Industrial and Pharmaceutical applications of Microorganisms

Introduction

- Industrial microbiology is a branch of biotechnology that applies microbial sciences to create industrial products in mass quantities.
- There are **multiple ways** to manipulate a microorganism to increase maximum product yields.
- Introduction of **mutations** into an organism many be accomplished by introducing them to mutagens. There are various types of microorganisms that are used for large-scale production of industrial items.
- The ability of specific microorganisms to produce specialized enzymes and proteins has been exploited for many purposes in industry.
- Industrial microorganisms are used to produce many things, including food, cosmetics, pharmaceuticals and construction materials.
- Microorganisms can be **genetically modified or engineered** to aid in large-scale production.

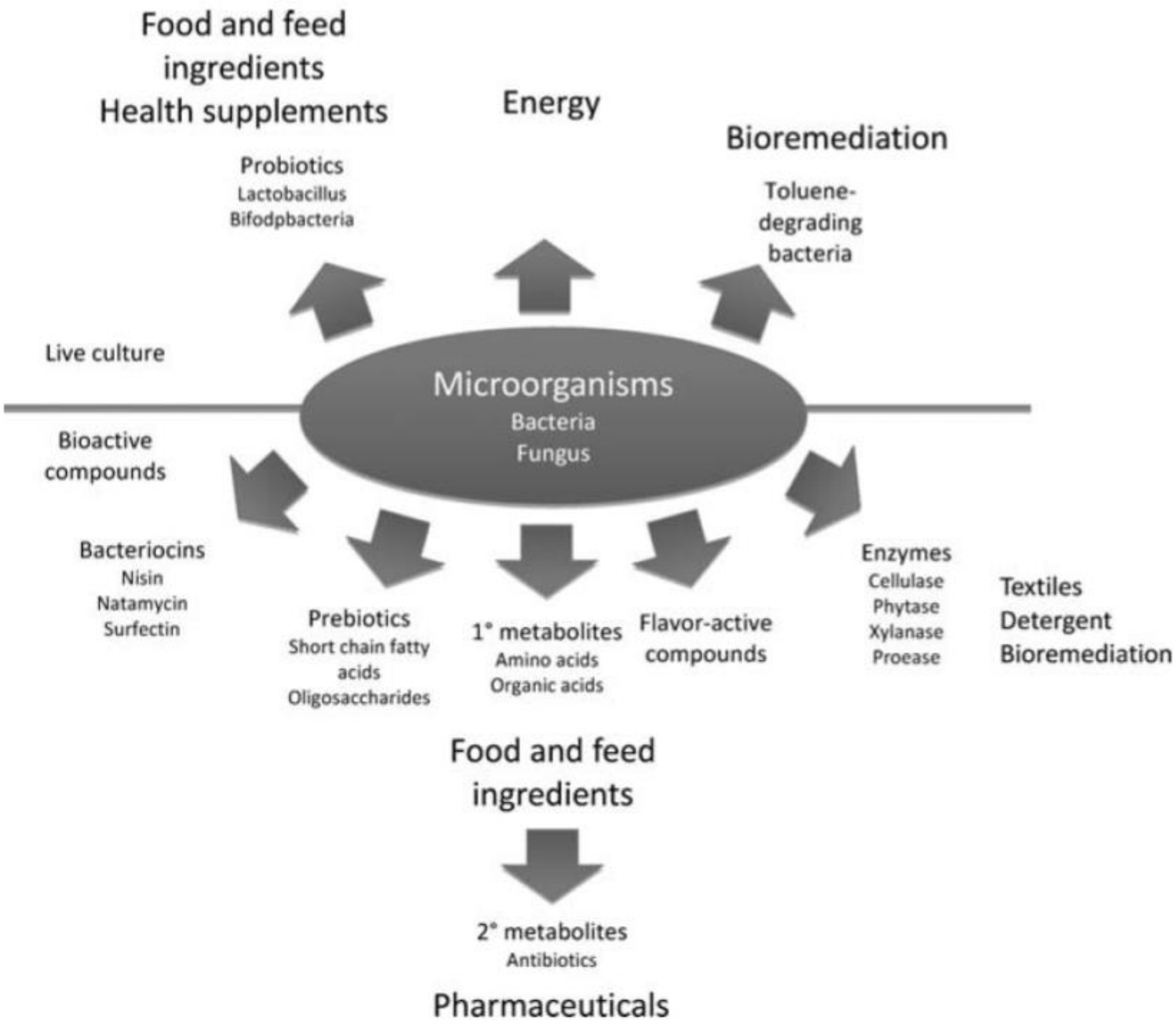
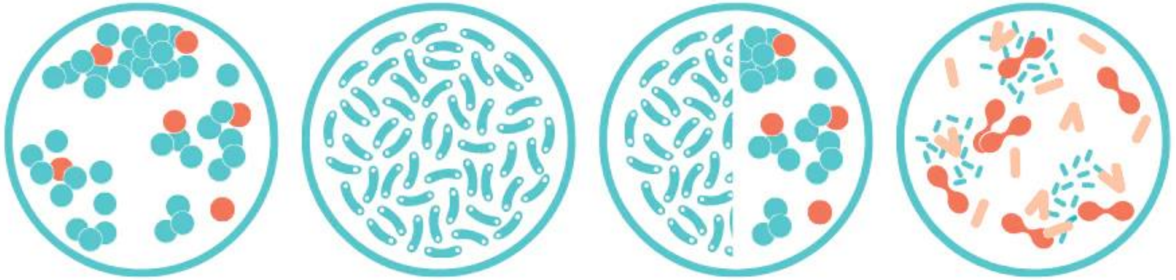


Fig. 1.1. Application of microorganisms in various industries.



Prebiotics

Non-digestible fiber compounds that stimulate the growth and activity of beneficial gut microorganisms.

Probiotics

Live microorganisms that inhabit the microbiome and confer health benefits when consumed in sufficient amounts.

Synbiotics

A combination of prebiotics and probiotics.

Postbiotics

Products of prebiotic and probiotic activity that mimic some of the same benefits as probiotics, but also offer additional health benefits.

OPTIMOZ
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PREBIOTICS

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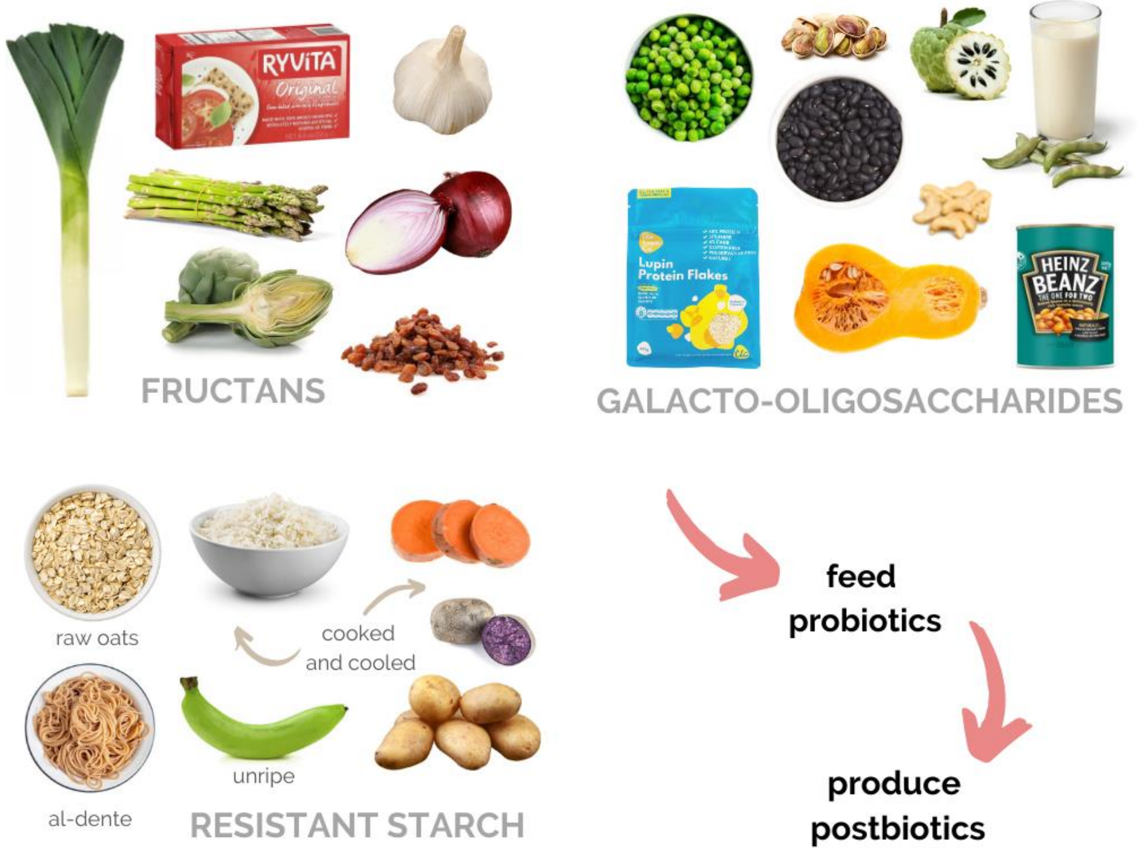

feed probiotics

produce postbiotics

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Isolation and Screening of Microorganisms

The success of an industrial fermentation process chiefly depends on the **microorganism strain used**. An ideal producer or economically important strain should have the following characteristics.

- It should be pure, and free from phage.
- It should be genetically stable, but amenable to genetic modification.
- It should produce both vegetative cells and spores; species producing only mycelium are rarely used.
- It should grow vigorously after inoculation in seed stage vessels.
- Should produce a single valuable product, and no toxic by-products.
- Product should be produced in a short time, e.g., 3 days.
- It should be amenable to long term conservation.
- The risk of contamination should be minimal under the optimum performance conditions

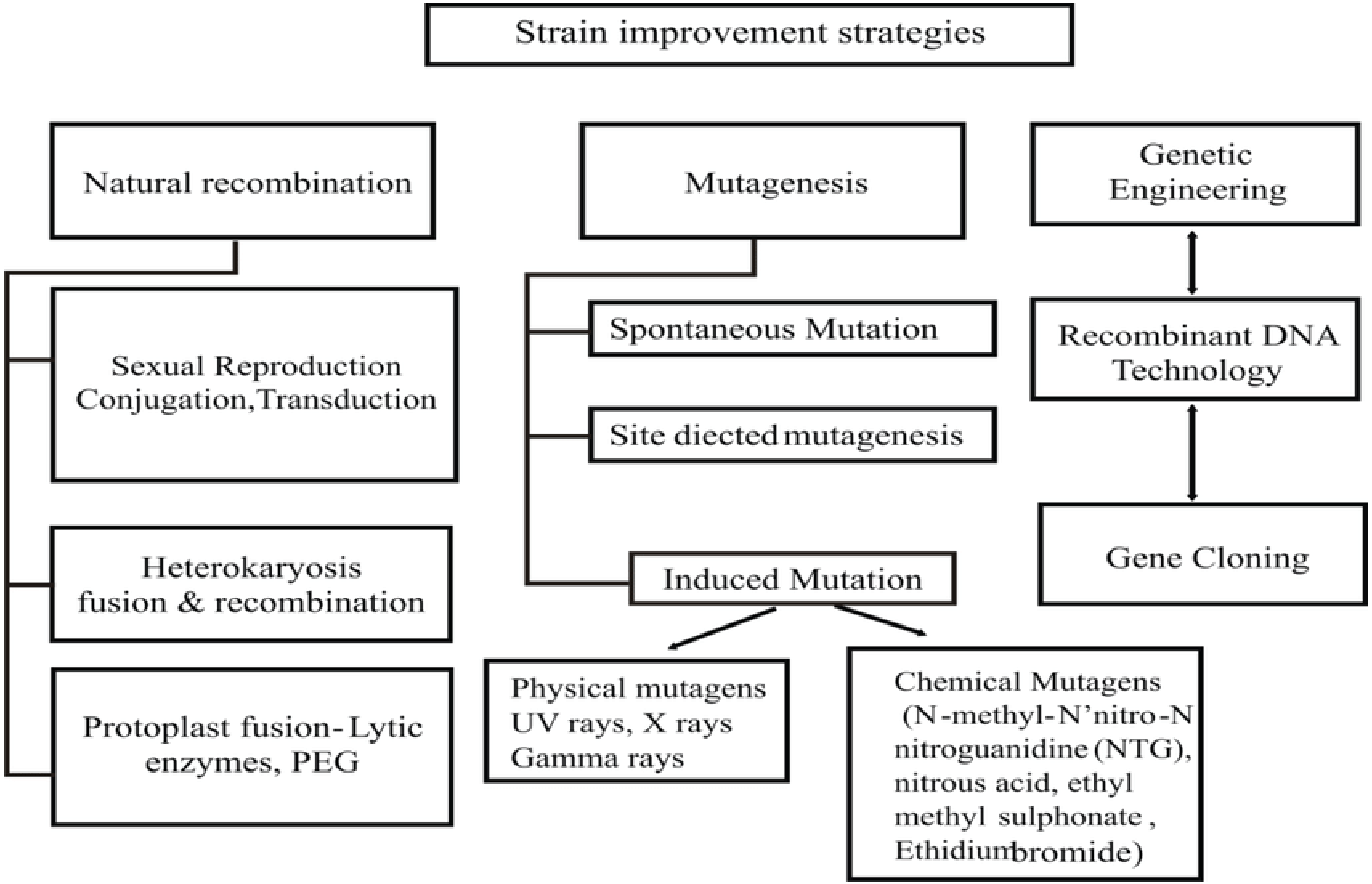
Common Contaminants

The most common contaminants of different industrial processes are considerably different. Some examples are given below.

- In canning industry, ***Clostridium butylicum*** is the chief concern. This obligate anaerobe can grow in sealed cans, and produce heat resistant spores and a deadly toxin. However, it is not a problem for catsup (too acidic), jam and jellies (too high sugar concentration) and milk (stored at low temperature).
- Organisms like ***Lactobacillus*** are a problem in production of wine.
- In antibiotic industry, potential contaminants are many, e.g., molds, yeast, and many bacteria, including ***Bacillus.***
- The most dreaded contaminants of fermentation industry are **phages**. The only effective protection against phages is to develop resistant strains.

Strain improvement

- Strain improvement is defined as **the process of improving the production and yielding capacity of a microorganism through certain (deliberate) technological, microbiological, biotechnological, or biochemical process.**
- Improvement of microbial strains for the overproduction of industrial products has been the hallmark of all commercial fermentation processes. Conventionally, strain improvement has been achieved through **mutation, selection, or genetic recombination.**
- After an organism producing a valuable product is identified, it becomes necessary to increase the product yield from fermentation to minimize production costs. Product yields can be increased by:
  - developing a suitable medium for fermentation,
  - refining the fermentation process and
  - improving the productivity of the strain.
- Strain improvement is based on the following three approaches:
  - mutant selection,
  - recombination, and
  - recombinant DNA technology.


Strain improvement strategies
Natural recombination
Sexual Reproduction Conjugation,Transduction
Heterokaryosis fusion & recombination
Protoplast fusion- Lytic enzymes, PEG
Mutagenesis
Spontaneous Mutation
Site diected mutagenesis
Induced Mutation
Physical mutagens UV rays, X rays Gamma rays
Chemical Mutagens (N-methyl-N'nitro-N nitroguanidine (NTG), nitrous acid, ethyl methyl sulphonate , Ethidium bromide)
Genetic Engineering
Recombinant DNA Technology
Gene Cloning

Types of microorganisms used

The following is a brief overview of the various microorganisms that have industrial uses, and of the roles they play.

Archaea are specific types of prokaryotic microbes that exhibit the ability to sustain populations in unusual and typically harsh environments.

- Those surviving in the most hostile and extreme settings are known as *extremophile archaea*.
- The isolation and identification of various types of *Archaea*, particularly the extremophile archaea, have allowed for analysis of their metabolic processes, which have then been manipulated and utilized for industrial purposes.
- Extremophile archaea species are of particular interest due to the enzymes and molecules they produce that allow them to sustain life in extreme climates, including very high or low temperatures, extremely acid or base solutions, or when exposed to other harmful factors, including radiation.
- **Specific enzymes which have been isolated and used for industrial purposes include thermostable DNA polymerases from the *Pyrococcus furiosus*. This type of polymerase is a common tool in molecular biology; it is capable of withstanding the high temperatures that are necessary to complete polymerase chain reactions.**
- **Additional enzymes isolated from *Pyrococcus* speciesinclude specific types of amylases and galactosidases which allow food processing to occur at high temperatures as well.**

Corynebacteria are characterized by their diverse origins.

- They are found in numerous ecological niches and are most often **used in industry for the mass production of amino acids and nutritional factors.**
- In particular, the amino acids produced by *Corynebacterium glutamicum* include the amino acid glutamic acid.
- Glutamic acid is used as a common additive in food production, where it is known as ***monosodium glutamate*** **(MSG).**
- *Corynebacterium* can also be used in steroid conversion and in the degradation of hydrocarbons. Steroid conversion is an important process in the development of pharmaceuticals.
- Degradation of hydrocarbons is key in the breakdown and elimination of environmental toxins.
- Items such as plastics and oils are hydrocarbons; the use of microorganisms which exhibit the ability to breakdown these compounds is critical for environmental protection.

Xanthomonas, a type of Proteobacteria, is known for its ability to cause disease in plants.

- The bacterial species which are classified under *Xanthomonas* exhibit the ability to produce the acidic exopolysaccharide commonly marketed as xanthan gum, used as a thickening and stabilizing agent in foods and in cosmetic ingredients to prevent separation.

Aspergillus: This genus includes several hundred types of mold.

- *Aspergillus* has become a key component in industrial microbiology, where it is used in the production of alcoholic beverages and pharmaceutical development.
- *Aspergillus niger* is most commonly used to produce citric acid, which is used in numerous products ranging from household cleaners, pharmaceuticals, foods, cosmetics, photography and construction.
- *Aspergillus* is also commonly used in large-scale fermentation in the production of alcoholic beverages such as Japanese sake.

Products obtained from the microbes

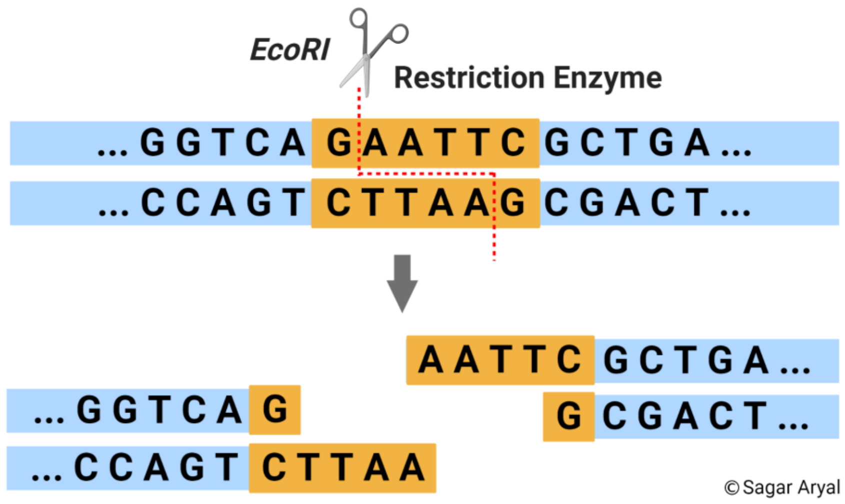
The following is a brief overview of some of the molecular products derived from microbes that allow for the performance of popular molecular biology techniques.

Taq Polymerase

- Taq polymerase is an enzyme that was first isolated from the microbe *Thermus aquaticus*.
- *T. aquaticus* is a specific type of bacterial species, a DNA polymerase, that is thermostable, i.e, it can withstand extremely high temperatures.
- The isolation of this polymerase has resulted in the ability to perform polymerase chain reactions (PCR), a process used to amplify DNA segments, in a single step.
- Prior to the isolation of Taq polymerase, a new DNA polymerase had to be added to the reaction after every cycle because of thermal denaturation.
- With the addition of Taq polymerase to the reaction tube, the cycle can be performed much more quickly, and less enzyme needs to be used.
- Currently, Taq polymerase is manufactured and produced on a large scale and is available for commercial sale.

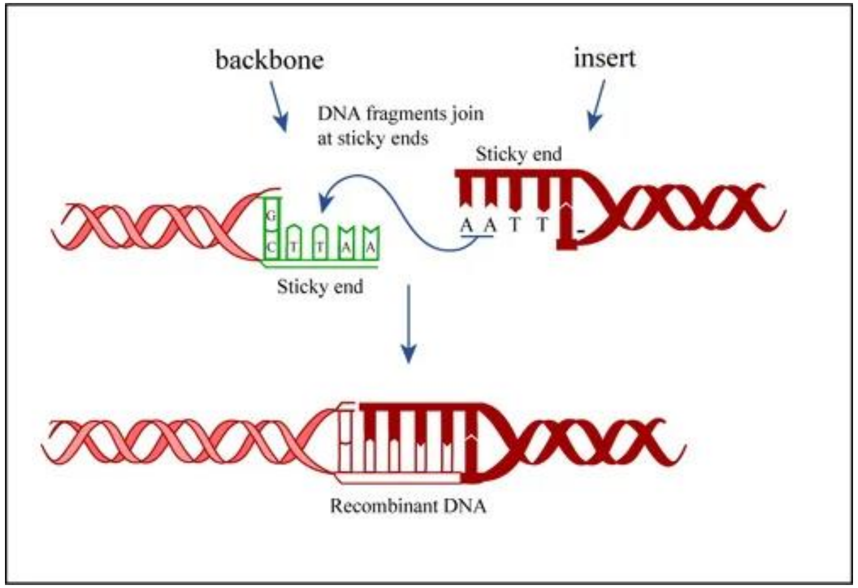
Restriction Enzymes

- Restriction enzymes are a specific class of enzymes isolated from various bacteria and archaea, in which they grow naturally as a means of protection against viral infection.
- These enzymes have the ability to cut DNA at specific recognition sequences and have served as invaluable tools in DNA modification and manipulation.
- The enzymes have the ability to recognize foreign DNA and cut it up.
- The bacteria and archaea from which these enzymes are isolated from have innate mechanisms to protect their own DNA sequences from these enzymes, such as methylation.
- The isolation of approximately 3000 restriction enzymes has allowed molecular biologists to utilize them in processes such as cloning and the production of recombinant DNA.



DNA Ligase

- Another enzyme that was isolated from *T. aquaticus* and that has been undeniably important to the field of molecular biology is DNA ligase.
- DNA ligase plays a key role in molecular biology processes due to its ability to insert DNA fragments into plasmids.
- The process of DNA ligation is defined as the ability of DNA ligase to covalently link, or ligate, fragments of DNA together.
- In molecular biology — specifically, during the process of developing recombinant DNA — DNA ligase can be used to ligate a fragment of DNA into a plasmid vector.
- The most commonly used DNA ligase is derived from the T4 bacteriophage and is referred to as T4 DNA ligase.



- Within the field of industrial microbiology, alcohol is one of the most common primary metabolites used for large-scale production.
- Specifically, alcohol is used for processes involving fermentation which produce products like beer and wine.
- **Additionally, primary metabolites such as amino acids– including L-glutamate and L-lysine, which are commonly used as supplements– are isolated via the mass production of a specific bacterial species, *Corynebacteria glutamicum.***
- Another example of a primary metabolite commonly used in industrial microbiology includes **citric acid.**
- Citric acid, produced by *Aspergillus niger*, is one of the most widely used ingredients in food production. It is commonly used in pharmaceutical and cosmetic industries as well.

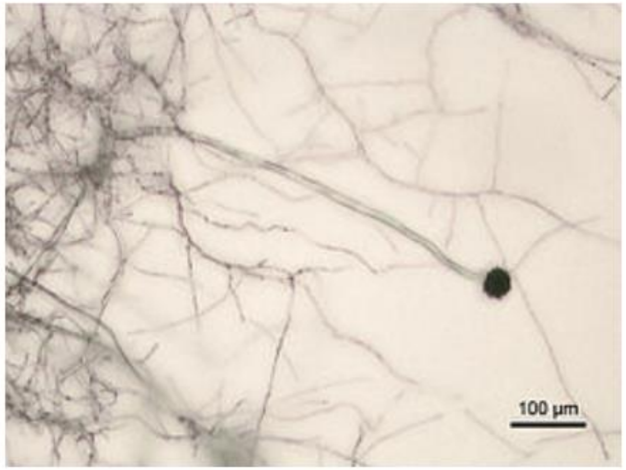


Figure: **Aspergillus niger**: Microorganisms such as Aspergillus niger are used in industrial microbiology for mass production of citric acid.

Importance of *Corynebacterium glutamicum*

- In 1950's , *Corynebacterium glutamicum* was shown to produce large quantities of glutamic acid (Kinoshita et al., 1957)
- Gram-positive rods and aerobic
- Produces amino acids such as L-glutamic acid ,L-lysine, L-threonine and L-isoleucine from aspartate
- Genome of *Corynebacterium glutamicum* ATCC 13032 was sequenced (Kalinowski et al., 2003).

Glutamic Acid

Ikeda in 1908, working on the flavouring component in kelps

- Discovered GLUTAMIC ACID (L-glutamate) after acid hydrolysis and fractionation of kelp and neutralization with caustic soda.
- These treatments enhance the taste of kelp
- Gave rise to the birth of:

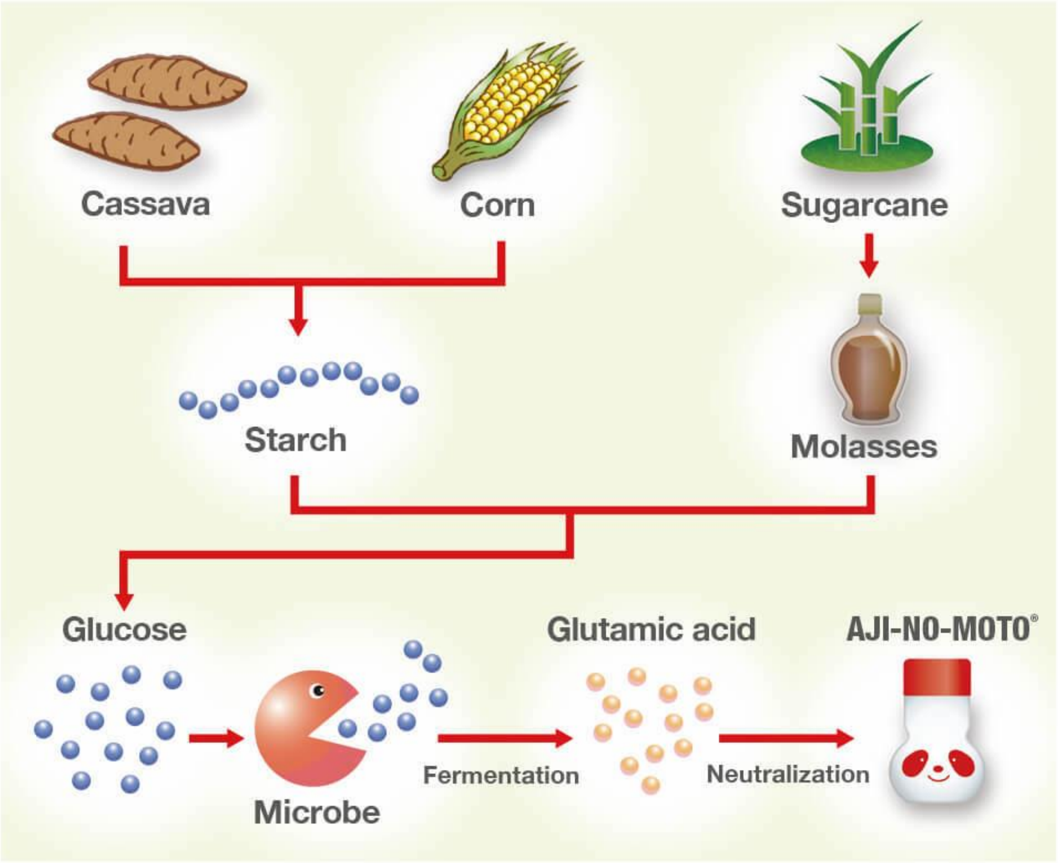
MONO SODIUM GLUTAMATE (MSG), flavor enhancing compound. It was extracted from soy and wheat. Now micro-organisms (*Corynebacterium glutamicum*) are used for MSG production.

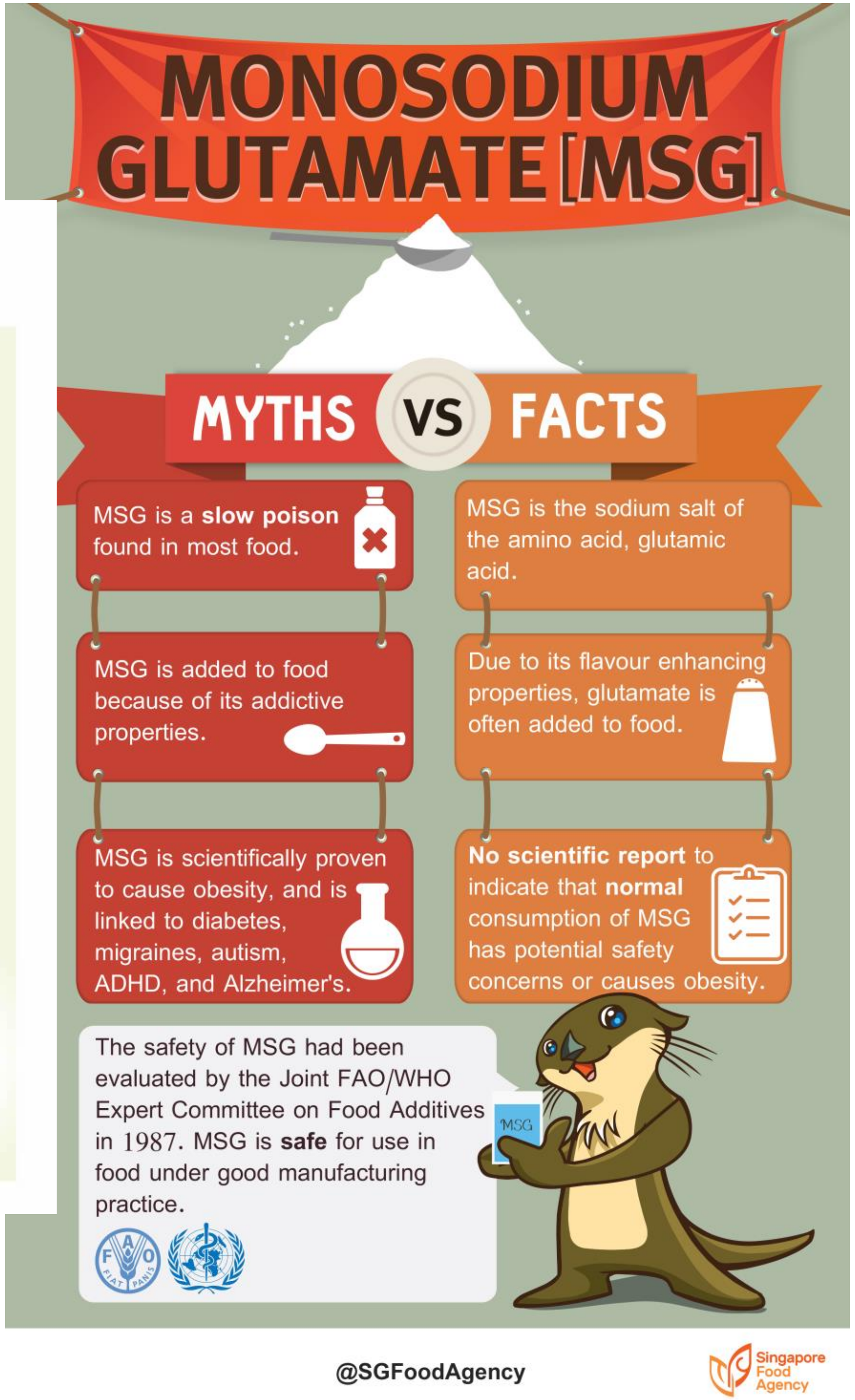
Commercial production of MSG is the largest and biggest industries world over.

Commercial Production
Glutamic acid > lysine > methionine > threonine > Aspartic acid

The market is growing steadily by about 5-10% per year.

Fermentation and MSG Production





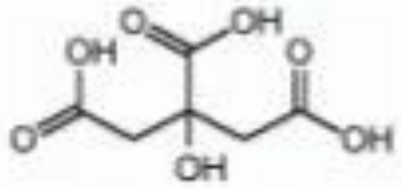
Lysine biosynthesis

- Two biosynthetic pathways exist for the amino acid, lysine
  - DAP pathway – diaminopimelic acid (DAP) is an intermediate - found in bacteria, algae, higher plants and Oomycota
    - Pyruvate + aspartate → → DAP → → lysine
  - AAA pathway - α-amino adipate (AAA)is an intermediate - found in fungi and euglenoids
    - Acetate + α-ketoglutarate → → AAA → → lysine
  - Animals do not synthesize lysine. It is an essential amino acid

INTRODUCTION

- **Citric acid**
  - Citric acid is a week organic acid found in citrus fruits
  - Molecular formula is $C_6H_8O_7$ and belongs to the carboxylic acids groups
  - Stronger acid compared to other typical carboxylic acid. Produced by fermentation and suitable pH is around 3-6

- **Application In Industry**
  - Beverages
  - Food
  - Pharmaceutical
  - Agriculture
  - Metal Industry



Structural Formula of Citric Acid

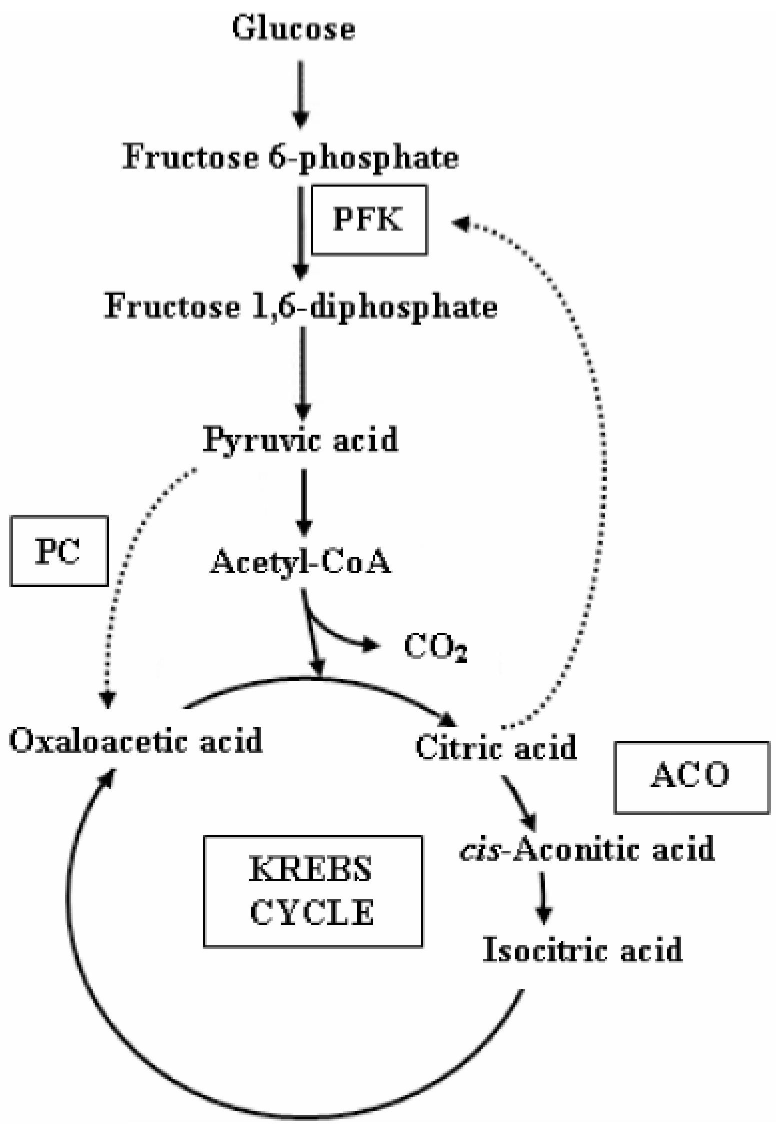


Figure 2. Schematic representation of the main metabolic reactions involved in the production of citric acid by *A. niger* (Manzoni, 2006). PFK = phosphofructokinase, PC = pyruvate carboxylase, ACO = aconitase.