Some facts about production of SCP by microorganisms

* The protein content and quality is largely dependent on the specific microorganism utilized and the fermentation process.

* Fast-growing aerobic microorganisms are primarily used due to their high yields and high productivity.

* Bacteria generally have faster growth rates and can grow at higher temperatures than yeasts or filamentous fungi, and normally contain more protein.

* Yeasts grow relatively rapidly and, like bacteria, their unicellular character gives somewhat fewer fermentation problems than do filamentous organisms.

* However, many filamentous fungi have a capacity to degrade a wide range of materials and, like yeasts, can tolerate a low pH, which reduces the risk of microbial contamination. They are also more easily harvested at the end of fermentation than yeasts or bacteria.

Table 14.2 Percentage composition (dry weight basis) of single cell proteins and soya meal

	Quorn protein *Fusarium venenatum*	Pekilo protein *Paecilomyces variotii*	Pruteen *Methylophilus methylotrophus*	Pronin *Methylococcus capsulatus*	Brewer's yeast *Saccharomyces cerevisiae*	Spirulina protein *Spirulina maxima*	Soya meal *Glycine max*
Crude protein	58	59	72	70	49	53	48
Nucleic acids	10*	10.6	16	–	12	–	–
Lipid	1.0	1.4	5–6	10	3	3	1–2
Ash	6.4	6.4	10–12	7	7	9	5–6

* Reduced to 2% after processing.

COMPARISION OF MICRO-ORGANISMS

	ADVANTAGES	DIS ADVANTAGES
FUNGI	Easy to grow & harvest	Lower growth rates & lower protein content
ALGAE	Easy to grow & harvest & high quality protein	Non –digestible cellulosic cell wall, concentrate heavy metals
YEAST	Larger in size, lower NA content , familiarity & acceptability	Poor digestibility, low protein content, slow growth rate
BACTERIA	High protein content, digestible cell wall	High NA content, small in size, low density

Usage of SCP

*Most SCP products are currently used as animal feed and not for human consumption downstream processing .

*Nevertheless, these products must meet stringent safety requirements. Obtaining regulatory approval for the production of proteins for human consumption is an even lengthier and more expensive process, and obviously influences the choice of production organism.

*A safety aspect that must be considered for all SCP products is nucleic acid content. Many microorganisms have naturally high levels and the problem is further exacerbated because fermentation conditions favoring rapid growth rates and high protein content also promote elevated RNA levels.

*This can be problematic as the digestion of nucleic acids by humans and animals leads to the generation of purine compounds.
Their further metabolism results in elevated plasma levels of uric acid, which may crystallize in the joints to give gout-like symptoms or forms kidney stones.

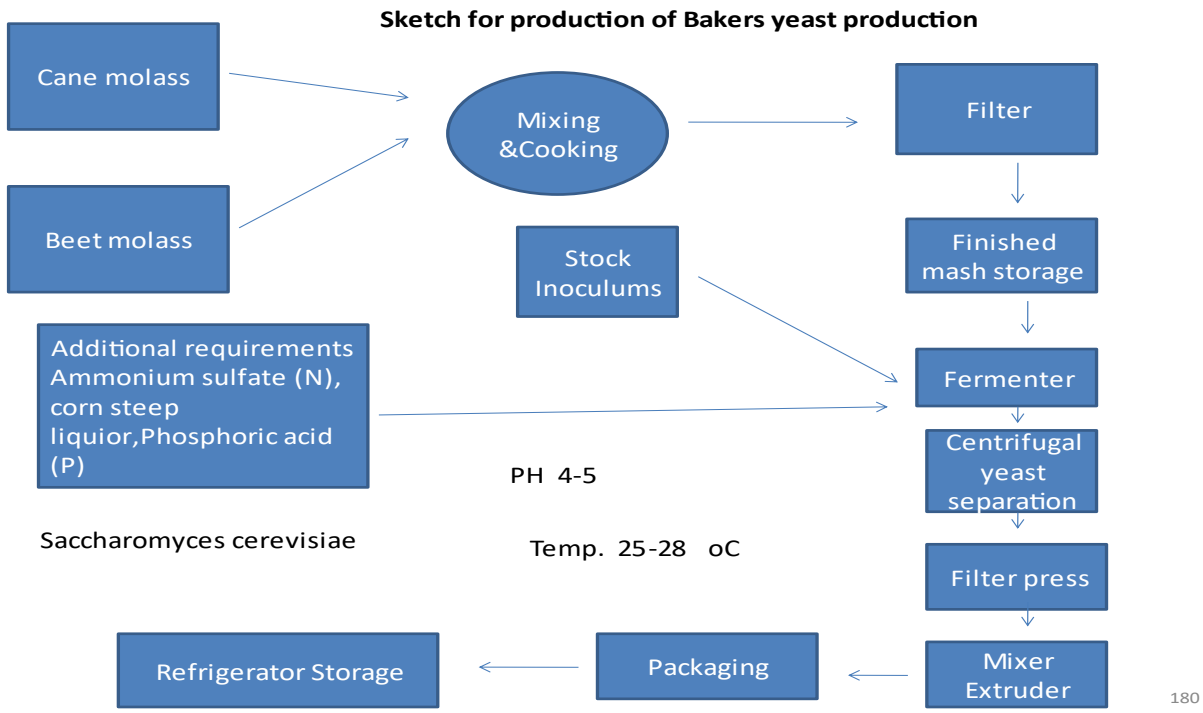
*Slow digestion or indigestion of some microbial cells within the gut and any sensitivity or allergic reactions to the microbial protein must also be examined.

*For filamentous fungi, the possibility of aflatoxin production must be eliminated. An additional concern is the absorption of toxic or carcinogenic substances, such as polycyclic aromatic compounds, which may be derived from certain growth substrates.

Baker yeast

Yeast strains used for the modern fast-rising dough have been developed with the following traditional and new physiological properties in mind.

(a) ability to grow rapidly at room temperature of about 20-25°C.
(b) easy dispensability in water.
(c) ability to produce large amounts of CO_2 in flour dough, rather than alcohol.
(d) good keeping quality, i.e., ability to resist autolysis when stored at 20°C.
(e) high potential glycolytic activity.
(f) ability to adapt rapidly to changing substrates.
(g) high invertase and other enzyme activity to hydrolyze the higher glucofructans rapidly.
(h) ability to grow and synthesize enzymes and coenzymes under the anaerobic conditions of the dough.
(i) ability to resist the osmotic effect of salts and sugars in the dough.
(j) high competitiveness i.e. high yielding in terms of dry weight per unit of substrate used.



Concentration of Sugar

Concentration of sugar should be suitable (0.5-1.5%).

Under anaerobic conditions glucose ferment quickly to ethanol and CO_2 and at aerobic conditions fermentation decreases by respiration .

Aeration causes a decline in glucose consumption and in the production of ethanol and CO_2 .

High concentration of sugar inhibit respiration even at the presence of oxygen , therefore material containing sugar source should be add at low concentration and gradually to prevent ethanol production.

Packaging

Baker's yeasts may be packaged as moist (compressed) yeasts or as dried active yeast.

(1) Compressed yeast

The yeast product obtained after harvesting, is mixed with fine particles of ice, starch, fungal inhibitors and processed vegetable oils (e.g. glyceryl monostearate) which all help to stabilize it. It is then compressed into blocks of small (1-5 Ib) blocks for household use or large (up to 50 Ib) for factory bakery operations, stored at – 7 to 0°C and transported in refrigerated vans.

(2) Active dry yeast

Dry yeast is more stable in that it can be used in areas or countries where refrigeration is not available. In many developing countries baker's yeast is imported from abroad in the form of active dry yeast.

For active dry yeast production special strains better suited for use and dry conditions may be used. It has been found that when regular strains are used they perform better as dry yeasts when they are subjected to a number of treatments.

These treatments include raising the temperature to 36°C (from about 30°C) towards the end of the fermentation, addition of alcohol-containing spent broth (resulting from centrifugation or finished yeast fermentation), synchronization of budding by alternate feeding and starving.

Yeast cream of 30-38% content from filter pressing is extruded through a screen to form continuous thread-like forms. These are then chopped fine and dried, using a variety of driers: tray driers, rotary drum driers, or fluidized bed driers. The final product has a moisture content of about 8%.

Antibiotic production

Classification and Nomenclature of Antibiotics

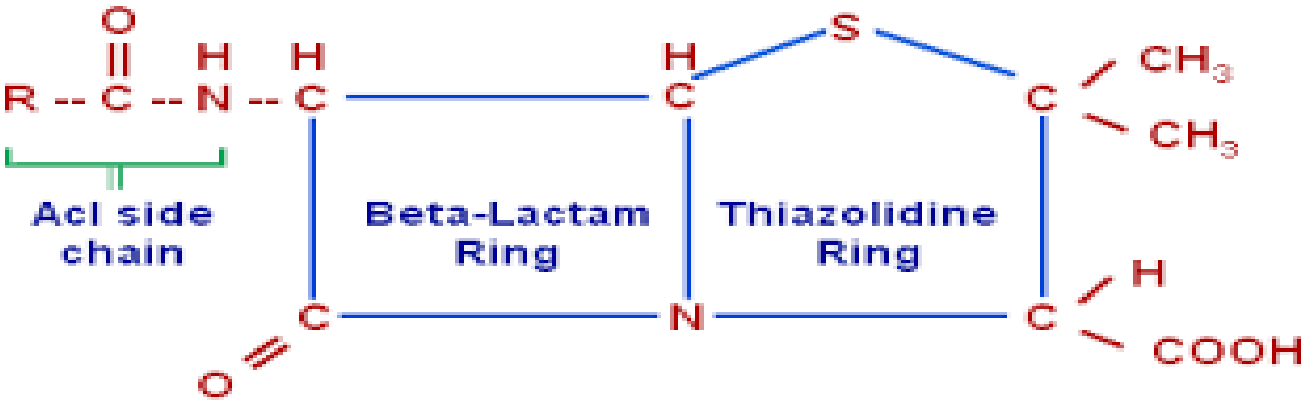
Several methods of antibiotic classification have been adopted by various authors. The mode of action has been used, e.g. whether they act on the cell wall, or are protein inhibitors, etc. Several mechanisms of action may operate simultaneously making such a method of classification difficult to sustain.

In some cases they have been classified on the basis of the producing organisms, but the same organism may produce several antibiotics, e.g. the production of penicillin N and cephalosporin by a Streptomyces sp. The same antibiotics may also be produced by different organisms.

Antibiotics have been classified by routes of biosynthesis; however, several different biosynthetic routes often have large areas of similarity.

The spectra of organisms attacked have also been used, e.g. those affecting bacteria, fungi, protozoa, etc. Some antibiotics belonging to a well known

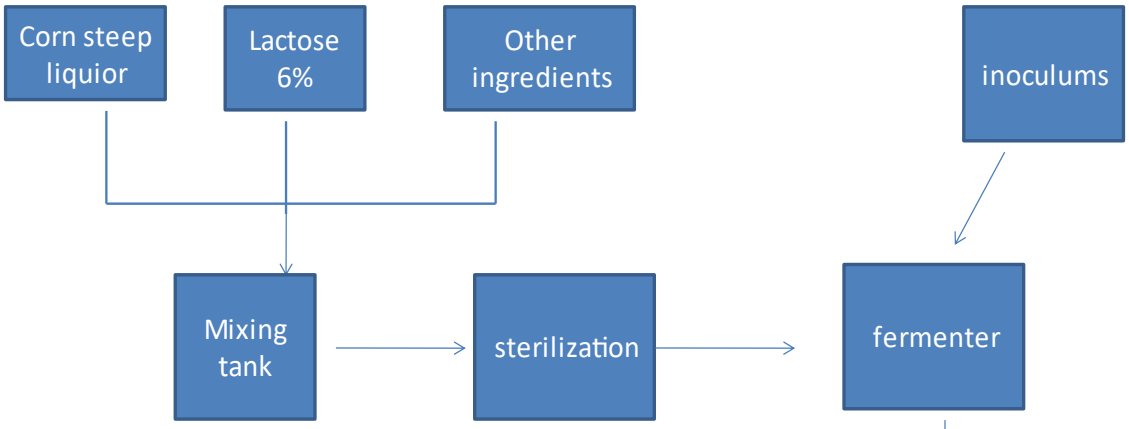
group e.g. aminoglycosides may have a different spectrum from the others. The classification to be adopted here therefore is based on the chemical structure of the antibiotics and classifies antibiotics into 13 groups. This enables the accommodation of new groups as they are discovered.



General Structure of Penicillins

Table 24.1 Grouping of antibiotics based on their chemical structures

Chemical Group	*Example*
Aminoglycosides	Streptomycin
Ansamacrolides	Rifamycin
Beta-lactams	Penicillin
Chloramphenicol and analogues	Chloramphenicol
Linocosaminides	Linocomycin
Macrolides	Erythromycin
Nucleosides	Puromycin
Puromycin	Curamycin
Peptides	Neomycin
Phenazines	Myxin
Polyenes	Amphothericin B
Polyethers	Nigericin
Tetracyclines	Tetracycline



Sketch for production of Penicillin by Penicillium chyrsogenum
PH 6.5-7.7 ,Temp. 25 -27oC , aeration

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Penicillin fermentation can be divided into three phases

The first phase (trophophase) during which rapid growth occurs, lasts for about 30 hours during which mycelia are produced.

The second phase (idiophase) lasts for five to seven days; growth is reduced and penicillin is produced.

In the third phase, carbon and nitrogen sources are depleted, antibiotic production ceases, the mycelia lyses releasing ammonia and the pH rises .

Commercial production of penicillin

Penicillin production is usually via a fed-batch process carried out aseptically in stirred tank fermenters of 40000–200000L capacity, although airlift systems are sometimes used. involves an initial vegetative growth phase followed by the antibiotic production phase.

Throughout the process, the oxygen level is very important and must be maintained at 25– 60mmol/L/h. However, this is not straightforward, because the oxygen transfer rate is affected by the viscosity, which increases as the fermentation progresses. These processes are maintained at 25–27°C and pH 6.5–7.7, the specific conditions depending upon the *Penicillium chrysogenum* strain used.

Various carbon sources have been adopted for penicillin production, including glucose, lactose, sucrose, ethanol and vegetable oils. About 65% of the carbon source is metabolized for cellular maintenance, 25% for growth and 10% for penicillin production. In the past, a mixture of glucose and lactose was used, the former producing good growth, but poor penicillin yields, whereas the latter had the opposite effect.

The mode of 'feeding' of a particular carbon source is vitally important, as it can influence the production of this secondary metabolite. Corn steep liquor is still used as a source of nitrogen, additional nutrients and side-chain precursors. Its acidic nature creates a requirement for calcium carbonate (1%, w/v) and a phosphate buffer to neutralize the medium, thereby optimizing its pH for penicillin production.

Ammonia, mineral salts and specific side-chain precursors, e.g. phenyl acetic acid or phenoxyacetic acid, may also be added. However, as some precursors are toxic, they must be fed continuously at non-inhibitory concentration.
Inoculum development is usually initiated by adding lyophilized spores to a small fermenter at a concentration of $5x10^3$ spores/ml. Fungal mycelium may then be grown up through one or two further stages until there is sufficient to

inoculate the production fermenter. Initially, there is a vegetative growth phase devoted to the development of biomass, which doubles every 6 h. This high growth rate is maintained for the first 2 days.

To ensure an optimum yield of penicillin in the following production phase, the mycelium must develop as loose pellets, rather than compact forms. During the following production phase, the carbon source is fed at a low rate and penicillin production increases. This continues for a further 6–8 days, provided that appropriate substrate feeds are maintained.
Penicillin is excreted into the medium and is recovered at the end of fermentation. Whole broth extraction may be performed, but can lead to downstream processing problems, as additional materials leach from the mycelium.

Recovery of Penicillin

*Usually, penicillin recovery follows removal of mycelium using rotary vacuum filters, the efficiency of which may be affected by the culture media composition, particularly its proteinaceous components.
Recovered mycelium is then washed to remove residual penicillin, prior to its use as animal feed or fertilizer.

*Antibiotic recovery is often by solvent extraction of the cell-free medium, which gives yields of up to 90%. This involves reducing the pH of the filtered medium to 2.0–2.5 by addition of sulphuric or phosphoric acid, followed by a rapid two-stage continuous counter current extraction at 0–3°C using amyl acetate, butyl acetate or methyl isobutyl ketone. The low temperature is necessary to reduce damage to penicillin due to the low pH.

*Alternatively, ion-pair extraction may be used at pH 5–7, in which range penicillin is stable. Any pigments and trace impurities are removed by treating with activated charcoal.

*The penicillin is then retrieved from the solvent by addition of sodium or potassium acetate. This reduces the solubility of the penicillin and it precipitates as a sodium or potassium salt.

*Resultant penicillin crystals are separated by rotary vacuum filtration.
*Solvent is recovered from the separated liquor and any other materials used, such as the charcoal, which is very important in terms of the overall economics of the process.

*Penicillin crystals are mixed with a volatile solvent, usually anhydrous ethanol, butanol or isopropanol, to remove further impurities.
The crystals are collected by filtration and air dried. At this stage the penicillin is 99.5% pure.

*This product may be further processed to form a pharmaceutical grade product or is used in the production of semisynthetic penicillin.

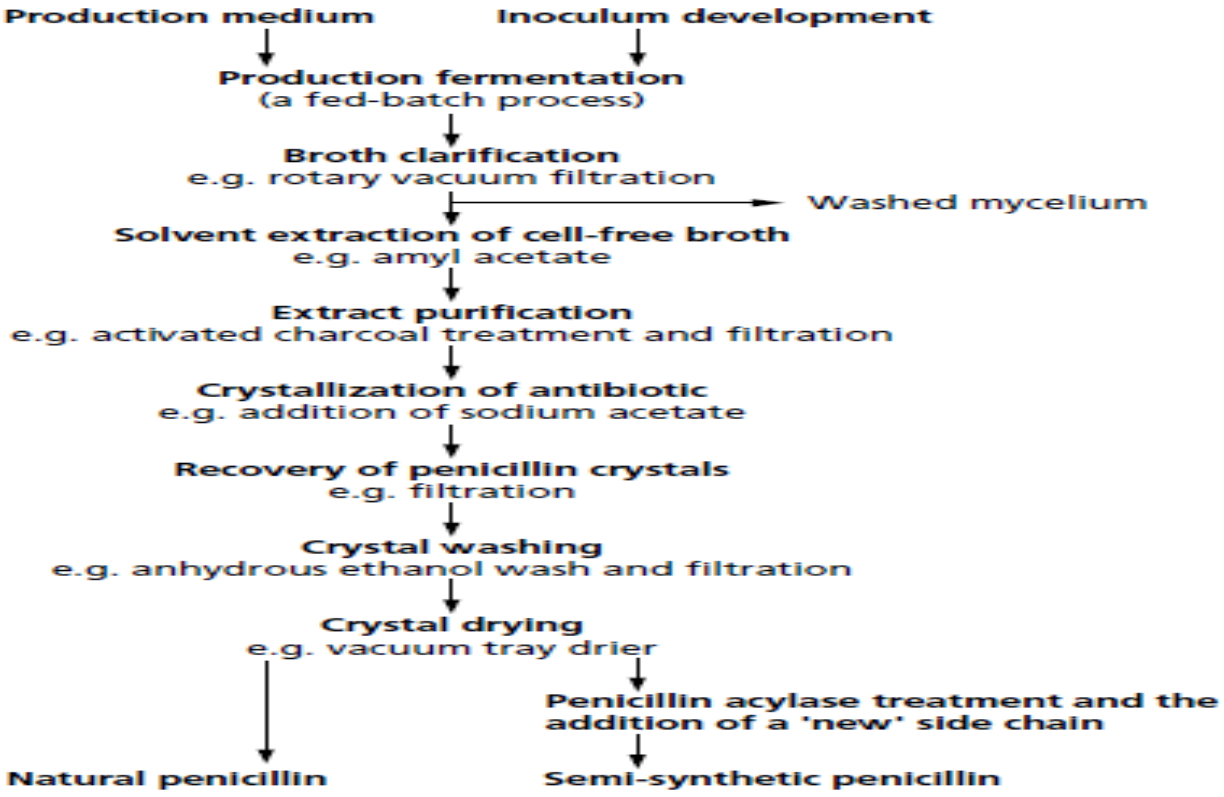

Fig. 11.1 Production of penicillin.