Metabolism of Starter Cultures (Proteins, Carbohydrates, Citrate)

The primary function of lactic acid bacteria (LAB) is to produce lactic acid by fermenting the milk sugar lactose. Fermentation of lactose is carried out by different pathways by different types of starter cultures. Microbial cells derive their energy requirements via Fermentations, Tri-carboxylic acid cycle, Cytochrome system for terminal electron transport.

Production of lactic acid

Lactose is a disaccharide constituting about 40% of milk solids. LAB use two strategies to hydrolyze lactose; β-D galactosidase (lactase or β-gal) and β-D phosphogalactosidase (β-p-gal)

Many lactic acid bacteria possess both β-gal and β-p-gal enzymes. Most lactobacilli have β-gal enzyme except *L. casei* which exhibits β-p-gal activity. Lactococci possess both but in starter cultures only β-p-gal activity is seen.

Lactose is transported into the lactic acid bacteria via two systems (through Permeases and Phosphoenolpyruvate dependent phosphotransferase system (PEP:PTS).

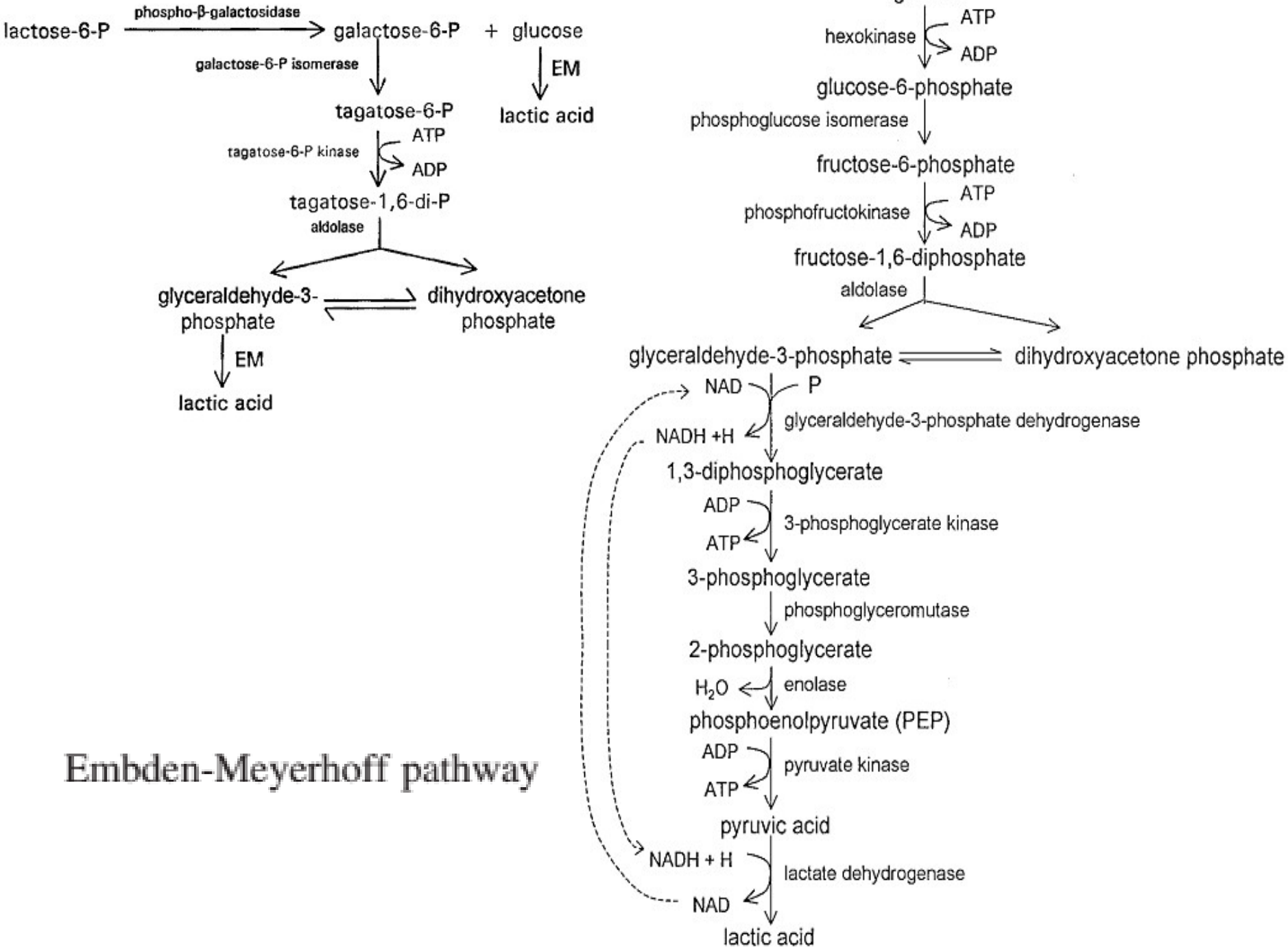
Lactose is broken down to glucose and galactose by β-D galactosidase by catalization of 1-4 β- galactosidic bond in lactose.

Anaerobic pathway leads to incomplete breakdown of glucose releasing small amounts of energy. TCA cycle is aerobic and in this glucose or acetate is oxidized to CO_2 and H_2O.

Lactic acid bacteria lack cytochrome or electron transport proteins, and therefore cannot derive energy via respiratory activity. Thus, substrate-level phosphorylation reactions that occur during glycolysis are the primary means by which ATP is obtained. Glucose is used in both aerobic and anaerobic organisms. Aerobic organisms have mechanisms to complete the catabolism of end products to CO_2 and H_2O.

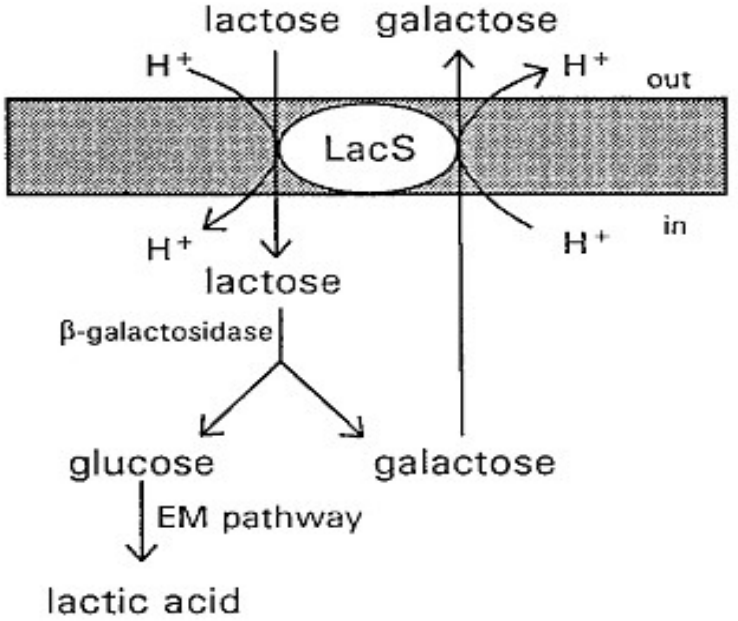
Homofermentation:

In homo-fermentative LAB, the lactose transport across the cell membrane involves the Phosphoenolpyruvate dependent phosphotransferase system wherein the lactose is phosphorylated to lactose-P during its translocation. Lactose- 6 P is hydrolyzed by phospho-β-galactosidase to D-glucose and galactose-6 phosphate. Glucose is metabolized to pyruvate via Embden Meyerhof Pathway (EMP). Galactose is first converted to glyceraldehyde -3 phosphate via D-tagatose-6 phosphate pathway. So two pathways are involved: EMP for the metabolism of glucose and tagatose 6-phophate pathway for metabolism of galactose.

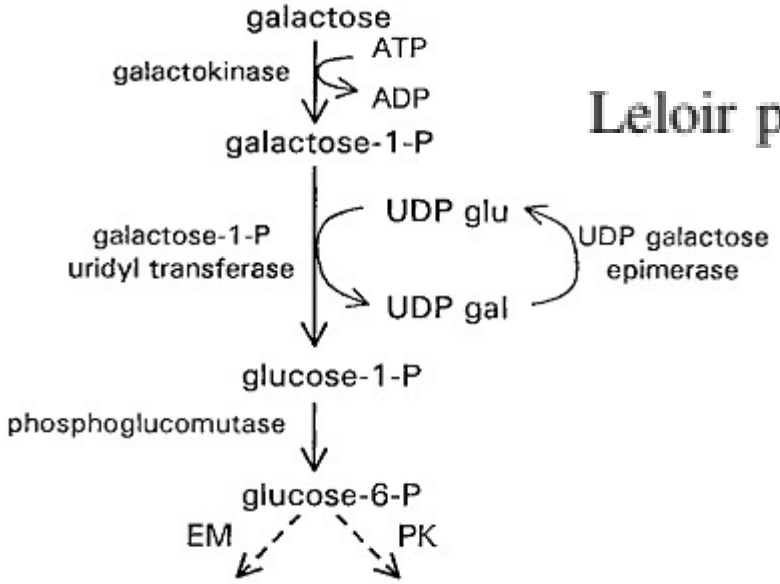


Embden-Meyerhoff pathway

Lactose in *S. thermophilus* and *Lb. bulgaricus* is transported into the cell by enzyme Permease. These organisms possess **β-D galactosidase (β-gal)** enzyme which hydrolyzes lactose into **β-D galactose** and **D-glucose**. Glucose is converted into lactic acid via EMP pathway. Galactose is excreted from the cell/may be utilized by other bacteria producing other than lactic acid. Dairy Leuconostocs also rely on a lactose permease for uptake of lactose. Some lactococci and lactobacilli have the ability to use both systems.



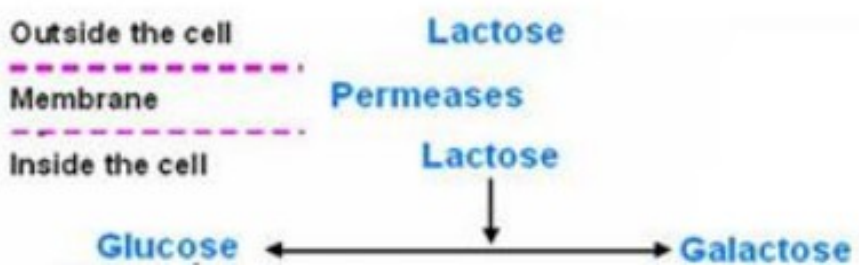
For the lactococci and some lactobacilli, free galactose appears to be transported by either a galactose-specific PTS or by a galactose permease. The intracellular product of the galactose PTS (galactose-6-phosphate) simply feeds into the tagatose pathway. When galactose accumulates via galactose permease, the intracellular product is free galactose. Subsequent metabolism occurs via the Leloir pathway, which phosphorylates galactose, and then converts galactose-1-phosphate into glucose-6 phosphate which then feeds into the glycolytic pathway. The Leloir pathway is used by lactococci, *Lb. helveticus*, *Leuconostoc* spp., and galactose-fermenting strains of *S.thermophilus*.

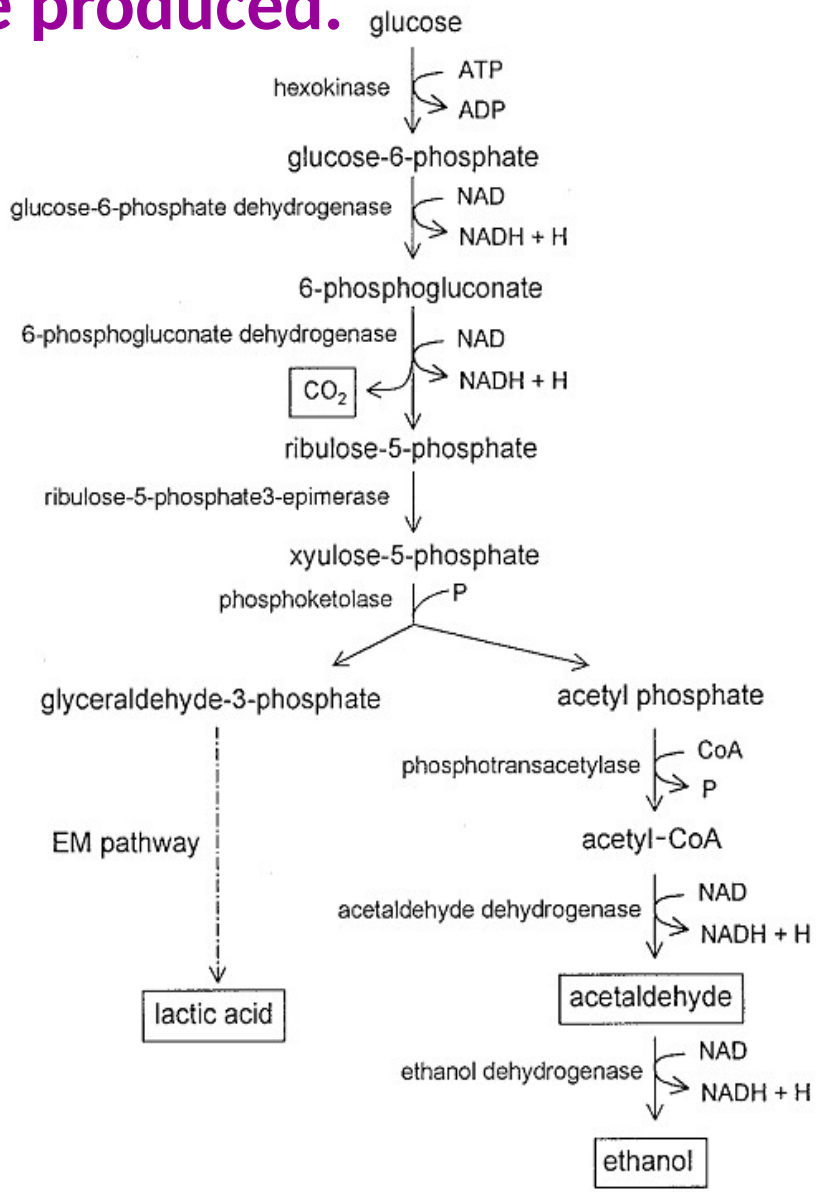


Leloir pathway in lactic acid bacteria.

In some instances, they will also encounter free extracellular galactose, especially if they are grown in the presence of galactose-nonfermenting strains. Subsequent galactose fermentation by *L. helveticus* and *Leuconostoc* occurs via the Leloir pathway.

Heterofermentation: In heterofermentative LAB, large quantities of ethanol, CO_2 as well as lactic acid when grown on lactose or glucose are produced.



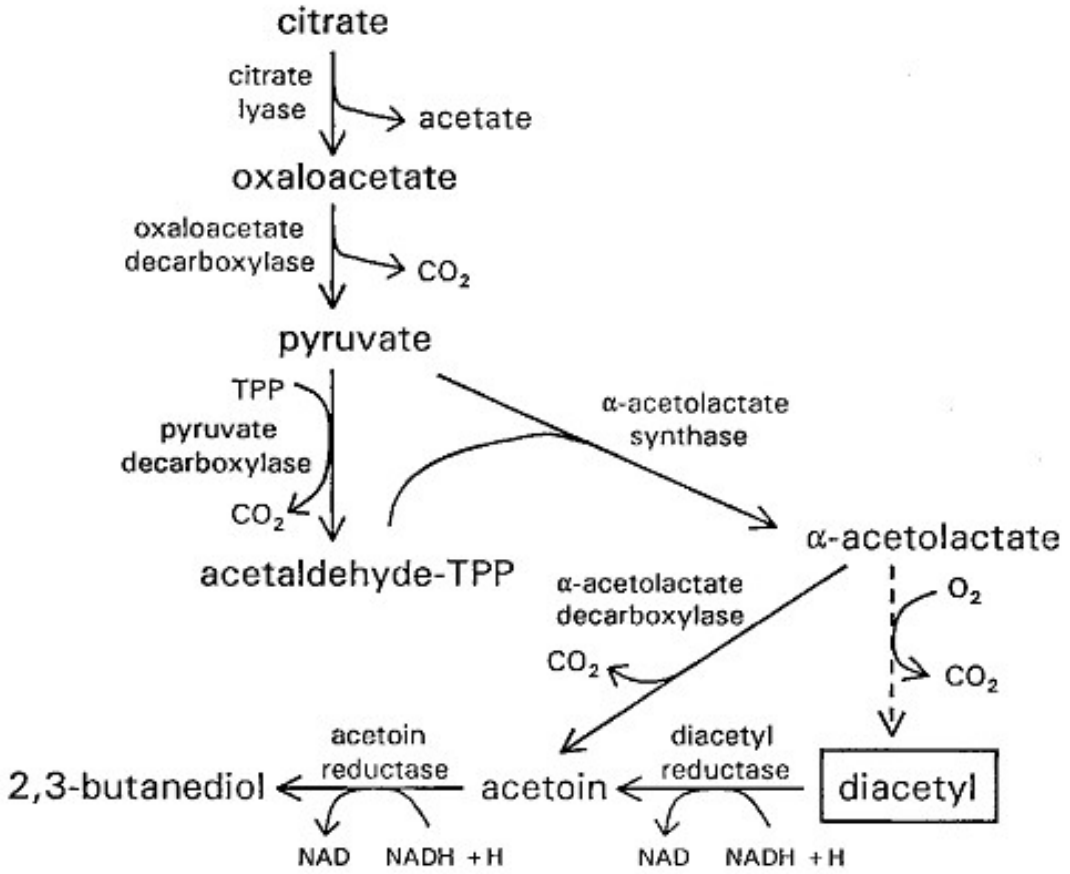


Citrate Metabolism

1. Citrate content of milk varies between 1.4-2.0 g per kg of milk. It is a normal constituent of milk and forms one of the main buffer systems that regulates the equilibrium between Ca2+ and H+ ions.
2. Citrate affects milk-processing characteristics because it interacts with other milk constituents to influence coagulation of milk protein and its fermentation products yield distinct aromatic flavor characteristic of fermented milk products.
3. The main role of citrate is maintenance of fluidity through its effects on structure of casein micelles.
4. Citrate utilization is most often associated with *Leuconostoc* spp. and selected strains of *Lactococcus* sp. In *L. lactis* subsp. *lactis* biovar *diacetylactis*, citrate fermentation is linked with 8 kb plasmid, whereas in *Leuconostoc*, citrate genes are associated with plasmids as large as 22 kb. These plasmids contain a cluster of genes that encode citrate permease (CitP) in *L. lactis* subsp. *lactis* biovar *diacetylactis* and CitP and citrate lyase in *Leu. paramesenteroides*.
5. Two pathways have been proposed where citrate is transported by the pH-dependent CitP optimum activity between pH 5 and 6.

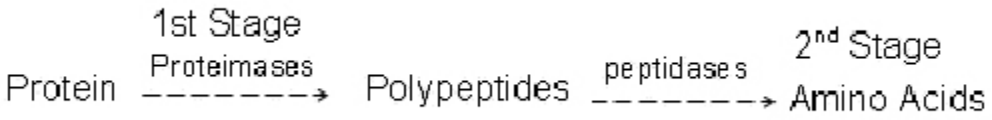
Diacetyl is an intermediate product. Concentration varies during manufacturing cultured dairy products with subsequent disappearance. There is relationship between the concentration of diacetyl and acidity of the product. To achieve the highest concentration of diacetyl in fermented milk, pH has to ≤ 4.6 for controlling diacetyl and the citric acid has to be completely fermented.

Dairy products require some diacetyl concentration for acceptable flavour. Active diacetyl reductase reduces flavour by converting diacetyl to acetoin. The potential source of the diacetyl reductase is the contaminating bacteria (Gram negative psychrotrophs, coliforms and yeasts.



Protein Metabolism

The hydrolysis of protein to yield amino acids can be accomplished in two major stages:



The range of products released by proteolysis is dependent on two main factors: firstly, the components of the milk protein fraction, and secondly, the types of proteolytic enzymes that the starter organisms may possess.

Enzymes acting on peptide bonds are known as peptide hydrolases. Peptide hydrolases are divided into two main groups, i.e. the peptidases and the proteinases. Peptidases (exopeptidases) specifically catalyze the hydrolysis of terminal α-amino or α-carboxyl groups of the peptide bonds. Proteinases catalyze the hydrolysis of the internal peptide linkages of protein.

Yoghurt starter cultures are weak in proteolytic fermentation. *S. thermophilus* and *L. bulgaricus* may produce proteolytic enzymes during fermentation, which cause a significant degree of proteolysis, and this activity needed for the following reasons: The enzymatic hydrolysis of milk proteins results in the liberation of peptides of varying sizes and free amino acids. The possible changes due to proteolytic activity can affect the physical structure of yoghurt. The liberation of amino acids into the milk is essential to the growth of *S. thermophilus*.

Vitamin Metabolism

- Milk and yoghurt contains both fat and water-soluble vitamins and various antimicrobial compounds synthesized by the starters. The content of these vitamins changes during the growth of starter cultures in fermented milk products preparation.
- Vitamins which increase during manufacture of yoghurt, are niacin and folic acid because they are actively synthesized by the starter cultures. The increases in folic acid and niacin in yoghurt (made from whole milk fortified with 2% SNF and incubated for 3 h at 42°C) amounts to 3.946 and 22 µg/100 g respectively.
- Though vitamin B12 decreases during yoghurt production, but some sp. of *Lactobacillus* and strains of yoghurt starter culture synthesize vitamin B_{12}.
- *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* synthesize less niacin and folic acid in comparison to vitamin B6 during the production of yoghurt.

- Excessive heat treatments of the milk (boiling for 5 mins) causes greater losses of the above vitamins. Vitamin B12 is reduced to 1.78 µg/l.
- The yoghurt starter bacteria utilize some of the vitamins present in milk during the fermentation period to meet their growth requirements. This factor contributes to a reduction of the nutritional properties of the product. However, the quantities consumed are dependent on the rate of inoculation, the strain of yoghurt starter and the conditions of fermentation.
- Some vitamins decrease during the storage of yoghurt at 4°C. During the storage of yoghurt at 5°C for 16 days, loss of folic acid and vitamin B12 is 28.6 and 59.9% respectively. A decrease in the biotin, niacin and pantothenic acid contents, and these losses were due to the combined effect of microbial catabolism during the incubation period and chemical decomposition of these vitamins during cold storage.

Biosynthesis of Folic acid (Folacin)

- Folate is a generic name given to around ten different compounds which share a basic structural unit connected to "conjugates" of different numbers of glutamic acid residues.
- Many organisms require folacin as a growth factor.
- It functions as a coenzyme in many different biochemical reactions, i.e. as an activator and carrier of carbon units during oxidation and it participates in the metabolism of purines, pyrimidines and some amino acids.

Biosynthesis of Niacin

- Niacin activity was exhibited by nicotinic acid and nicotinamide. The former compound constitutes part of the structure of the two important coenzymes i.e. NAD and nicotinamide adenine dinucleotide phosphate (NADP). These two coenzymes are composed of adenylic acid and nicotinamide ribotide linked through their phosphate groups.
- As NAD and/or NADP are essential for many oxidative/reductive biochemical reactions, the niacin synthesized by *S. thermophilus* and *L. bulgaricus* may originate from the nicotinamide fraction arising during the formation of NAD and/or NADP.

- **The biosynthesis of these nucleotides involves the steps:**

Firstly, the synthesis of a sugar moiety.

Secondly, the synthesis of the pyrimidine/purine base.

Alternatively, after this formation of NAD and/or NADP, the nicotinamide fraction could be released as a result of the degradation of these nucleotides.

- **Nicotinic acid is derived by a few bacteria from the metabolism or breakdown of tryptophan, a pathway which is dependent on the availability of certain vitamins, e.g. thiamine, riboflavin and vitamin B6, to activate the required enzymes. *S. thermophilus* and *L. bulgaricus* utilizes these vitamins and tryptophan does not accumulate during yoghurt production, it is possible that these organisms use the vitamins for the synthesis of niacin.**

Biosynthesis of Vitamin B6

- The activity of vitamin B6 is exhibited equally by the following compounds: pyridoxine, pyridoxal and pyridoxamine. The basic structure of these compounds is similar which consists of a pyridine ring, but they differ in the respect of the radical components as follows:

Pyridoxine	($C_8N_{11}NO_3$)	R= CH_2OH
Pyridoxal	($C_8N_9NO_3$)	R= CHO
Pyridoxamine	($C_8N_{12}N_2O_2$)	R= CH_2NH_2

- No information is available on the biosynthesis of the pyridine ring in microorganisms, plants or animals; however, the different forms of vitamin B6 are inter convertible by microorganisms.