 30 minutes and 2 hours after the ingestion of lactose (Matthews, 2005; Panesar, 2007; Lomer et al., 2008; Harrington and Mayberry, 2008; Chen et al., 2009). The existence of lactose intolerance is an important clinical syndrome, though its accurate prevalence is not known then also many individuals with real or perceived lactose intolerance avoid
milk and other dairy products, and consume inadequate amounts of calcium (Ca) and vitamin D; concerned a significant risk factor for retarded growth and development with low bone density, suffer from poorer health and bone formation, and higher risk of osteoporosis (Suchy et al., 2010; Savaiano, 2011). In most cases, there is no necessity to eliminate dairy consumption completely by individuals though lactose-intolerant individual approaches with and without dairy foods and supplementation strategies are required based on nutritional facts to ensure appropriate consumption of Ca and other nutrients (Suchy et al., 2010). According to earlier reported literatures, the prevalence of lactose intolerance varies from <5% to ~100% among different populations in the world (Figure 5). As such, the majority of adults are lactose intolerant in vast regions of the world. Prevalence of lactase persistence is high in most regions of Europe and Germany (80–95%); even so this condition also observed in only 20–40% of Indian adults, 30% of adults in Mexicans, Americans and Africans, and <10% of adults in Southeast Asia (Scrimshaw and Murray, 1988; Itan et al., 2010).

Figure 5: Interpolated map of world’s prevalence of lactose intolerance and lactase persistence phenotype frequencies. Dots represent locations of lactase persistent data collection. Colors signify frequencies estimated by surface interpolation where green and blue color indicates majority of adults are lactose intolerant (Modified; Itan et al., 2010).
2.7 DIAGNOSIS OF LACTOSE INTOLERANCE

The primary lactase deficiency ought to be exceptional in response to removal of lactose from the diet, and in consequences two diagnostic methods have been developed. For instance either by direct method, as in the jejunal biopsies test (i.e., the measurement of disaccharides activities in intestinal biopsies) and/or indirect method, such as in the hydrogen breath test (HBT) or the lactose tolerance test (LTT) (Arola 1994). Other than these diagnostic methods, a genetic test have also been developed for the determination of lactose intolerance that is clinically straightforward with a few difficulties wherein subjects have self-diagnosed being lactose intolerant (Erica et al., 2014).

2.7.1 Jejunal biopsies test

This biopsy technique considered as ‘golden standard method’ for diagnosis of adult-type hypolactasia, to test for lactase activity. Although, it is not suitable for routine diagnosis next to highly invasive and alternate method usually preferred (Vonk et al., 2001). As such, it is suggested when the lactase activity <10 units per gram (U/g) protein and lactase/sucrase ratio < 0.3 is reported; samples screening sucrase activity < 40 U/g protein and maltase activity < 150 U/g protein, when lactase activity was < 20 U/g protein, signifying minor causes of hypolactasia are excluded from the study (Rasinpera et al. 2004; Enattah et al. 2007a).

2.7.2 Hydrogen Breath Test

Hydrogen Breath Test (HBT) is a non-invasive test to measure hydrogen (H₂) production by colonic bacteria using a breath gas analyser; commonly used for detecting lactase non-persistence (Metz et al. 1976; Howell et al. 1981; Lomer et al., 2008). Initially HBT check with a basal H₂ level recorded in majority of an individual, followed by an oral dosage of 50g lactose, as found in about a liter of milk (Lomer et
al., 2008). If this ingested lactose is hydrolyzed by the enzyme (lactase) in the small intestine thereafter breath excretions are taken at 30 min intervals from the time of ingestion for the following 3 h and submitted to gas chromatography analysis to measure H₂ (in ppm). Noticeably increase in H₂ Conc. exceeding 20 ppm is considered as a positive response which indicates much of the lactose consumed is undigested and is being fermented by intestinal bacteria, releasing large quantities of H₂ that is partially absorbed into the bloodstream and later excreted in the breath. If no changes observed in breath hydrogen then an individual can be classified as a lactose digester/lactose tolerant (Argnani et al., 2008; Ghoshal et al., 2009).

A study by Gupta et al., (2007) provides substantial evidence that, prior to the lactose load, peak level of breath hydrogen was similar between patients of IBS and healthy subjects. In contrast, 50g of lactose load afterward, a 24 h period of carbohydrate restriction observed followed by 12 h of fasting. This significantly reduces false-negative errors and also, antibiotics should be avoided for several weeks before the test when they affect intestinal bacterial levels, evidently stated in that study (Avallone et al., 2010). HBT is cost-effective and relatively reliable, although reliability is questionable if certain protocols are not observed to the extent that 20% of intolerant individuals have been known to provide false-negatives if conditions are not carefully followed.

2.7.3 Lactose Tolerance Test

Lactose Tolerance Test (LTT) is based on the measurements of blood glucose level after an oral dose of 50g lactose for testing tolerance to lactose by using glucometer (Peuhkuri et al., 2000). As the lactose digested in the intestinal tract, the resulting glucose and galactose sugars are absorbed into the bloodstream. A baseline
measurement of blood glucose is taken at various time intervals (usually every 30 min) for the following 2 h prior to ingestion of a lactose load. If there is increase in blood glucose indicates lactose digestion and no increase is indicative of a lactose non-digester/mal-digester or intolerant phenotype. A small rise in blood glucose concentration (< 1.1 mmol/l) and (> 1.7 mmol/l) indicates lactase non-persistence (hypolactasia) and lactase persistence (normolactasia) respectively (Seppo et al., 2008). Normally, failure of blood sugar to rise by >20 mg/dL above the fasting level 30 minutes after lactose ingestion is considered as a positive LTT, evidently stated in that study (Gupta et al., 2007; Ghoshal et al., 2009; Law et al., 2010). A study by Argnani et al., (2008) have been suggested that in the clinical setting, the use of the 12.5g lactose tolerance test should be probably preferred to the 25g test, as it may help to identify those patients who would profit from dietary restriction of lactose-containing food. The reliability of the LTT can also be slightly increased by adding a small amount of ethanol (300 mg/kg of body weight) to the load unfortunately for the test subjects, LTT involve a lactose load, which can lead to extreme discomfort for intolerant individuals.

2.7.4 Genetic Test

The genetic tests might be constructive in assessing primary lactose intolerant individuals; indeed to diagnose lactase non-persistence by polymerase chain reaction (PCR) restriction fragment length polymorphism (Buning et al., 2005; Hogenauer et al., 2005; Mattar et al., 2008; Deng et al., 2015). This method of diagnosis is simple, non-invasive, and more often than not incites symptoms of lactose intolerance and is less delicate (Buning et al., 2005). The persistence of Lactase activity in adults is associated with two polymorphisms: -C/T 13910 and -G/A 22018 located in the MCM6 gene, reported in that study (Enattah et al., 2002). Conversely, Tag et al., (2008) stated that reverse hybridization strip assay based on multiplex DNA amplification and ready-to-
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use membrane test strips that detect LCT polymorphic variants (-C/G 13907, -C/T 13910, -T/C 13913, -G/A 13914, -T/G 13915, and -G/A 22018) and certainly helps in overcoming the interference of different melting profiles of the real-time PCR kit by the other polymorphic variants.

2.8 SOURCES OF LACTASE

Lactase is widely distributed in nature, found in numerous plants especially in almonds, peaches, apricots and apples (Pomeranz, 1964; Richmond et al., 1981). In animals, lactase is found in the intestines of dogs, rabbits, calves, sheep, and rats. Moreover lactase is certain normal component of the human intestinal secretion naturally (Wallenfels and Malhotra, 1960). And can also be obtained from microorganisms including yeast, fungi and bacteria (Panesar et al., 2006; Husain, 2010). Some of these sources are quite extensively used as potent lactase producers as listed below in Table 2. Microorganisms offer various advantages over the animal and plant sources such as; easy handling, higher multiplication rate and high production yield. However, Lactic acid bacteria are preferable among the other bacterial sources owing to ease of fermentation, high activities of enzyme and good stability (Nam and Ahn, 2011).

Table 2: Diversity of lactase producers

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sources</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td><em>Kluyveromyces lactis</em></td>
<td>Kim et al., 2004; Tello-Solis et al., 2005; Teles de Faria et al., 2012</td>
</tr>
<tr>
<td>2.</td>
<td><em>Kluyveromyces marxianus</em></td>
<td>Furlan et al., 2000; Martins et al., 2002; Pinheiro et al., 2003; Ribeiro et al., 2007</td>
</tr>
<tr>
<td>3.</td>
<td><em>Guehomyces pullulans</em></td>
<td>Nakagawa et al., 2006</td>
</tr>
</tbody>
</table>
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### Fungi
5. *Aspergillus nidulans* -do-  

### Bacteria
10. *Bifidobacterium longum* Hsu *et al.*, 2005  
11. *Bacillus sp.* Jayashree *et al.*, 2012  
12. *Bacillus licheniformis* Akcan, 2011  
13. *Lactobacillus reuteri* Sung *et al.*, 2003  
15. *Lactobacillus bulgaricus* -do-  
16. *Lactobacillus delbrueckii* -do-  
17. *Lactobacillus brevis* Bhalla *et al.*, 2015  

### Plants

### Animal
25. Mice Portugall *et al.*, 2004
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2.8.1 Lactic Acid Bacteria

The lactic acid bacteria (LAB) constitute a diverse group of *Lactococci*, *Streptococci* and *Lactobacilli*, and have made a contribution towards the scientific studies for three reasons: firstly, LAB are found in fermenting vegetative matter and milk, and are major contributors to the production and flavoring of fermented dairy products such as yogurts and cheeses (Marshall, 1987; Smit *et al.*, 2005). Secondly, they are generally regarded as safe (GRAS) so that enzyme derived from them can be applied directly in the food industry without extensive purification process; exhibited a remarkably higher storage and temperature stability in the crude enzyme extract (Splechtna *et al.*, 2007; Iqbal *et al.*, 2010). Last but not least, some LAB strains are also found in the human gut to facilitate probiotic activity such as improved digestion of lactose by the action of enzyme (lactase), and are associated with positive effects to the health (Guarner and Malagelada, 2003; Hempel *et al.*, 2012).

Generally, LAB can be characterized as Gram-positive rods or coccobacilli, non-motile, non-spore forming; catalase-negative, aerobic to facultative anaerobes requires specific media which produces lactic acid as the major end product from the energy yielding fermentation of sugars (Wood and Holzapfel 1995, Axelsson 1998., Carr *et al.*, 2002). According to Nobuta *et al.*, (2009) a traditional Japanese fermented vegetable i.e. Suguki (*Brassica rapa*) used for lactic fermented beverage by *Lactobacillus brevis* KB290 that improves gut health; and also stimulates immune function reported in previous literature (Kishi *et al.*, 1996). The potential probiotic strains were examined in traditional fermented vegetables reported in that study (Kawahara and Otani, 2006; Murooka and Yamshita 2008). In recent times, several research ongoing for the production β-galactosidase (lactase) enzyme i.e. widely used in the dairy industry and is produced by most of *Lactobacillus sp* as mentioned earlier in Table 2.
2.9 SOURCE OF LACTOSE SUBSTRATE

Whey is the watery part of milk generated after curd formation as a by-product of cheese manufacturing which is produced in bulk quantity. The main solute is lactose in cheese whey at a concentration of about 4.5-5% and additional components are proteins, salts and vitamins that are present in less quantity. The low concentration of these components makes their recovery inefficient. As a result of its high organic content, dumping directly to the environment causes pollution problems. The resolution is bioconversion of whey into lactase has been performed in several countries (Nahvi and Moeini, 2004). These changes are cradle for the production of new products such as lactose hydrolyzed milk (Mahoney, 1985). In whey 4.8% content of lactose and relatively high levels of other nutrients that make it suitable as a microbial culture medium.

2.10 APPLICATION STUDIES

Lactase is employed for the chemical reaction of milk sugar that is one amongst the foremost vital biotechnological processes within the food and dairy farm trade. It’s probably helpful effects on the assimilation of foods containing milk sugar, additionally because the attainable technological and environmental benefits of business applications (Gekas and Lopez-Leiva, 1985; Jurado et al., 2002). These applications square measure listed below:

1. Lactase has catalytic property to change disaccharide lactose into its derivative sugars i.e., glucose and galactose. This recognition of hydrolysis that has been widely used for milk and fermented dairy products (Jurado et al. 2002).
2. Lactose is employed for up sweetness, the solubility of the milk product, broader fermentation potentialities and reduced milk sugar concentration with associated shrivel chance of milk sugar crystallization (Zadow, 1992).

3. Low disaccharide milk and its product once consumed typically decrease the intolerance symptoms (Johnson et al., 1993).

4. Enhanced biodegradability of whey after lactose hydrolysis (Santos et al., 1998).

5. Formation of GOS (galactooligosaccharides) throughout milk sugar reaction to favor the development of intestinal bacterial microflora (Mahoney, 1998).

Lactase or β-galactosidase, an enzyme has concerned interest of the researchers throughout the last three decades owing to its wide area of applications in beverages, confectionary, baking and dairy industries (Panesar et al., 2010; Kara, 2004). A large number of microbes have been assessed as potential sources of β-galactosidase, as a viable significance (Panesar et al., 2010). Moreover, the above mentioned microorganisms employed to produce the lactase have varying nutritional requirements and in consequence they produce enzymes excluding β-galactosidases such as proteolytic and lipolytic enzymes which can produce lesser organoleptic properties and/or other quality defects in milk and dairy products (Grieve et al., 1983; Kumar, 2005).