Industrial microorganisms

Microoorganisms are used extensively to provide a vast range of products and services (Table 4.1). They have proved to be particularly useful because of the ease of their mass cultivation, speed of growth, use of cheap substrates (which in many cases are wastes) and the diversity of potential products. Their ability to readily undergo genetic manipulation has also opened up almost limitless further possibilities for new products and services from the fermentation industries.

Traditional fermentations were originally performed(and still are in some cases) by a mixture of wild microorganisms emanating from the raw materials or the local environment, e.g. some food and alcoholic beverage fermentations. Initial attempts to improve the microorganisms involved occurred little more than 120 years ago, when they were first isolated from

these processes as pure cultures from which the most useful strains were then selected. Those fermentation processes developed during the first 80 years of the 20th century have mostly used monocultures. The specific microorganisms employed were often isolated from the natural environment, which involved the random screening of a large number of isolates. Alternatively, suitable microorganisms were acquired from culture collections . Most of these microorganisms irrespective of their origins, were subsequently modified by conventional strain improvement strategies, using mutagenesis or breeding programmes, to improve their properties for

industrial use. Several processes developed in the last 20 years have involved recombinant microorganisms and genetic engineering technology has increasingly been used to improve established industrial strains.

In most cases, regulatory considerations are of major importance when choosing microorganisms for industrial use. Fermentation industries often prefer to use established GRAS (generally regarded as safe) microorganisms (Table 4.2), particularly for the manufacture of food products and ingredients. This is because requirements for process and product approval using a new' microorganism are more stringent and associated costs are much higher. Where pathogens and some genetically manipulated microorganisms (GMMs) are used as the producer organism, additional safety measures must be taken. Special containment facilities are employed and it may be possible to use modified ('crippled') strains that cannot exist outside the fermenter environment.

Isolation of suitable microorganisms from the environment:

Strategies that are adopted for the isolation of a suitable industrial microorganism from the environment can be divided into two types, '**shotgun**' and **objective** approaches. In the shotgun approach, samples of free living microorganisms, biofilms or other microbial communities are collected from animal and plant material, soil, sewage, water and waste streams, and particularly from unusual man-made and natural habitats. These

isolates are then screened for desirable traits. The alternative is to take a more objective approach by sampling from specific sites where organisms with the desired characteristics are considered to be likely components of the natural microflora. For example, when attempting to isolate an organism that can degrade or detoxify a specific target compound, sites may be sampled that are known to be contaminated by this material. These environmental conditions may select for microorganisms able to metabolize this compound.

Table 4.2 Examples of microorganisms classified as GRAS

(generally regarded as safe)

Bacteria: *Bacillus subtilis, Lactobacillus bulgaricus , Lactococcus lactis*

Leuconostoc oenos.

Yeasts: *Candida utilis, Kluyveromyces marxianus, Kluyveromyces lactis*

Saccharomyces cerevisiae

Filamentous fungi: *Aspergillus niger, Aspergillus oryzae*

Mucor javanicus (Mucor circinelloides f. *circinelloides)*

Penicillium roqueforti

Culture collections:

Microbial culture collections provide a rich source of microorganisms that are of past, present and potential future interest. There are almost 500 culture collections around the world; most of these are small, specialized collections that supply cultures or other related services only by special agreement. Others, notably national collections, publish catalogues listing the organisms

held and provide extensive services for industrial and academic organizations (Table 4.3). In the UK for example, the National Culture Collection (UKNCC) is made up of several collections. They are housed in

separate institutions and tend to specialize in bacteria, yeasts, filamentous fungi or algae of either industrial or medical importance; whereas in the USA there is a main centralized collection, the American Type Culture Collection (ATCC), which holds all types of microorganisms.

The prime functions of a culture collection are to maintain the existing collection, to continue to collect new strains and to provide pure, authenticated culture samples of each organism. Problems of culture maintenance have been aided by the development and use of cryopreservation and freeze-drying (lyophilization) techniques, along with miniaturized storage methods. One convenient method involves adsorption of cells to glass beads (2 mm diameter) that may be placed in frozen storage, from which individual beads may be removed without thawing the whole sample.

Use of microorganisms selected from a culture collection obviously provides significant cost savings compared with environmental isolation and has the

advantage that some characterization of the microorganism will have already been performed. However, the disadvantage is that competitors have access to the same microorganism.

Industrial strains and strain improvement:

Irrespective of the origins of an industrial microorganism, it should ideally exhibit:

1- genetic stability;

2- efficient production of the target product, whose route of biosynthesis should preferably be well characterized;

3- limited or no need for vitamins and additional growth factors;

4- utilization of a wide range of low-cost and readily available carbon sources;

5 -amenability to genetic manipulation;

6- safety, non-pathogenicity and should not produce toxic agents, unless this is the target product;

7- ready harvesting from the fermentation;

8- ready breakage, if the target product is intracellular;

9 production of limited byproducts to ease subsequent purification problems.