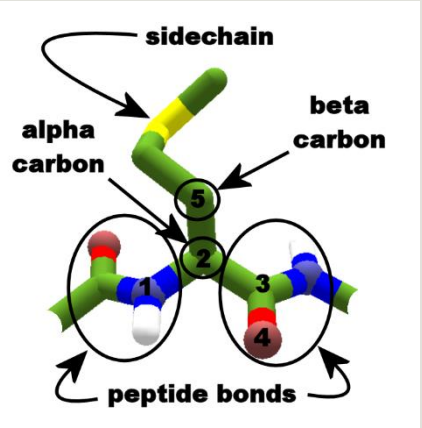
Enzymes Production



All enzymes have infrastructural backbones of protein. In some enzymes only proteins exist, while in others, covalently attached carbohydrate groups may be present; often these carbohydrate groups may play no part in the catalytic activity of the enzyme, though they may contribute to the stability and solubility of the enzyme. Metal ions known as co-factors and low molecular weight organic compounds, known as coenzymes may also be present. Co-factors and co-enzymes are important for the stability and activity of the enzyme. They have a tendency to be detached and it is important to provide conditions which ensure their retention.

Advantages

(a) Plants and animals grow slowly in comparison with microorganisms;

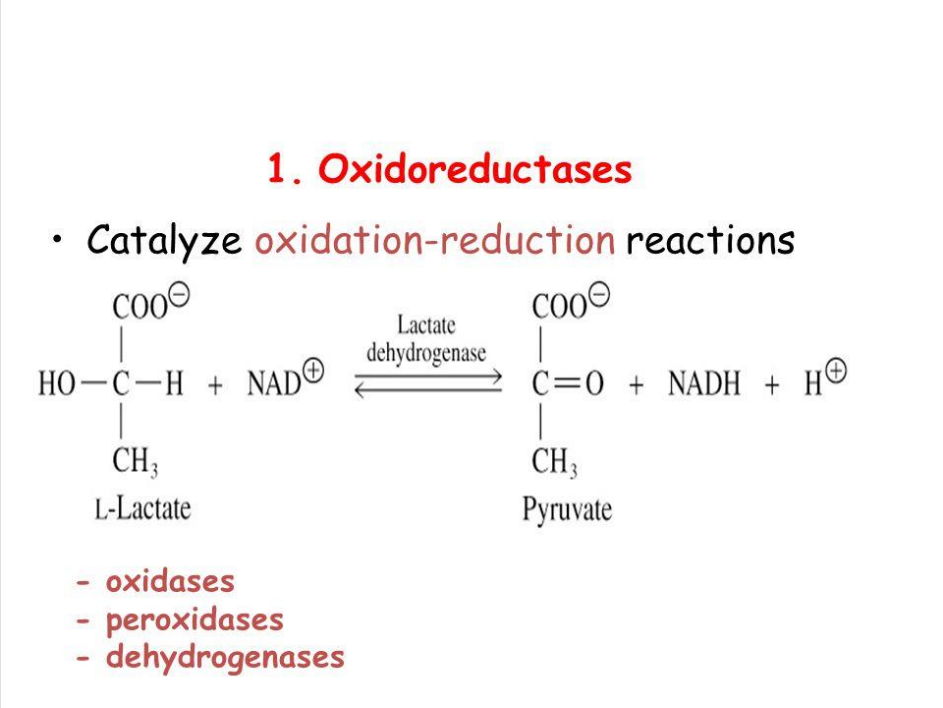
(b) Enzymes form only small portions of the total plant or animal and large tracts of land as well as huge numbers of animals would be necessary for substantial productions. These limitations make plant and animal enzymes expensive. Microbial enzymes on the other hand are not subject to the above constraints and may be produced at will in any desired amount.

(c) By far the greatest attraction for the production of microbial enzymes, however, is the great diversity of enzymes which reflects the diversity of microbial types in nature. Thus largely, though not entirely, because of the widely varying environmental conditions in nature, microbial enzymes have been isolated which operate under extreme environmental conditions. For example microorganisms produce amylases functioning at temperatures as high as 110°C and proteases operating at pH values as high as 11 or as low as 3.

(d) Finally, following from greater understanding of the genetic basis for the control of physiological function in micro-organisms it is now possible to manipulate microorganisms to produce virtually any desired metabolic product, including enzymes.

The six major EC groups are as follows.

1. Oxidoreductases catalyze a variety of oxidation-reduction reactions. Common names include dehydrogenase, oxidase, reductase and catalase.



2. Transferases catalyze transfers of groups (acetyl, methyl, phosphate, etc.). Common names include acetyltransferase, methylase, protein kinase, and polymerase. The first three subclasses play major roles in the regulation of cellular processes.

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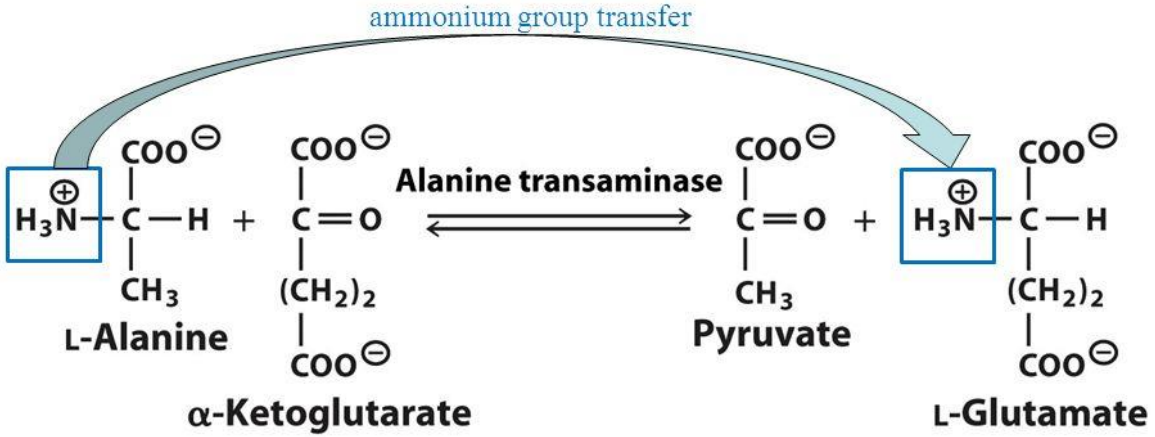
2. Transferases catalyze group transfer reactions

-These enzymes usually require a coenzyme be present.

-coenzyme: complex organic molecule (often a vitamn) needed for catalysis



3. Hydrolases catalyze hydrolysis reactions where a molecule is split into two or more smaller molecules by the addition of water. Some examples are:

Proteases: Proteases split protein molecules. They are further classified by their optimum pH as acid, alkaline or neutral. They may also be classified on the basis of their active

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centers into the following:

- (i) *Serine proteases*: These have a residue in their active center and are specifically inhibited by diisopropyl phosphofluoridate and other organophosphorus

derivates.

- (ii) *Thiol proteases*: The activity of these depends on the presence of an intact-SH group in their active center. They are specifically inhibited by thiol reagents such asheavy metal ions and their derivatives, as well as alkylating and oxidizing agents.
- (iii) *Metal proteases*: These depend on the presence of more of less tightly bound divalent cations for their activity.
- (iv) *Acid proteases*: Acid proteases contain one or more side chain carboxyl groups intheir active center.

4. Lyases catalyze the cleavage of C-C, C-O, C-S and C-N bonds by means other than hydrolysis or oxidation. Common names include decarboxylase and aldolase.

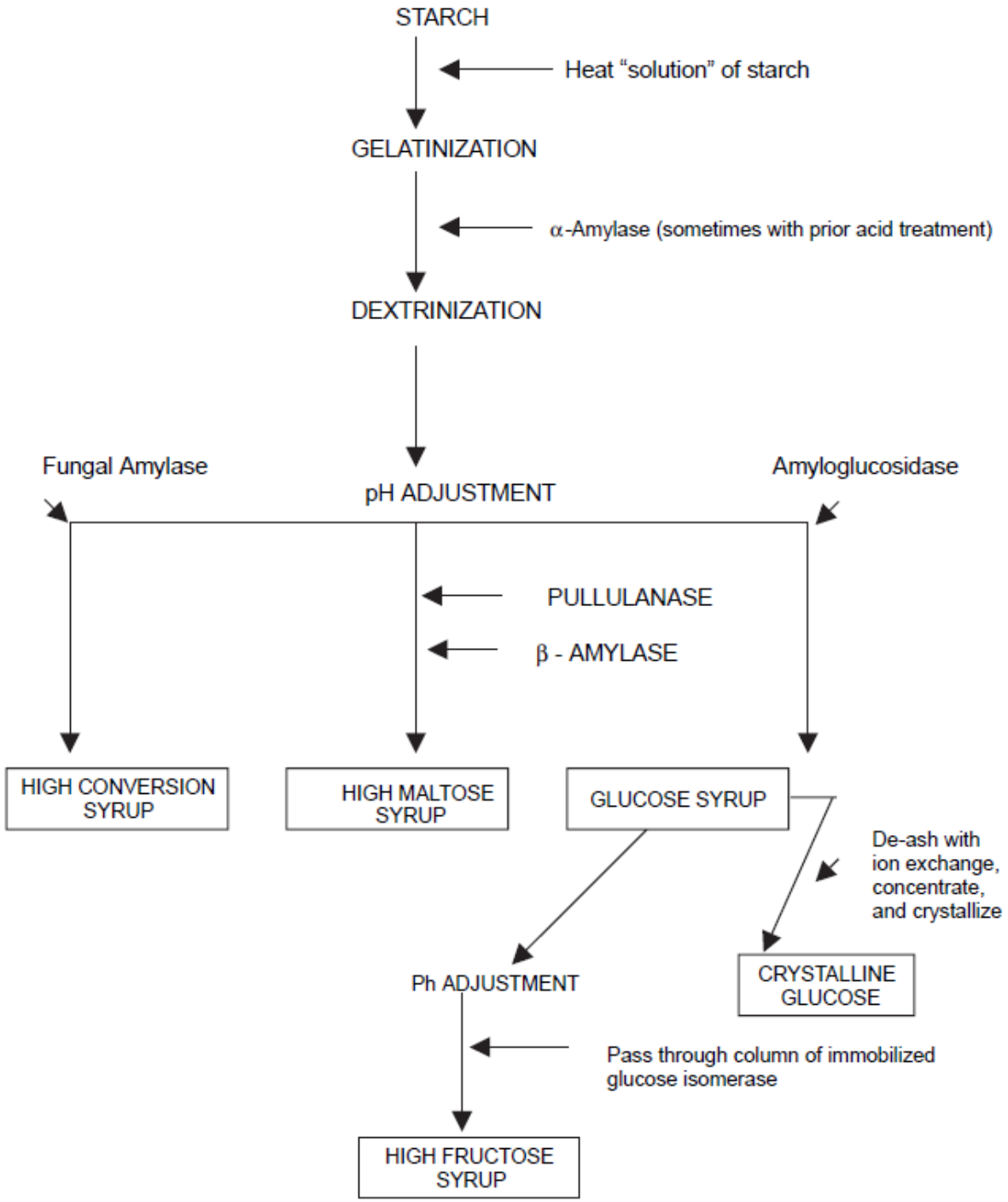
5. Isomerases catalyze atomic rearrangements within a molecule. Examples include rotamase, protein disulfide isomerase (PDI), epimerase and racemase.

6. Ligases catalyze the reaction which joins two molecules. Examples include peptide synthase, aminoacyl-tRNA synthetase, DNA ligase and RNA ligase.

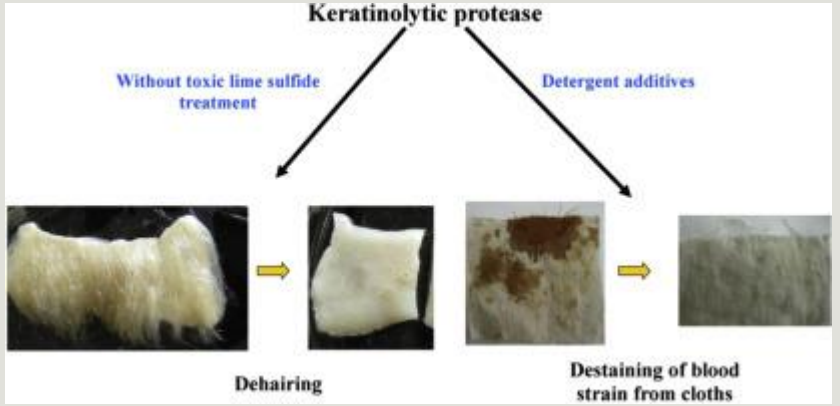
USES OF ENZYMES IN INDUSTRY

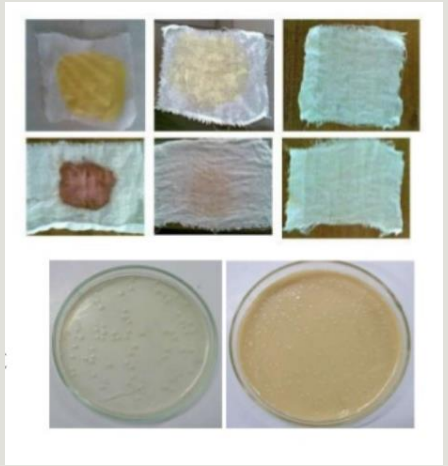
(i) ***Production of nutritive sweeteners from starch*:**

The sweeteners
which have been produced from starch are high conversion (or high DE) syrup, high maltose syrup, glucose syrup, dextrose crystals and high fructose syrup. These sweeteners are often called corn syrups because they are produced from maize, although starch from any source (e.g. cassava, sorghum, or potatoes) may be used. The processes of production of sweeteners from corn consists of the gelatinization of starch production of water-soluble dextrins with -amylase, the subsequent application of a de-branching enzyme (e.g. pullulanase) and, depending on the sugar sought, the application of a third enzyme. -Amylase from *B. licheniformis* is particularly suitable for dextrinization because its optimum temperature is 110°C, a convenient temperature on account of the need to boil starch to gelatinize it. If high maltose syrup is sought -amylase is applied, while gluco-amylase is applied if glucose syrup is sought


STARCH
Heat "solution" of starch
GELATINIZATION
α-Amylase (sometimes with prior acid treatment)
DEXTRINIZATION
Fungal Amylase
pH ADJUSTMENT
Amyloglucosidase
PULLULANASE
β - AMYLASE
HIGH CONVERSION SYRUP
HIGH MALTOSE SYRUP
GLUCOSE SYRUP
De-ash with ion exchange, concentrate, and crystallize
Ph ADJUSTMENT
CRYSTALLINE GLUCOSE
Pass through column of immobilized glucose isomerase
HIGH FRUCTOSE SYRUP

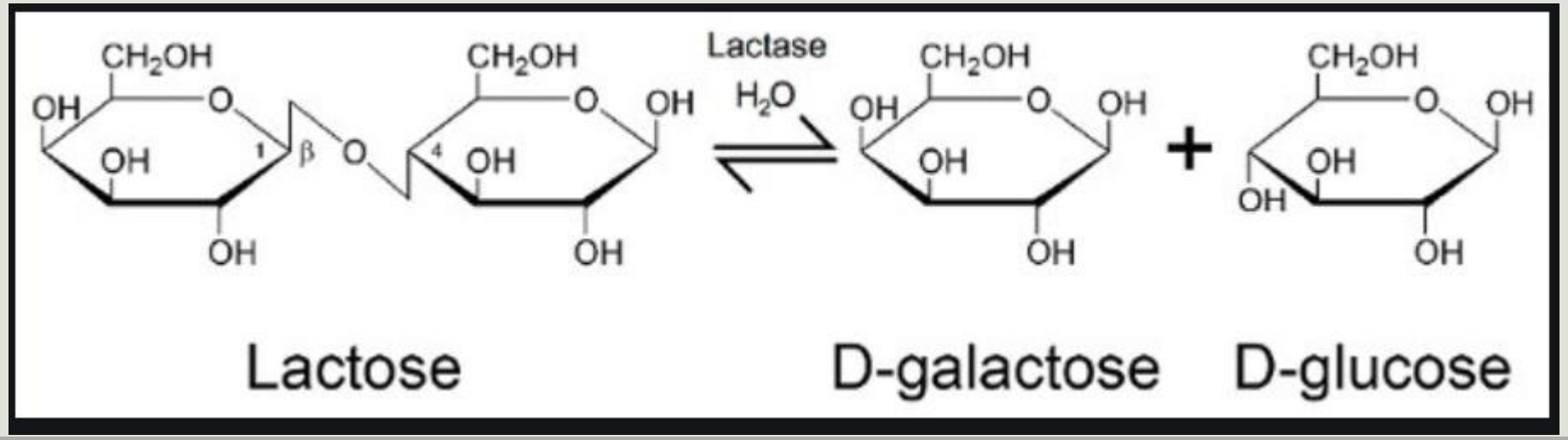
(ii) *Proteolytic enzymes in the detergent industry*: The detergent industry is at present one of the greatest consumers of enzymes, and uses mostly proteases. Blood and pus stains from hospital linen and other protein dirt precipitate and coagulate on clothes and are ordinarily difficult to remove. The inclusion of proteolytic enzymes in a detergent or washing soap greatly facilitates the removal of such stains. The proteolytic enzymes used for this purpose should have a high pH optimum of 9-11, which is the pH of detergents, and a high temperature optimum of 65-70°C since hot water facilitates laundering. Furthermore, the enzyme should be able to cleave peptide bonds randomly and facilitate the dissolution of the protein. Such proteolytic enzymes have been produced mainly by alkalophilic and aerobic spore-formers such as strains of *Bacillus licheniforms* and *Bacillus amyloliquifaciencs.* The latter has the advantage of producing -amylases as well.

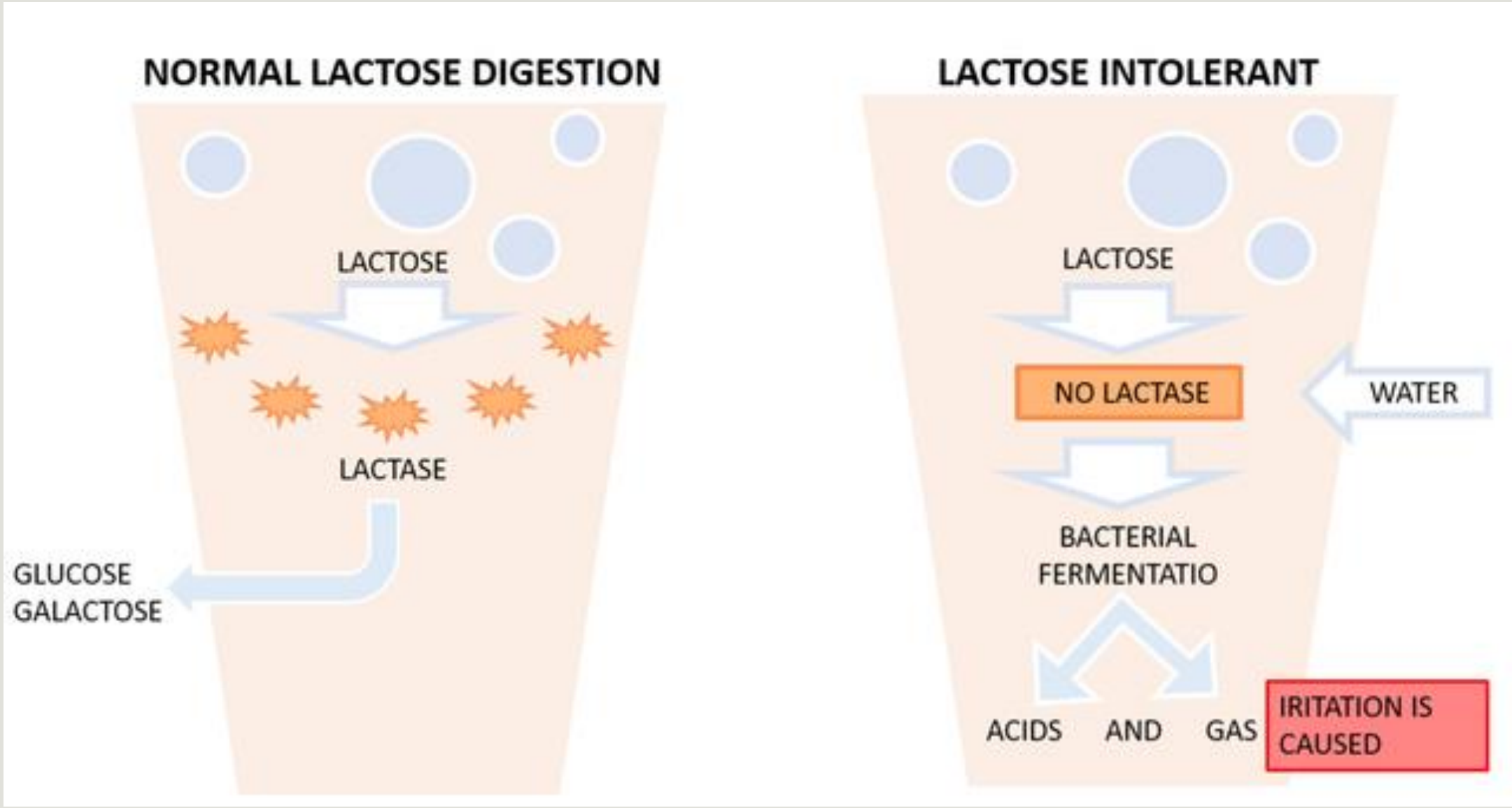




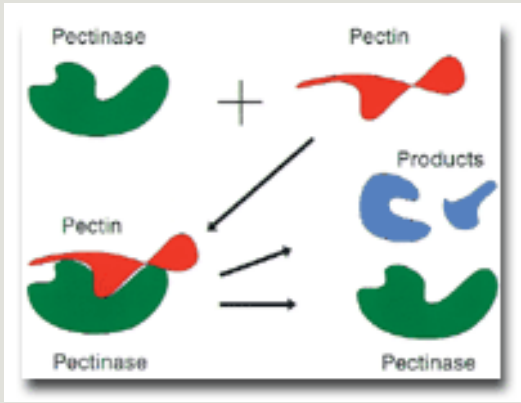
(iii) *Microbial rennets*: Rennin is an acid protease found in gastric juice of young mammals where it helps to digest milk. It is used in the manufacture of cheese and functions by hydrolyzing a polypeptide fragment from milk protein-kappa casein to leave paracasein; this then forms an insoluble complex with cations to give a firm curd. The commercial form of rennin known as rennets is obtained from the fourth stomachs of young calves. It is therefore expensive and tedious to produce since it involves the maturation, gestation and delivery of cows. Due to this, a search for substitutes ensued. Strains of *Mucor miehi, M. pusillus, Endothia parasitica, Bacillus polymyxa, B. subtilis* and *Aspergilus* are used to produce acid proteases which successfully substitute for rennin. Indeed, microbial rennets constitute about the third largest use of microbial enzymes.

(iv) *Lactase*: Lactase hydrolyzes the disaccharide lactose into its component galactose and glucose, both of which are sweeter than lactose and correspond to the addition of 0.9% sucrose. Thus, dairy products containing lactose, such as yoghurt, and ice cream, can be sweeter and more acceptable to consumers without the extra expense of extraneously added sugar. Galactose and glucose are also metabolized by a far wider range or organisms than can attack lactose. The result is that lactase-hydrolyzed whey can be used to produce alcohol or soft drinks. Furthermore, milk in which lactose is hydrolyzed is preferred by individuals in some parts of the world where intestinal lactase is low. Finally, when lactose occurs in high concentrations, such as is in ice cream, it tends to crystallize out giving the impression during consumption that grains of sand are present in the product. The addition of lactase prevents such crystallization.Lactase is now produced commercially from *Kluyveromyces fragilis, Saccharomyces lactis or Aspergillus niger.*




NORMAL LACTOSE DIGESTION
LACTOSE
LACTASE
GLUCOSE
GALACTOSE
LACTOSE INTOLERANT
LACTOSE
NO LACTASE
WATER
BACTERIAL
FERMENTATIO
ACIDS
AND
GAS
IRITATION IS
CAUSED

(vi) ***Pectinases for use in fruit juice and wine manufacture:*** Pectinases are enzymes which attack pectic substances, a group of complex acidic polysaccarides. Pectic substances are high molecular weight substances made up of poly – D – galacturonic acid. As the carboxylic acid groups of the sugar units are partially esterified with methanol, they are regarded as poly-uronides. They are the cementing material holding plant cells together.



(vii) ***Naringinase*** is used for removing the bitter tasting substance from citrus fruits, especially grape fruits. Naringin is a flavonoid found in grapefruits, and gives grapefruit its characteristic bitter flavor. Flavonoids are a group of polyphenolic secondary metabolites secreted by plants and found widely among plants. They are present in many plant-based foods such as tea and soybeans, and are generally believed to be beneficial to health. Although naringin is supposed to have some beneficial effect such as stimulating our perception of taste by stimulating the taste buds (for which reason some people eat grape fruits before a meal), the bitter taste is undesirable in fruit juices. Therefore, grapefruit processors attempt to select fruits with a low naringin content, or use naralginase produced by strains of *Aspergillus* spp. to remove it.

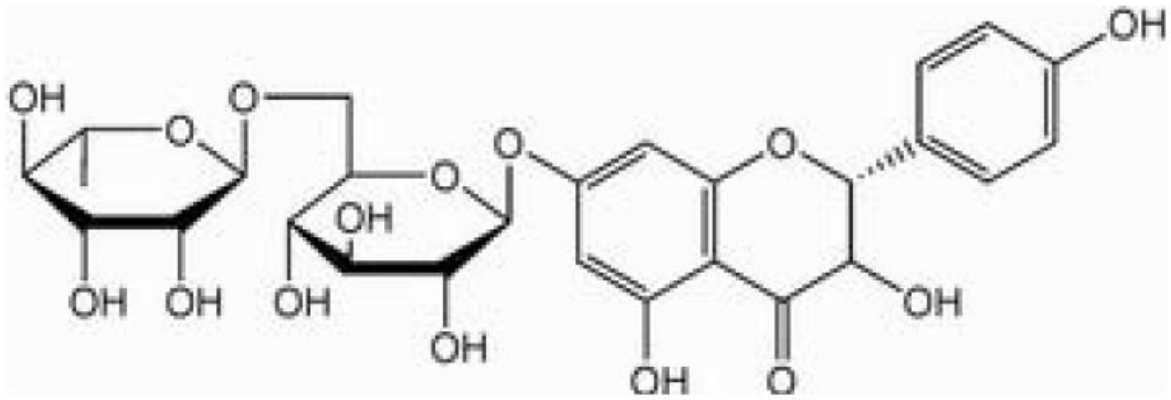
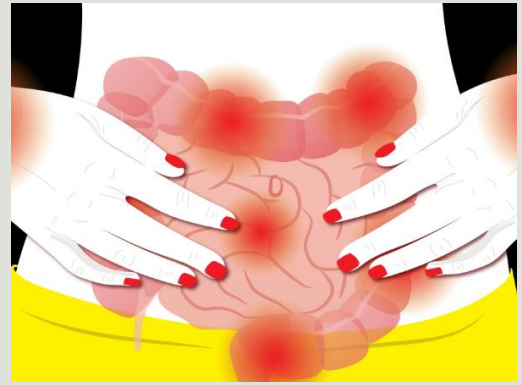


Fig. 22.3 Structure of Naringin

(xi) *Some medical uses of microbial enzymes*: At present, the most successful medical applications of enzymes are the use of proteolytic enzymes from *Bacillus* spp. and other bacteria for the treatment of burns and skin cancers, and the treatment of life-threatening disorders within the blood circulation using hemolytic enzymes produced from Bhemolytic streptococci. Other uses are given below:



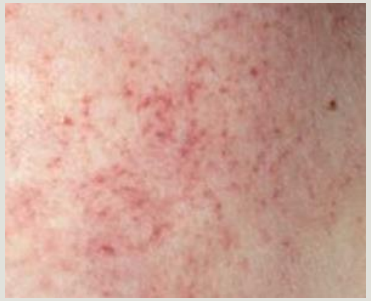
(a) Fungal acid proteases may be used to treat alimentary dyspepsia, because of the acid resistance of the enzyme. Fungal amylases may also be used to help digestion.

(b) Dextrans deposited on the teeth by *Streptococcus mutans* may be removed with the use of fungal dextranase often introduced into the toothpaste, thus helping to fight dental decay.

(c) L-asparaginase produced from *E. coli* and other gram-negative bacteria may be used in the treatment of certain kinds of leukemia.

(d) Penicillinases produced by many organisms are sometimes used in emergency cases of penicillin hypersensitivity.

(e) Rhodanase which catalyses the reaction, $S_2O_3^{2-} + CN^- \longrightarrow SO_3^{2-} + SCN^-$ has been used to combat cyanide poisoning. Rhodanase is produced by the thermoacidophilic bacterium *Sulfobacillus sibiricus.*

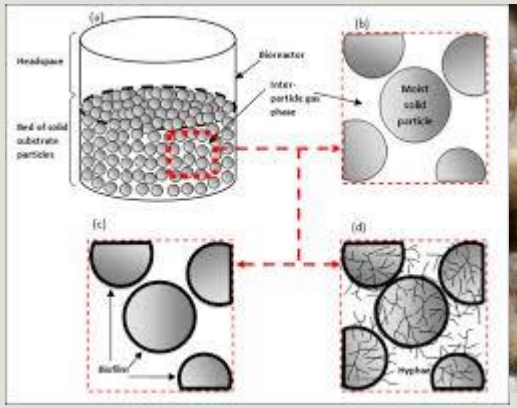


The above are only a few of some of the uses to which microbial enzymes have been put in the medical area.

PRODUCTION OF ENZYMES

Semi solid medium

This system, also known as the ‘Koji’ or ‘moldy bran’ method of ‘solid state’ fermentation is still widely used in Japan. The medium consists of moist sterile wheat or rice bran acidified with HCl; mineral salts including trace minerals are added. An inducer is also usually added; 10% starch is used for amylase, and gelatin and pectin for protein and pectinase production respectively. The organisms used are fungi, which appear amenable to high enzyme production because of the low moisture condition and high degree of aeration of the semi-soluble medium.

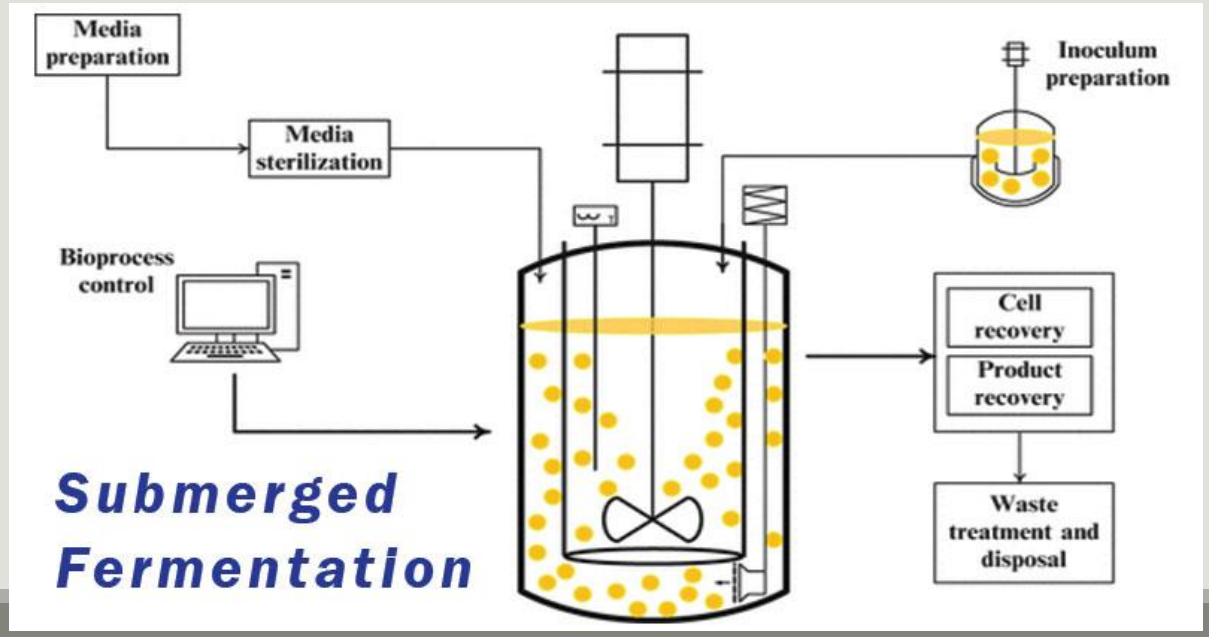


The moist bran, inoculated with spores of the appropriate fungi, is distributed either in flat trays or placed in a revolving drum. Moisture (about 8%) is maintained by occasionally spraying water on the trays and by circulating moist air over the preparation. The temperature of the bran is kept at about 30°C by the circulating cool air.

The production period is usually 30-40 hours, but could be as long as seven days. The optimum production is determined by withdrawing the growth from time to time and assaying for enzyme. The material is dried with hot air at about 37°C–40°C and ground. The enzyme is usually preserved in this manner. If it is desired, the enzyme can be extracted. Growth in a semi-solid medium seems sometimes to encourage an enzyme range different from that produced in submerged growth. Thus, *Aspergillus oryzae* on semi-solid medium will produce a large number of enzymes, primarily amylase, glucoamylose, and protease. In submerged culture amylase production rises at the expense of the other enzymes. Similarly, if *Aspergillus oryzae* producing takadiastase (a commercial powder containing amylase and some protease) is grown in submerged culture four protease components are formed whereas on semi-solid medium not only are two proteases formed, but these are less heat resistant than those produced in submerged fermentation.

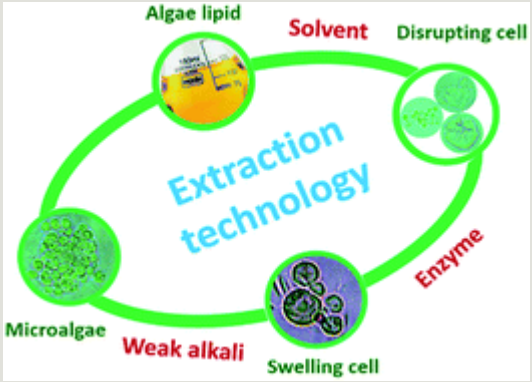
Submerged production

Most enzyme production is in fact by submerged cultivation in a deep fermentor. Submerged production has replaced semi-solid production wherever possible because the latter is labor intensive and therefore expensive where labor is scarce, and because of the risk of infection and the generally greater ease of controlling temperature, pH and other environmental factors in a fermentor. The medium must contain all the requirements for growth, including adequate sources of carbon, nitrogen, various metals, trace elements, growth substances, etc. However, a medium adequate for growth may not be satisfactory for enzyme production. For the production of inducible enzymes, the inducers must be present. Thus, pectic substances need to be in the medium when pectinolytoc enzymes are being sought. Similarly, in the production of microbial rennets soy bean proteins are added into the medium to induce protease production by most fungi. The inducer may not always be the substrate but sometimes a breakdown or end-product may serve. For example, cellobiose may stimulate cellulose production.



Enzyme Extraction

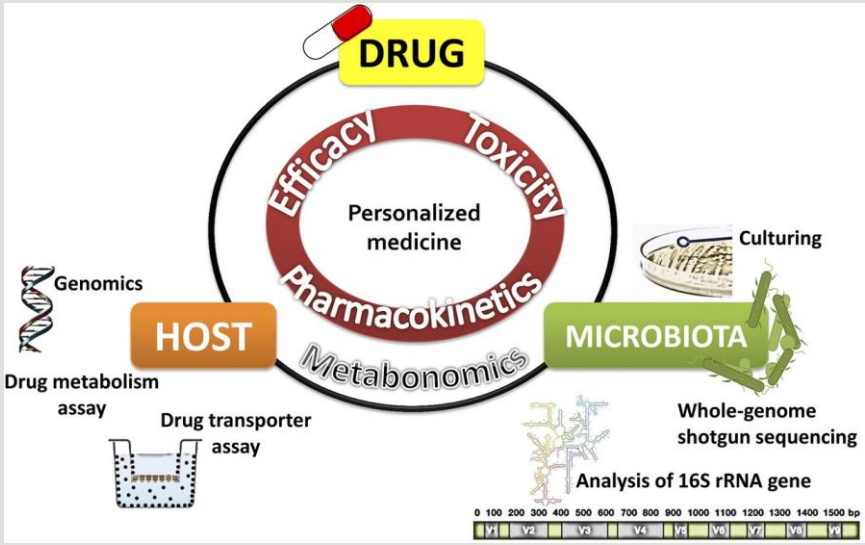
The procedures for the extraction of fermentation products described are applicable to enzyme extraction. Care is taken to avoid contamination. In order to limit contamination and degradation of the enzyme the broth is cooled to about 20°C as soon as the fermentation is over. Stabilizers such as calcium salts, proteins, sugar, and starch hydrolysates may be added and destabilizing metals may be removed with EDTA. Antimicrobials if used at all are those that are normally allowed in food such as benzoates and sorbate. Most industrial enzymes are extra-cellular in nature. In the case of cell bound enzymes, the cells are disrupted before centrifugation and/or vacuum filtration. The extent of the purification after the clarification depends on the purpose for which the enzyme is to be used. Sometimes enzymes may be precipitated using a variety of chemicals such as methanol, acetone, ethyl alcohol or ammonium sulfate. The precipitate may be further purified by dialysis, chromatography, etc., before being dried in a drum drier or a low temperature vacuum drier depending on the stability of the enzymes to high temperature. Ultra-filtration separation technique based on molecular size may be used.



Drug Discovery in Microbial Metabolites: The Search for Microbial Products with Bioactive Properties

Microbial metabolites of pharmaceutical and clinical

Organized search for microbial metabolites of pharmaceutical and clinical importance began in the late 1960s when methods were developed for the isolation of enzyme inhibitors of microbial origin. This led to the discovery of many drugs of clinical importance. One such enzyme inhibitor is an beta-lactamase inhibitor which is administered with Beta-lactam antibiotics; the other is an inhibitor of cholesterol accumulation, while a third is the immunosupressant, cyclosporin A.



β-lactam antibiotics

- Penicillins
  - Ampicillin
  - Piperacillin
- Beta-lactam/beta-lactamase inhibitors
  - Ampicillin/sulbactam
  - Amoxicillin/clavulanate
  - Ticarcillin/clavulanate
  - Piperacillin/Tazobactam