Chapter 2
Barley

Abstract Barley has been a staple in the agricultural world for many years due its many health benefits, e.g., better control of glucose levels, blood pressure, bad cholesterol, and weight gain (Whole Grains Council in Health benefits of barley, 2013). Suggested medical applications for barley currently under investigation include use as a low-cost biological catalyst (Nagaoka in Biotechnol Prog 20(1):128–133, 2004), use as a host for the production of endotoxin-free recombinant proteins (Magnusdottir et al. in Trends Biotechnol 31(10):572–580, 2013) and use as a potential source of improved water sanitation through algal inhibition (Xiao et al. in Environ Microbiol 16(5):1238–1251, 2013).

Introduction

Hordeum vulgare L., more commonly known as cultivated barley (Fig. 2.1), has been a crop of great importance in world agriculture since approximately 10,000 years ago when it was first domesticated from the wild-type *Hordeum spontanuem* in the Fertile Crescent. Cultivated barley is notable for its ability to grow in areas where other cereals cannot, e.g., colder, saltier, and drier environments; while originally grown on temperate climates, barley may also be grown in many tropical regions and is an annual plant in comparison with its other perennial relatives (Badr et al. 2000; Global Crop Diversity Trust 2013). There are two forms of this cereal crop, 2-row and 6-row barley. The 2-row barley has two rows of seeds per head, while 6-row barley has six rows of seeds per head; the latter has been the chosen strain for domestication (Barley World 2013). Barley is used primarily for animal feed, human consumption, and malting (Gramene 2013).

As a whole grain and a source of human nutrition, barley has a variety of health benefits: better control of blood sugar levels, lowering glucose levels, reducing blood pressure, lowering bad cholesterol, and better weight control (Whole Grains Council 2013). The worldwide production of barley was about 132 million metric tons (MT) in 2012 utilizing a harvesting area of approximately

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Fig. 2.1 Barley field (*left*) and barley fruit (*right*) (from Wikimedia commons: http://commons.wikimedia.org/wiki/File:Barley.jpg and http://commons.wikimedia.org/wiki/File:Barley_fruit.jpg, respectively)

50 million hectare (ha), and placing barley production only behind wheat, rice, and maize in the category of cereal crops. Developing countries make up around 18 % of this barley production, while Russia, France, and Germany were the largest producers of barley in 2012 (Global Crop Diversity Trust 2013; Food and Agricultural Organization of the United Nations 2013). In 2012, Bolivia produced 49,000 MT of barley harvested over an area of 50,000 ha with production remaining mostly constant since 2002 until the present day (Food and Agricultural Organization of the United Nations 2013; Index Mundi 2013).

Medical Applications

Necessity for Biological Catalysts

Biocatalysts are substances, e.g., enzymes, which initiate or alter chemical reactions in living systems (Collins English Dictionary 2013). Biological catalysts are vital to the world of medicine due to their impact on chemical synthesis. They have been used to increase production of new products as well as to reduce costs of products in high demand. Biocatalysts have been utilized in the creation of corn syrups, aspartame, semisynthetic penicillin, and a variety of cancer-treating drugs (Scouten and Petersen 2000). Innovation in the availability of new sources and types of biocatalysts will surely help shape the future of medicine.

Low-Cost Biological Catalysts

Biomaterial biocatalysts (Fig. 2.2) allow for more easily obtained, stereoselective (Fig. 2.3), regioselective, and substrate-specific biocatalysts with the ability

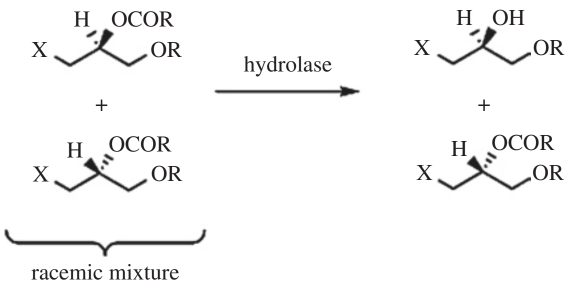

Fig. 2.2 Kinetic resolution of a racemic mixture: use of enzyme results in higher proportion of one enantiomer (from Wikimedia commons: http://en.wikipedia.org/wiki/File:Kineticresolution.gif)

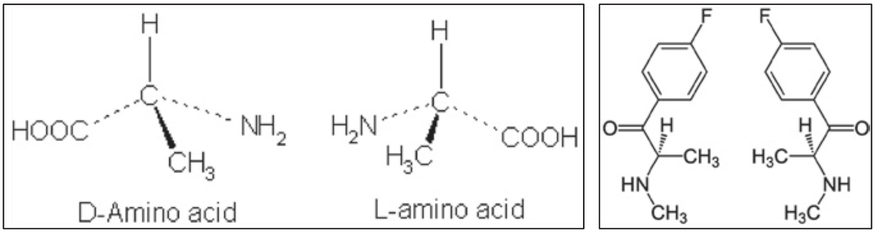

Fig. 2.3 Amino acid optical isomers (chiral molecules) (*left*) and structural enantiomers (*right*) (from Wikimedia commons: http://commons.wikimedia.org/wiki/File:Op_isomer.png and http://commons.wikimedia.org/wiki/File:(%C2%B1)-Flephedrone_4-isomer_Enantiomers_Structural_Formulae.png, respectively)

to act as asymmetric reagent bases. These biomaterials are notable not only for what they can do, but also for their ability to function under more moderate conditions to synthesize asymmetric organic compounds over their organic metal catalyst counterparts (Nagaoka 2004). Transition metal catalysts may be harmful and require harsher, not necessarily environmentally friendly, reaction conditions to synthesize chiral compounds (Sheldon 2000).

Biomaterials currently being investigated for their use as biocatalysts include microbial cells (including cells with overexpressed enzymes) (Kataoka et al. 1999), enzymes derived from animal tissue (Matumoto et al. 1995), and cultured plants (Naoshima and Akakabe 1991). Cultured plants do not require the bacterial medium common for many of the other natural biocatalysts although they do require long preincubation periods. Young barley leaves have recently been investigated for their use as providers of low cost, easy to obtain, stereoselective, regioselective, and substrate-specific natural biocatalysts with the ability to function under more moderate conditions in order to synthesize asymmetric compounds. Natural biomaterials, such as barley, have the advantage of already being produced in large scales in many countries where there is already experience with their genetic modification, and therefore established knowledge of their genetics and growth cycles. Recent findings support the use of natural, plant-based biomaterials in their ability to resolve enantiomers of varying methyl carbinols, to synthesize optically active alcohols in environmentally conscious settings, and to allow for the oxidation and reduction of different reactions. Findings in Nagaoka's 2004 study support the use of immobilized young barley leaves—as well as other already genetically modified crops—as successful sources of NAD(P)-E for the chiral resolution of *para*-substituted *rac*-ArCH(OH)Me (Nagaoka 2004). Chiral resolution is an important aspect of modern medicine as this separation of racemic compounds into their different enantiomers allows for the increased sensitivity and specificity of drug development.

Biocatalysts' importance in the field of bulk chemical synthesis, pharmaceuticals, agriculture, and food ingredients cannot be over emphasized. As limitations are reached within the current process of microbial synthesis of biocatalysts, researchers are expanding into other areas—e.g., genetically modified crops for biotransformations—in order to remove these limitations (Boyd 2000; Schoemaker et al. 2003).

Water Sanitation

Bolivia has seen improvements regarding actions taken to reduce water pollution. Open defecation rates decreased from 46 % in 1990 to 19 % of the population in 2011. This improvement, however, was not evenly distributed; 57 % of the 2011 total population in urban areas saw improvements in their use of sanitation facilities compared to the much lower 24 % of the 2011 total population in rural areas that did the same (World Health Organization and UNICEF 2013). In certain regions, maintenance of water quality can be a serious concern. The Pulitzer Center has reported on the contamination of the Seco River. The Seco begins in the Andean glaciers, runs through the fast-growing city of El Alto, and heads toward Lake Titicaca—the largest lake in South America. The poverty and overtaxation of sewage treatments in El Alto have contributed to the dumping of untreated wastewater into the river. The resulting presence of blood, algae, oil, mineral processing, feces, and garbage disposal in the Seco River has the river doubling as a trashcan and bathroom. The Seco flows alongside lakeside communities where families typically raise livestock that drinks from the river (Shahriari 2011). The river algae could present a problem as nutrient pollution from human activities has been found to worsen algal blooms (Fig. 2.4). Algal blooms may produce toxins that could negatively impact the health of people and animals drinking the water, create dead zones in the water, raise water treatment costs, and affect the water-dependent economy of the area (United States Environmental Protection Agency 2013). Other potential consequences of the river's contamination are still being studied. While treating the symptoms may not necessarily cure the cause, it is certainly worth investigating as a first step toward health improvement. Biomaterials offer the opportunity to treat water without contributing to pollution.

Fig. 2.4 Algal bloom (*left*) and toxic algae bloom in Lake Erie (*right*) (from Wikimedia commons: http://commons.wikimedia.org/wiki/File:Algal_Bloom_-_%E0%B4%AA%E0%B4%BE%E0%B4%AF%E0%B5%BD_06.JPG and http://commons.wikimedia.org/wiki/File:Toxic_Algae_Bloom_in_Lake_Erie.jpg, respectively)

Algae Inhibition

Barley straw has been investigated for its ability to inhibit cyanobacteria and algal growth, for the past 30 years. While previously the mechanism for algal inhibition remained unknown, current studies have identified chiral flavonolignans—salcolin A and salcolin B—as likely responsible. Salcolin A and salcolin B differ somewhat in their interaction with cyanobacteria. In a recent study, the effects of barley-isolated salcolin A and salcolin B were tested on Microcystis, a freshwater cyanobacteria, and while both enantiomers were found to have inhibitory effects, their mechanisms of action differed. Salcolin A induced increased levels of intracellular reactive oxygen species (ROS) and inhibited esterase activity, thereby inhibiting algal growth, while salcolin B induced leakage of the cyanobacterial cytoplasms, thereby acting as a sort of poison to already growing cyanobacteria. The allelopathic inhibition of cyanobacteria by barley shows promise in the area of controlling algal blooms in undesired locations, especially as the incidences of these algal blooms grow due to the effects of human pollution (Xiao et al. 2013).

Research into barley inhibition of cyanobacteria is notable not only for its direct results but for what it can help avoid. Presently, varying approaches have been taken to reduce undesired algal blooms; however, on occasion, the cure can be just as harmful as the problem. Many techniques currently used have short-lived effects and, when improper physical and chemical care is taken in their execution, can aggravate an already fragile ecosystem. To address the mentioned concern, recent studies have begun to assess barley's potential to inhibit algal growth even after it has been degraded for full removal of nitrogen and phosphorous. The removal of nitrogen and phosphorous from barley would prevent the additional contribution of these and other nutrients to the waters being treated for cyanobacterial inhibition. Excess addition of nutrients to already nutrient-rich waters can cause problems such as "smelly" waters as well as increases in water-bred mosquitoes. Results from Cui et al.'s 2013 study revealed the effectiveness of degraded barley (containing neither nitrogen nor phosphorous) on waters containing varying algal species—e.g., *Microcystis aeruginosa*, *Aphanizomenon flosaquae*, *Oscillatoria* (L.), and *Scenedesmus* (L.)—and found the plant crop to still have a significant inhibitory effect on algal growth. Study findings support barley's value as a source of cheap and effective algal control that as of yet has not shown signs of secondary pollution effects (Cui et al. 2013).

Endotoxin-Free Recombinant Proteins

Engineers are often faced with certain considerations when designing pathways for the cellular manufacturing of desired molecules. Bhatia (2013) describes three aspects of concern during metabolic engineering: metabolic flux, metabolic burden, and genetic instability. Imbalances in the molecular turnover rate through a metabolic pathway, metabolic flux, can arise from insufficient regulatory mechanisms in engineered pathways ultimately contributing to an increased amount of toxic intermediates and reduced product yield. Overexpression of enzymes in engineered cells increases their metabolic burden. The overconsumption of needed cellular materials, such as amino acids and nucleotides, for the production of engineered proteins stresses the cell and slows its growth. Genetic instability may be found in engineered protein-expressing plasmid-containing cells as a result of the greater pressures they are under in comparison with those cells without engineered plasmid DNA (Bhatia 2013). Engineers are constantly looking for the correct balance to strike in terms of product yield and cellular stability, leading to the search for cellular hosts with the more optimal results.

A shift from bacterial hosts to plant hosts for the production of recombinant proteins is being sought due to the reduced production cost, reduced storage costs, easy scalability, and decreased presence of endotoxins (bacterial contaminants), viruses, and human pathogens found in plant systems. There is a need to reduce the number of recombinant proteins used for medical purposes, e.g., growth factors, which are often significantly contaminated by endotoxins. Bacterial hosts such as *E. coli* present the problem of having more rudimentary translational mechanisms, compared to the eukaryotic mechanisms present in the desired protein's original environment, along with no posttranslational modification. Mammalian cell hosts present the problem of having a rather high production cost along with possible low yields. While low protein yields and the recentness of using plant systems as sources of recombinant proteins present this emerging area with its greatest challenges, the low cost of initial clinical trials presents the field with an optimistic future (Magnusdottir et al. 2013).

Barley is being considered as an adequate source for human recombinant proteins. The greatest opposition to barley stems from public dislike of genetically modified organisms and the crop's resistance to genetic modification. However, there is also much support for barley as its grain provides good long-term protein storage, it has been studied at length, there is ample familiarity with its structure and cycle, it is an approved generally recognized as safe (GRAS) organism with a low known presence of toxic compounds, it has no endotoxin contamination, and it does not contain known pathogens or viruses harmful to mammals. Barley's ability to grow in non-ideal environments also makes it easier to be regulated and contained as other crops are not likely to be grown nearby, making it all the more attractive to prospective developers who may be concerned with genetically modified feed coming into contact with other crops (Magnusdottir et al. 2013).

Collagen is an example of a useful protein that could be amenable to production in barley. Collagen type I makes up 90 % of the organic extracellular matrix in mature human bone (Reffitt et al. 2003). Collagen is incredibly important in maintaining an adequate cellular structure as its structural support influences cell growth, proliferation, and differentiation. While animal-derived collagen is one of the major sources of this protein for medical purposes, alternative sources are being investigated in order to help prevent the immune response often observed with the established source. Researchers are studying the possibility of creating recombinant collagen capable of maintaining collagen's triple helical structure as well as the same amino acid sequence found in human collagen in order to prevent possible dangerous immune responses. The discovery and implementation of new collagen sources have the potential to positively impact the tissue-engineering field as advances are looked to be made in bone, ligament, tendon, and skin repair (Yang et al. 2004). Barley has been demonstrated to be a suitable candidate for the production of correctly structured recombinant human proteins. For every kilo of grain, barley can store and produce as much as 1 g of recombinant proteins in its endosperm (Horvath et al. 2000). Barley is currently being investigated for its potential for collagen production. Collagen has been particularly difficult to produce in the usual animal/plant systems used for human protein production due to the eight enzymes required for its proper formation (triple helical, completely folded) and complete retention of function. Furthermore, some of these enzymes appear to be unique to collagen production. In order to test barley's capacity as a producer of procollagen (collagen's precursor), research is being conducted to develop vectors containing the homotrimeric hydroxylated procollagen I genes necessary for barley grain production of the collagen predecessor. Presently, while barley transformants have been created, research still remains to be completed regarding the structural and functional analysis of the proteins generated. The ultimate goal is the production of recombinant collagen type I in barley endosperm (Osorio 2004).

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