Modification of genetics

Cutting and pasting DNA: Special enzymes, obtained from bacteria, are an essential tool of the molecular biologist. In nature, these enzymes help bacteria fend off viral attack by precisely dissecting the foreign DNA of invading viruses. In this way, the proliferation of the viruses is restricted. Restriction enzymes (as they are known) recognise and cut DNA molecules at specific locations. Many hundreds of restriction enzymes have been isolated from different microbes and are available commercially. With restriction enzymes almost any section of DNA, and consequently any single gene, can be excised at will. The end of one DNA molecule will readily link to that of another that has been cut with the same enzyme. To join two DNA molecules permanently it is necessary to form chemical bonds along the DNA's sugar-phosphate backbone. An enzyme called DNA ligase can do this job. The function of these 'cut and paste' enzymes in assembling novel DNA molecules is obvious, but the genetic engineer's tool kit would be incomplete without one or two other enzymes. To understand their role it is necessary to appreciate how proteins are made.

A genetic intermediary: The genetic information encoded in DNA lies within the nucleus of the cell. However, proteins are not made in the nucleus, but elsewhere, at special structures called ribosomes. Before a particular protein can be made, a copy of the appropriate instructions must first be transcribed from the DNA and then ferried to the ribosomes. The copied instructions are made from mRNA (messenger ribonucleic acid). This mRNA is virtually a mirror image of the sequence of bases on one DNA strand, according to the basepairing rules. Upon arrival at the ribosomes the base sequence within the mRNA directs the construction of proteins from amino acids. A sequence of three adjacent bases in the mRNA molecule is needed to determine each amino acid in the protein. Cells that are producing a particular protein will have many identical copies of that protein's mRNA inside them. It is often easier to search for genes among the small mRNA molecules rather than along the entire length of the cell's DNA. Once a desired length of mRNA has been isolated, two additional enzymes are needed.

DNA from RNA: The enzyme reverse transcriptase assembles a single strand of complementary DNA alongside a corresponding piece of mRNA. A second enzyme (DNA polymerase) can then be used to construct a double-stranded helix using the first DNA strand as a template. DNA made in this way is called copy or complementary DNA (cDNA). A copy of a gene from a donor cell is used in genetic modification.

Gene synthesis: By the judicious use of restriction and other enzymes, molecular biologists are able to assemble DNA molecules which contain one or more genes of interest. Where a particular piece of DNA is difficult to isolate, it is sometimes possible to make it artificially using a DNA synthesiser. Under computer control, these devices string together the biochemical precursors needed to make short stretches of DNA. Of course, to programme the synthesiser it is necessary to know the sequence of bases present in the desired gene, this too can be determined automatically using a DNA sequencer. It is also possible to copy specific genes using the polymerase chain reaction (PCR). The PCR has been likened to a 'genetic photocopier'. From a very small amount of DNA millions of copies of a specific section of DNA can be made quickly. The PCR lies behind many of the spectacular successes of forensic genetic fingerprinting, where criminals have been identified from the DNA in just a few drops of blood or even a couple of cells on a cigarette butt.

Plasmids: Once a suitable DNA molecule has been constructed, it must be moved into a cell in which it can be expressed and duplicated so that it passes from one cell division to the next. For micro-organisms, one of the most successful methods involves the use of plasmids as a vehicle for transferring genes. Plasmids are rings of DNA that are found in some cells. They carry a limited set of genes and

depends on the optical purity of the compounds, mainly (S)-enantiomer. For instance, (S)-ibuprofen ((S)-2(4-isobutylphenyl) propionic acid) is 160 times more effective than (R)- isomer in the inhibition of prostaglandins synthesis. Optically, pure profens can be synthesised by asymmetric chemical synthesis, catalytic kinetic resolution, and chiral chromatography, but enzymatic enantioselective esterification seems to be the best method. Discussed reaction was carried out in saturated cyclohexane with 1-propanol or 2-propanol as acyl agents and completed with good conversion degree and excellent enantiomeric excess of (S)-ibuprofen.

Also, enantiomerically pure amines constitute a class of compounds with possible biological properties and industrial applications. Candida antarctica lipase B is one of the most effective catalyst in the preparation of enantiomerically pure nitrogenated compounds (e.g. amines, amides, amino acids, amino alcohols, etc.). This is achieved by enantioselective acetylation. For example, resolution of amino-alkylpyridines was most effective (conversion 50%, time 4h, ee of product, and substrate >99%) with the use CAL-B and ethyl acetate as an acyl donor in the tert-butyl methyl ether (TBME-medium).

CAL-A isoform of *C. antarctica* lipase is able to selectively acylate cyclic, sterically hindered structures via kinetic resolution alicyclic β-aminocarboxylic acids esters—building blocks for the synthesis of various pharmaceutical important heterocycles are synthesised this way. The best activity and enantioselectivity were observed in diethyl ether or diisopropyl ether with 2,2,2-trifluoroethyl hexanoate as an acyl donor. CAL-A is also active towards sterically hindered tertiary alcohols. This feature is quite unique among hydrolases. The first example of enantioselective kinetic resolution of racemic mixture of tertiary alcohol was acylation of 2-phenylbut-3-yn-2-ol. The reaction was quite enantioselective, but the yield was rather moderate (25%) because of the steric hindrance. Another interesting application of CAL-A is selective acylation of sterols, furyl substituted allyl alcohol, or cyanohydrins.

Yeast's Invertase

Invertase (β-fructofuranosidase- EC 3.2.1.26) catalyses hydrolysis of the glycoside bond from the terminal nonreducing beta-fructofuranoside side in disaccharide. It is also widely distributed in the environment, mainly in plants and micro-organisms. The most important application of invertase is production of invert syrup—equimolar mixture of fructose and glucose, released from sucrose, which is used in food and beverage industries. Monosaccharides mixture is sweeter than sucrose and hygroscopic, it mainly is used for production of soft-centered candies and fondants. Invertase is also applied for the manufacture of artificial honey, plasticising agents for cosmetics, pharmaceutical and paper industries, and enzyme electrodes for the detection of sucrose. Additionally, it can be applied for the synthesis of probiotic oligosaccharides like non-digestible oligosaccharides (NDO), e.g. lactosucrose. Commercially invertase is produced mainly by *Saccharomyces cerevisiae* (Baker's yeast) or *Saccharomyces* carlsbergensis. In yeast cells, invertase is produced either in intracellular or extracellular form.

Yeast's Oxidoreductases

Enantiometrically pure alcohols including α- and β-hydroxyesters are important and valuable intermediates in the synthesis of pharmaceuticals and other fine chemicals. Enantioselective ketone reductions are one of the most common methods applied for optically pure alcohols productions. Because reactions catalysed by dehydrogenases/reductases require cofactors (NADH or NADPH), the use of whole cells rather than isolated enzymes is preferred, to decrease the cost of enzyme purification and cofactor regeneration. However, isolated dehydrogenases employment decreased product purification problems.

a model strain with a perfect heterothallic life cycle. In contrast, brewer's yeast is refractory to the genetic procedures used with laboratory strains. The main reason is its low sexual fertility. Like most other industrial yeast, brewing strains do not sporulate or do so with low efficiency. Even in those cases that they show a suitable sporulation frequency, most spores are not viable.

Strain Types

There are basically two kinds of yeast used in brewing that correspond to the ale and lager types of beer. Ale beer is produced by a top-fermenting yeast that works at about room temperature, ferments quickly, and produces beer with a characteristic fruity aroma. The bottom-fermenting lager yeast works at lower temperatures, about 10–14°C, ferments more slowly and produces beer with a distinct taste. The vast majority of beer production worldwide is lager. It is difficult to make generalisations concerning the yeast strains used for the industrial production of beer, since they are generally ill characterised and very few comparative studies have been reported. Bottom fermenting, lager strains are usually labelled *Saccharomyces carlsbergensis*. Although strains from different sources show differences regarding cell size, morphology and frequency of spore formation, it is unlikely that these differences reflect a significant genetic divergence. Only one strain, Carlsberg production strain 244, has been extensively analysed and most of the studies described in the following sections have been conducted with this strain.

Genetic Crosses

Early attempts to carry out conventional genetic analysis with brewer's yeast faced the problems of poor sporulation and low viability. To overcome this difficulty, several researchers hybridised brewing strains with laboratory strains of *S. cerevisiae*. Notwithstanding the poor performance of brewing strains, viable spores were recovered from them. Some of the spores had mating capability and could be crossed with *S. cerevisiae* to generate hybrids easier to manipulate. The meiotic offspring of the hybrids was repeatedly backcrossed with laboratory strains of *S. cerevisiae* to bring particular traits of the brewing strain into an organism amenable to analysis. This procedure was followed to study flocculence, an important character in brewing. Gjermansen and Sigsgaard carried out a detailed analysis of the meiotic offspring of *S. carlsbergensis* strain 244. They obtained viable spore clones of both mating types. Celllines with opposite mating type were crossed pairwise to generate a number of hybrids that were tested for brewing performance. One of them was as good as the original strain. Additionally, the clones derived from strain 244 with mating capability served as starting material for further genetic analysis which are described in the following section.

The kar mutations have been particularly useful tools to investigate cytoplasmic inheritance. Additionally, the kar mutations supplied new genetic techniques. For instance, the chromosome number of virtually any *Saccharomyces* strain can be duplicated upon mating with a *kar*2 partner. These new tools and techniques opened a new way for the characterisation of the brewer's yeast. Since the brewing strain does not mate normally, the strain used in *kar* crosses was a meiotic derivative of strain 244 with mating capability. When disomic strains for chromosome III (also referred to as chromosome addition strains) were crossed to haploid *S. cerevisiae* strains, normal spore viability was obtained, allowing tetrad analysis. In this process, one of the two copies of chromosome III can be lost. If the original *S. cerevisiae* copy is lost, the result is a 'chromosome substitution strain' carrying a complete *S. cerevisiae* chromosome set, except chromosome III, which comes from *S. carlsbergensis*. Meiotic analysis of crosses between chromosome III addition strains and laboratory strains of *S. cerevisiae* revealed two important facts: (i) the functional equivalence of chromosome III for the brewing strain and *S. cerevisiae*, since ascospore

2. Germination, during which the contents of the starchy endosperm are substantially degraded ('modification') resulting in a softening of the grain.
3. Kilning, in which the moisture is reduced to a level low enough to arrest modification.

Mashing: The Production of Sweet Wort

Sweet wort is the sugary liquid that is extracted from malt (and other solid adjuncts used at this stage) through the processes of milling, mashing and wort separation. Larger breweries will have raw materials delivered in bulk (rail or road) with increasingly sophisticated unloading and transfer facilities as the size increases. Smaller breweries will have malt, etc. delivered by sack.

The conscientious brewer will check the delivery and the vehicle it came in for cleanliness and will representatively sample the bulk. The resultant sample will be inspected visually and smelled before unloading is permitted. Most breweries will spotcheck malt deliveries for key analytical parameters to enable them to monitor the quality of a supplier's material against the agreed contractual specification. Grist materials are stored in silos sized according to brewhouse throughput.

Milling

Before malt or other grains can be extracted, they must be milled. Fundamentally the more extensive the milling, the greater the potential there is to extract materials from the grain. However, in most systems for separating wort from spent grains after mashing, the husk is important as a filter medium. The more intact the husk, the better the filtration. Therefore, milling must be a compromise between thoroughly grinding the endosperm while leaving the husk as intact as possible. There are fundamentally two types of milling: dry milling and wet milling. In the former, mills may be either roll, disk or hammer. If wort separation is by a lauter tun (discussed later), then a roll mill is used. If a mash filter is installed, then a hammer (or disk) mill may be employed because the husk is much less important for wort separation by a mash filter. Wet milling, which was adopted from the corn starch process, was introduced into some brewing operations as an opportunity to minimise damage to the husk on milling. By making the husk 'soggy', it is rendered less likely to shatter than would a dry husk.

Mashing

Mashing is the process of mixing milled grist with heated water in order to digest the key components of the malt and generate wort containing all the necessary ingredients for the desired fermentation and aspects of beer quality. Most importantly it is the primary stage for the breakdown of starch. The starch in the granules is very highly ordered, which tends to make the granules difficult to digest. When granules are heated (in the case of barley starch beyond 55–65°C), the molecular order in the granules is disrupted in a process called gelatinisation. Now that the interactions (even to the point of crystallinity) within the starch have been broken down, the starch molecules become susceptible to enzymic digestion. It is for the purpose of gelatinisation and subsequent enzymic digestion that the mashing process in brewing involves heating. Although 80–90 per cent of the granules in barley are small, they only account for 10–15 per cent of the total weight of starch. The small granules are substantially degraded during the malting process, whereas degradation of the large granules is restricted to a degree of surface pitting. (This is important, as it is not in the interests of the brewer (or maltster) to have excessive loss of starch, which is needed as the source of sugar for fermentation.) The starch in barley (as in other plants) is in two molecular forms: amylose, which has very long linear chains of glucose units, and amylopectin, which comprises shorter chains of glucose units that are linked through side chains.