Small bugs, big business:  
The economic power of the microbe  
Arnold L. Demain*  

* Corresponding author. Fax: 1-617-253-8699.  
E-mail address: demain@mit.edu (A.L. Demain).  

Abstract  
The versatility of microbial biosynthesis is enormous. The most industrially important primary metabolites are the amino acids, nucleotides, vitamins, solvents, and organic acids. Millions of tons of amino acids are produced each year with a total multibillion dollar market. Many synthetic vitamin production processes are being replaced by microbial fermentations. In addition to the multiple reaction sequences of fermentations, microorganisms are extremely useful in carrying out biotransformation processes. These are becoming essential to the fine chemical industry in the production of single-isomer intermediates. Microbially produced secondary metabolites are extremely important to our health and nutrition. As a group, they have tremendous economic importance. The antibiotic market amounts to almost 30 billion dollars and includes about 160 antibiotics and derivatives such as the â-lactam peptide antibiotics, the macrolide polyketide erythromycin, tetracyclines, aminoglycosides and others. Other important pharmaceutical products produced by microorganisms are hypcholesterolemic agents, enzyme inhibitors, immunosuppressants and antitumor compounds, some having markets of over 1 billion dollars per year. Agriculturally important secondary metabolites include coccidiostats, animal growth promotants, anthelmintics and biopesticides. The modern biotechnology industry has made a major impact in the business world, biopharmaceuticals (recombinant protein drugs, vaccines and monoclonal antibodies) having a market of 15 billion dollars. Recombinant DNA technology has also produced a revolution in agriculture and has markedly increased markets for microbial enzymes. Molecular manipulations have been added to mutational techniques as means of increasing titers and yields of microbial processes and in discovery of new drugs. Today, microbiology is a major participant in global industry. The best is yet to come as microbes move into the environmental and energy sectors. © 2000 Elsevier Science Inc. All rights reserved.  

Keywords: Biotechnology; Primary metabolites; Secondary metabolites; Economics

© 2000 Elsevier Science Inc. All rights reserved.
1. Introduction

For thousands of years, microorganisms have been used to supply us with products such as bread, beer, wine, distilled spirits, vinegar, cheese, pickles and other fermented materials. These processes were originally developed for the preservation of fruits, vegetables and milk, but developed into sophisticated products satisfying the palate and psyche of humans. A second phase of biotechnology began during World War I which resulted in a quantum jump in the economic importance of microbes. In England, Chaim Weizmann developed the acetone-butanol fermentation and in Germany, the glycerol fermentation was formulated by Neuberg. Both acetone and glycerol were needed for manufacture of munitions to support the war efforts of the respective opposing nations. These events were followed after the war by development of fermentation, bioconversion, and enzymatic processes yielding many useful products with large annual markets. These include amino acids, nucleotides, vitamins, organic acids, solvents, vaccines and polysaccharides. A major segment of the second phase was represented by secondary metabolites such as antibiotics. Ever since the discovery of penicillin in 1929 and its commercial development starting at the beginning of World War II, antibiotic molecules have had major beneficial effects on human and animal health. Many secondary metabolites which have antibiotic activity (meaning killing or growth inhibition of bacteria and/or fungi) are used for other purposes. These include hypocholesterolemic agents, immunosuppressants, anticancer agents, bioherbicides, bioinsecticides, coccidiostats, animal growth promotants, and ergot alkaloids. Other important secondary metabolites do not have antibiotic activity; these include the antihelmintic ivermectin, the bioinsecticide spinosad, and the plant growth stimulants, the gibberellins.

In the early 1970s, a phenomenal third phase began with the birth of recombinant DNA technology. Traditional industrial microbiology merged with molecular biology to yield many new products of the modern biotechnology era. Recombinant DNA technology has impacted the production of primary and secondary metabolites, bioconversions and especially the enzyme industry. In this article, I hope to impress the reader with the positive economic importance of the microbial world.

2. Why are microorganisms used in industry?

The importance of the fermentation industry resides in five important characteristics of microorganisms: (1) a high ratio of surface area to volume, which facilitates the rapid uptake of nutrients required to support high rates of metabolism and biosynthesis; (2) a tremendous variety of reactions which microorganisms are capable of carrying out; (3) a facility to adapt to a large array of different environments, allowing a culture to be transplanted from nature to the laboratory flask or the factory fermentor, where it is capable of growing on inexpensive carbon and nitrogen sources and producing valuable compounds; (4) the ease of genetic manipulation, both in vivo and in vitro, to increase production of the products, to modify structures and activities, and to make entirely new products; and (5) an ability to make specific enantiomers, usually the active ones, in cases where normal chemical synthesis yields a mixture of active and inactive enantiomers.

Microorganisms are important to us for many reasons, but one of the principal ones is that they produce things of value to us. These may be very large materials such as proteins, nu-
cleic acids, carbohydrate polymers, or even cells, or they can be smaller molecules which we usually separate into metabolites essential for vegetative growth and those inessential (i.e. primary and secondary metabolites, respectively). The power of the microbial culture in the competitive world of commercial synthesis can be appreciated by the fact that even simple molecules (i.e. L-glutamic acid and L-lysine), are made by fermentation rather than by chemical synthesis. Although a few products have been temporarily lost to chemical synthesis (e.g. solvents such as acetone and butanol), it is obvious that most natural products are made by fermentation technology. Despite the efficiency of the chemical route to riboflavin, much of the production of this compound is carried out by fermentation; chemical processes to vitamin C and steroids still employ microbial bioconversion steps. Most natural products are so complex and contain so many centers of asymmetry that they probably will never be made commercially by chemical synthesis.

Although microbes are extremely good in presenting us with an amazing array of valuable products, they usually produce them only in amounts that they need for their own benefit; thus they tend not to overproduce their metabolites. Regulatory mechanisms have evolved in microorganisms that enable a strain to avoid excessive production of its metabolites so that it can compete efficiently with other forms of life and survive in nature. The fermentation microbiologist, however, desires a ‘wasteful’ strain which will overproduce and excrete a particular compound that can be isolated and marketed. During the screening stage, the microbiologist is searching for organisms with weak regulatory mechanisms. Once a desired strain is found, a development program is begun to improve titers by modification of culture conditions, mutation and recombinant DNA technology. The microbiologist is actually modifying the regulatory controls remaining in the original culture so that its ‘inefficiency’ can be further increased and the microorganism will excrete tremendous amounts of these valuable products into the medium.

The main reason for the use of microorganisms to produce compounds that can otherwise be isolated from plants and animals or synthesized by chemists is the ease of increasing production by environmental and genetic manipulation. Thousand-fold increases have been recorded for small metabolites. Of course, the higher the specific level of production, the simpler is the job of product isolation.

3. Production of primary metabolites

Primary metabolites are the small molecules of all living cells that are intermediates or end products of the pathways of intermediary metabolism, or are building blocks for essential macromolecules, or are converted into coenzymes. The most industrially important are the amino acids, nucleotides, vitamins, solvents, and organic acids. Primary metabolites vary in size from hydrogen gas (2 Da) and methane (16 Da) to vitamin B\textsubscript{12} (1355 Da). It is not unexpected that amino acids and vitamins are used in human and animal nutrition, that ethanol, acetone, and butanol are used as fuels and/or solvents, and that citric and acetic acids are used as acidulants. However, many of these general metabolites are used in novel ways: the sodium salts of glutamic, 5’-inosinic and 5’-guanylic acids as flavor enhancers, sodium gluconate as a sequestering agent to prevent the deposition of soap scums on cleaned surfaces, and fumarate in the manufacture of polyester resins. Organisms producing such products are fantastic in their degree of overproduction.
About 1 million tons of amino acids with a market of $3 billion are produced annually (Table 1) (Hols et al., 1999). In amino acid production, feedback regulation is bypassed by isolating an auxotrophic mutant and partially starving it of its requirement. A second means to bypass feedback regulation is to produce mutants resistant to a toxic analogue of the desired metabolite (i.e. an antimetabolite). Combinations of auxotrophic and antimetabolite resistance mutations are common in primary metabolite-producing microorganisms. About 1.6 billion pounds of monosodium glutamate, a potent flavor enhancer, are made annually by fermentation using various species of the genera Corynebacterium and Brevibacterium (e.g. Corynebacterium glutamicum, Brevibacterium flavum and Brevibacterium lactofermentum). Today, these glutamate producers are classified as subspecies of C. glutamicum (e.g. C. glutamicum ssp. flavum and C. glutamicum ssp. lactofermentum). Monosodium glutamate sells for about $0.95 per pound (Wilke, 1999).

The bulk of the cereals consumed in the world are deficient in the essential amino acid, L-lysine. Lysine supplementation converts such cereals into balanced food or feed. Lysine is a member of the aspartate family of amino acids. It is produced in bacteria by a branched pathway that also produces methionine, threonine, and isoleucine. This pathway is controlled very tightly in an organism like Escherichia coli; this organism contains three aspartate kinases, each of which is regulated by a different end-product. In addition, after each branch point, the initial enzymes are inhibited by their respective end-products and no overproduc-

Table 1
Estimated annual markets and tonnage of some primary metabolites

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>World market ($ millions)</th>
<th>Production (tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosodium glutamate</td>
<td>915</td>
<td>800 000</td>
</tr>
<tr>
<td>L-Lysine-HCl</td>
<td>600</td>
<td>300 000</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>198</td>
<td>13 000</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Flavor nucleotides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S’IMP + S’GMP</td>
<td>350</td>
<td>2500</td>
</tr>
<tr>
<td>Organic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric</td>
<td>1400</td>
<td>400 000</td>
</tr>
<tr>
<td>Lactic</td>
<td>150</td>
<td>100 000</td>
</tr>
<tr>
<td>Gluconic</td>
<td>93</td>
<td>40 000</td>
</tr>
<tr>
<td>Itaconic</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Succinic(^a)</td>
<td></td>
<td>15 000</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin(^b)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>B(_{12}) (cyanocobalamin)</td>
<td>71</td>
<td>3</td>
</tr>
<tr>
<td>C (ascorbic acid)(^c)</td>
<td>60</td>
<td>60 000</td>
</tr>
<tr>
<td>Riboflavin(^d)</td>
<td></td>
<td>2000</td>
</tr>
</tbody>
</table>

\(^a\)Totally synthetic process.
\(^b\)Mainly synthetic.
\(^c\)Partly synthetic process (chemical + bioconversion).
\(^d\)Mainly by fermentation.
tion occurs. However, in lysine fermentation organisms (e.g. various mutants of *C. glutamicum* and its relatives), there is a single aspartate kinase which is regulated via concerted feedback inhibition by threonine plus lysine. By genetic removal of homoserine dehydrogenase, a glutamate-producing wild-type *Corynebacterium* is converted into a lysine-overproducing mutant that cannot grow unless methionine and threonine are added to the medium. As long as the threonine supplement is kept low, the intracellular concentration of threonine is limiting and feedback inhibition of aspartate kinase is bypassed.

Recombinant DNA techniques have made their way into the amino acid production area. *E. coli* and *Serratia marcescens* strains have been constructed with plasmids bearing amino acid biosynthetic operons. Plasmid transformation has been accomplished in *C. glutamicum*, so that recombinant DNA technology is now used to improve these commercial amino acid-producing strains. The major manipulations have involved gene cloning to increase the levels of feedback-resistant aspartate kinase and dihydrodipicolinate synthase. As a result, lysine industrial production yields 170 g per L and 0.54 g L-lysine HCl per g glucose used (molar yield of 0.54 moles of L-lysine per mole of glucose used) (Eggeling and Sahm, 1999; Kawaihara et al., 1990). L-lysine is produced at an annual level of 300 000 tons with a market of $600 million (McCoy, 1999). It sells for about $1 per pound (Wilke, 1999; Reisch, 2000).

Recombinant technology as well as traditional mutagenesis and selection have been major influences in constructing bacterial strains capable of producing 100 g per L of L-threonine, 40 g per L of L-isoleucine, 34 g per L of L-leucine, 31 g per L of L-valine, 28 g per L of L-phenylalanine, 58 g per L of L-tryptophan, 26 g per L of L-tyrosine, 100 g per L of L-proline, 65 g per L of L-arginine and 40 g per L of L-histidine. Some of the production figures and/or market values for a number of these amino acids are as follows: L-phenylalanine: 13 000 tons, $198 million; L-aspartic acid: $43 million (McCoy, 1999); L-isoleucine: 400 tons. The price of L-threonine is $4 per pound and L-tryptophan $20 per pound (Wilke, 1999).

Commercial interest in nucleotide fermentations is due to the activity of two purine ribonucleoside 5'-monophosphates, namely guanylic acid (5'-GMP) and inosinic acid (5'-IMP) as enhancers of flavor (Demain, 1978; Kuninaka, 1996). Some 2500 tons of GMP and IMP are produced annually in Japan alone with a combined market of $350 million per year (McCoy, 1999). Three main processes are used: (1) hydrolysis of yeast RNA by fungal nuclease to AMP and GMP followed by enzymatic deamination of AMP to IMP; (2) fermentative production of the nucleosides inosine and guanosine by *Bacillus subtilis* mutants followed by chemical phosphorylation; and (3) direct fermentation of sugar to IMP by *C. glutamicum* mutants plus conversion of guanine to GMP by salvage synthesis using intact cells of *Brevibacterium ammoniagenes*. Titers of IMP by direct fermentation have reached 27 g per L (Kuninaka, 1996). The key to effective purine accumulation is the limitation of intracellular AMP and GMP. This limitation is best effected by restricted feeding of purine auxotrophs. Thus, adenine-requiring mutants lacking adenylosuccinate synthetase accumulate hypoxanthine or inosine that results from breakdown of intracellularly accumulated IMP. Certain adenine-auxotrophs of *B. subtilis* excrete over 10 g of inosine per L. These strains are still subject to GMP repression of enzymes of the common path. To minimize the severity of this regulation, the adenine auxotrophs are further mutated to eliminate IMP dehydrogenase. These adenine-xanthine double auxotrophs show a twofold increase in specific activity of some common-path enzymes and accumulate up to 15 g inosine per L under conditions of
limiting adenine and xanthine (or guanosine). Further deregulation is achieved by selection of mutants resistant to purine analogues. Thus, mutants requiring adenine and xanthine and resistant to azaguanine produce over 20 g inosine per L. Insertional inactivation of the IMP dehydrogenase gene in another *B. subtilis* strain yielded a culture producing 35 g inosine per L (Miyagawa et al., 1989). Genetic engineering of the inosine monophosphate dehydrogenase gene in a *B. subtilis* strain, which was producing 7 g per L guanosine and 19 g per L inosine, changed production to 20 g per L guanosine and 5 g per L inosine (Miyagawa et al., 1986). Other *B. subtilis* mutants produce as much as 23 g per L guanosine (Kuninaka, 1996). With regard to pyrimidine production, another recombinant strain of *B. subtilis* produces 18 g per L of cytidine and a mutant lacking homoserine dehydrogenase (which increased the concentration of the precursor aspartate in the cell) produces 30 g per L (Asahi et al., 1996).

Riboflavin (vitamin B<sub>2</sub>) was produced commercially for many years by both fermentation and chemical synthesis (Demain, 1972) but today, fermentation is the major route. Riboflavin overproducers include two yeast-like molds, *Eremothecium ashbyii* and *Ashbya gossypii*, which synthesize riboflavin in concentrations greater than 20 g per L. Production of riboflavin amounted to 4 million pounds in 1992 (Vandamme, 1992). A riboflavin-overproducer such as *A. gossypii* makes 40 000 times more vitamin than it needs for its own growth. The biochemical key to riboflavin overproduction appears to involve insensitivity to the repressive effects of iron. New processes using *Candida* species or recombinant *B. subtilis* strains have been developed in recent years which produce 20–30 g riboflavin per L.

Vitamin B<sub>12</sub> (cyanocobalamin) is produced industrially with *Propionibacterium shermanii* or *Pseudomonas denitrificans* (Spalla et al., 1989; Kusel et al., 1984). Such strains make about 100 000 times more vitamin B<sub>12</sub> than they need for their own growth. The key to the fermentation is avoidance of feedback repression by vitamin B<sub>12</sub>. Of major importance in the *P. denitrificans* fermentation is the addition of betaine (Kusel et al., 1984). Vitamin B<sub>12</sub> overproduction is totally dependent upon betaine but the mechanism of control is unknown. Production of vitamin B<sub>12</sub> has reached a level of 150 mg per L (Spalla et al., 1989). Some 3 tons of cyanocobalamin are produced per year with a market of $71 million (McCoy, 1999).

In production of biotin, feedback repression is caused by the enzyme acetyl-CoA carboxylase biotin holoenzyme synthetase, with biotin 5-adenylate acting as corepressor (Barker and Campbell, 1981). Strains of *S. marcescens* obtained by mutagenesis, selected for resistance to biotin antimetabolites and subjected to molecular cloning, produce 600 mg per L in the presence of high concentrations of sulfur and ferrous iron (Masuda et al., 1995). Such a titer is high enough to economically compete with the traditional chemical process. The biotin market is $100 million per year (Shaw et al., 1999). Traditionally, it has been produced chemically but new biological processes are becoming economical. Engineering of *E. coli* genes into *Agrobacterium/Rhizobium* HK4 led to production of 110 mg per L (Shaw et al., 1999).

Vitamin C (L-ascorbic acid) has been produced chemically for many years. It is manufactured at the rate of 60 000 tons per year with a market of $60 million (Wilke, 1999). A novel process involves the use of a genetically engineered *Erwinia herbicola* strain containing a gene from *Corynebacterium* sp. The engineered organism converts glucose into 2-ketogulonic acid, which can be easily converted by acid or base to ascorbic acid (Pramik, 1986). Another process devised independently converts 40 g per L glucose into 20 g per L 2-keto-L-
gulonate (Grindley et al., 1988). This process involves cloning of the gene encoding 2,5-diketo-D-gluconate reductase from Corynebacterium sp. into Erwinia citreus. Plasmid cloning of the genes encoding L-sorbose dehydrogenase and L-sorbose dehydrogenase from Gluconobacter oxydans back into the same organism yielded a strain capable of converting 150 g per L of D-sorbitol into 130 g per L of 2-keto-L-gulonate (Saito et al., 1997).

Microbes have been widely used for the commercial production of organic acids. About 1 billion pounds of citric acid are produced per year with a major market of $1.4 billion. This organic acid is produced via the Embden-Meyerhof pathway and the first step of the tricarboxylic acid cycle. The major control of the process involves the feedback inhibition of phosphofructokinase by citric acid. The commercial process employs the fungus Aspergillus niger in media deficient in iron and manganese. Manganese deficiency has two beneficial effects in the citric acid fermentation: (1) it leads to high levels of intracellular NH₄⁺ which reverses citric acid inhibition of phosphofructokinase, and (2) it brings on the formation of small mycelial pellets which are the best morphological form for citric acid production. The morphological effect is due to a change in cell wall composition caused by growth in low Mn⁺². A high level of citric acid production is also associated with a high intracellular concentration of fructose 2,6-biphosphate, an activator of glycolysis (Harmsen et al., 1992). Other factors contributing to high citric acid production are the inhibition of isocitrate dehydrogenase by citric acid, and the low pH optimum (1.7–2.0). Higher pH levels (e.g. 3.0) lead to production of oxalic and gluconic acids instead of citric acid. The low pH inactivates glucose oxidase which normally would yield gluconic acid (Kubicek and Röhr, 1986). In approximately 4–5 days, the major portion (80%) of the sugar is converted to citric acid, titers reaching about 100 g per L. Citric acid is easily assimilated, palatable and has low toxicity. Consequently, it is widely used in the food and pharmaceutical industry. It is employed as an acidifying and flavor-enhancing agent, as an antioxidant for inhibiting rancidity in fats and oils, as a buffer in jams and jellies, and as a stabilizer in a variety of foods. The pharmaceutical industry uses approximately 16% of the available supply of citric acid. Processes have been developed for the production of citric acid by Candida yeasts, especially from hydrocarbons or oils. Such yeasts are able to convert n-paraffins to citric and isocitric acids in extremely high yields (150–170% on a weight basis). Production of citric acid instead of isocitric acid is favored by selecting mutants which are deficient in the enzymeaconitase. Citric acid titers as high as 225 g per L have been reached with these yeasts (Kubicek and Röhr, 1986).

Other valuable organic acids include acetic, lactic, malic, gluconic, itaconic, tartaric and succinic acids. Succinic acid can be produced by the rumen organism Actinobacillus succinogenes at 110 g per L (Zeikus and Jain, 1999). The projected price at the 75 000 tons/year level is $0.55 per kg. Present production is 15 000 tons per year, all made petrochemically at a price of $5.90–8.80 per kg. The global market for lactic acid is over 100 000 tons per year (Skory and Bothast, 1999) and its price is $1.05 per pound (Wilke, 1999). Products in development are the non-chlorinated solvent, ethyl lactate, and bioplastic, polylactide. Rhizopus oryzae is favored for production since it only makes stereoisomerically pure L-(+)-lactic acid. Lactic acid was made at one time by chemical synthesis but this process has been totally eliminated in favor of fermentation. It is produced anaerobically with a 95% (w/w) yield based on charged carbohydrate, a titer of over 100 g per L and a productivity of over 2 g per
Lactic acid is being polymerized into polylactide which could become a major bioplastic in the future. Although microbial processes exist for the other acids, they have not been exploited commercially on a large scale. An interesting application of genetic engineering to the acetic acid fermentation was the cloning of the aldehyde dehydrogenase gene from *Acetobacter polyoxogenes* on a plasmid vector into *Acetobacter acetii* subsp. *xylinum*. This manipulation increased the rate of acetic acid production by over 100% (from 1.8 to 4 g per Lh) and titer by 40% (from 68 to 97 g per L) (Fukaya et al., 1989). Gluconic acid has a market of $93 million, a price of $0.85 per pound and a production of 40 000 tons per year (Wilke, 1999). Itaconic acid has an annual market of $68 million (McCoy, 1999).

Ethyl alcohol is a primary metabolite that can be produced by fermentation of a sugar, or a polysaccharide that can be depolymerized to a fermentable sugar. Yeasts are preferred for these fermentations but the species used depends on the substrate employed. *Saccharomyces cerevisiae* is employed for the fermentation of hexoses, whereas *Kluyveromyces fragilis* or *Candida* species may be utilized if lactose or pentoses respectively are the substrates. Under optimum conditions, approximately 10–12% ethanol by volume is obtained within 5 days. Such a high concentration slows down growth and the fermentation ceases. With special yeasts, the fermentation can be continued to alcohol concentrations of 20% by volume. However, these concentrations are attained only after months or years of fermentation. Although synthetic ethanol production from the petrochemical ethylene was once the predominant source of industrial ethanol, today it is mainly manufactured by fermentation at a level of 4 million tons per year; synthetic ethanol amounts to only 1 million tons. Bacteria such as clostridia and *Zymomonas* are being reexamined for ethanol production after years of neglect. *Clostridium thermocellum*, an anaerobic thermophile, can convert waste cellulose directly to ethanol. Other clostridia produce acetate, lactate, acetone and butanol and will be utilized as petroleum becomes depleted in the world. Fuel ethanol produced from biomass would provide relief from air pollution caused by the use of gasoline and would not contribute to the greenhouse effect. Because of the elimination of lead from gasoline, ethanol is being substituted as a blend to raise gasoline’s octane rating. The US demand for fuel ethanol in 1995 was 1 billion gallons per year mainly as a fuel oxygenate (O’Brien and Craig, 1996) and was expected to reach 2 billion gallons by 2001. *E. coli* has been converted into an excellent ethanol producer (43%, v/v) by recombinant DNA technology (Ingram et al., 1987). Alcohol dehydrogenase II and pyruvate decarboxylase genes from *Zymomonas mobilis* were inserted in *E. coli* and became the dominant system for NAD regeneration. Ethanol represents over 95% of the fermentation products in the genetically engineered strain, whereas the original *E. coli* strain carried out a mixed acid fermentation. With regard to beverage ethanol, some 60 million tons of beer and 30 million tons of wine are produced each year.

Polysaccharides are also important commercial products made by microorganisms. The most well known is xanthan which is produced at 30 000 tons per year with a market of $408 million (Wilke, 1999; McCoy, 1999).

In addition to the multiple reaction sequences of fermentations, microorganisms are extremely useful in carrying out biotransformation processes in which a compound is converted into a structurally related product by one or a small number of enzymes contained in the cells (Rosazza, 1982). Bioconverting organisms are known for practically every type of chemical reaction. Transformed steroids have been very important products for the pharmaceutical
industry. One of the earliest and most famous is the biotransformation of progesterone to 11α-hydroxyprogesterone. The reactions are stereospecific, the ultimate in specificity being exemplified by such steroid bioconversions. This specificity is exploited in the resolution of racemic mixtures, when a specific isomer rather than a racemic mixture is desired. Bioconversion is becoming essential to the fine chemical industry in that their customers demand single-isomer intermediates (Rogers, 1999). Bioconversions are characterized by extremely high yields (i.e. 90–100%). Other attributes include mild reaction conditions and the coupling of reactions using a microorganism containing several enzymes working in series. There is a tremendous interest in immobilized cells to carry out such processes. These are usually much more stable than either free cells or enzymes and are more economical than immobilized enzymes. Recombinant DNA techniques have been useful in developing new bioconversions. For example, the cloning of the fumarase-encoding gene in *S. cerevisiae* improved the bioconversion of malate to fumarate from 2 g per L to 125 g per L in a single manipulation (Neufeld et al., 1991). The conversion yield using the constructed strain was near 90%.

4. Production of secondary metabolites

Microbially produced secondary metabolites (Table 2) are extremely important to our health and nutrition (Demain and Fang, 1995). As a group that includes antibiotics, other medicinals, toxins, pesticides and animal and plant growth factors, they have tremendous economic importance. In batch or fed-batch culture, secondary metabolites are produced usually

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>World market ($ millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>28 000</td>
</tr>
<tr>
<td>Penicillins G &amp; V</td>
<td>11 000</td>
</tr>
<tr>
<td>Erythromycins</td>
<td>4400</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>3500</td>
</tr>
<tr>
<td>Vancomycin &amp; Teichoplanin</td>
<td>1400</td>
</tr>
<tr>
<td>Rocephin (semisynthetic cephalosporin)</td>
<td>1000</td>
</tr>
<tr>
<td>Antihelmintics</td>
<td></td>
</tr>
<tr>
<td>Avermectins</td>
<td>1000</td>
</tr>
<tr>
<td>Hypcholesterolemic agents</td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>8400</td>
</tr>
<tr>
<td>Immunosuppressants</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>1500</td>
</tr>
<tr>
<td>Antitumor agents</td>
<td></td>
</tr>
<tr>
<td>Taxol&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1000</td>
</tr>
<tr>
<td>Plant growth enhancers</td>
<td></td>
</tr>
<tr>
<td>Gibberellins</td>
<td>120</td>
</tr>
<tr>
<td>Bioinsecticides</td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt; toxin</td>
<td>125</td>
</tr>
</tbody>
</table>

<sup>a</sup>Made from plants.
after growth has slowed down. They have no function in growth of the producing cultures, are produced by certain restricted taxonomic groups of organisms, and are usually formed as mixtures of closely related members of a chemical family. In nature, secondary metabolites are important for the organisms that produce them, functioning as: (1) sex hormones; (2) ionophores; (3) competitive weapons against other bacteria, fungi, amoebae, insects and plants; (4) agents of symbiosis; and (5) effectors of differentiation (Demain, 1996).

The best known of the secondary metabolites are the antibiotics. This remarkable group of compounds form a heterogeneous assemblage of biologically active molecules with different structures and modes of action. They attack virtually every type of microbial activity such as DNA, RNA, and protein synthesis, membrane function, electron transport, sporulation, germination, and many others. Since 1940, we have witnessed a virtual explosion of new and potent antibiotic molecules which have been of great use in medicine, agriculture, and basic research. However, the search for new antibiotics continues in order to combat evolving pathogens and naturally resistant bacteria and fungi, and previously susceptible microbes that have developed resistance; improve pharmacological properties; combat tumors, viruses, and parasites; and discover safer and more potent compounds. From 1990 to 1994, over 1000 new secondary metabolites were characterized from actinomycetes alone (Sanglier et al., 1996). About 6000 antibiotics have been described, 4000 from actinomycetes, and they still are being discovered at a rate of about 500 per year. *Streptomyces griseus* strains produce over 40 different antibiotics and *B. subtilis* over 60. Strains of *Streptomyces hygroscopicus* make almost 200 antibiotics. One *Micromonospora* strain can produce 48 aminocyclitol antibiotics. The antibiotics vary in size from small molecules like cycloserine (102 daltons) and bacilysin (270 daltons) to polypeptides such as nisin which contains 34 amino acid residues. In the pursuit of effective antibiotics, many of the new products are made chemically by modification of natural antibiotics; this process is called ‘semisynthesis.’ As early as 1974, there were over 20 000 semisynthetic pencillins, 4000 cephalosporins, 2500 tetracyclines, 1000 rifamycins, 500 kanamycins, and 500 chloramphenicols which had been prepared. The antibiotic market includes about 160 antibiotics and derivatives such as the β-lactam peptide antibiotics, the macrolide polyketide erythromycin, tetracyclines, aminoglycosides and others (Strohl, 1997; Brown, 1996). The worldwide market for antibiotics in 1996 was over $28 billion (Bentley, 1999). The β-lactam antibiotics are the natural penicillin G and the biosynthetic penicillin V with a combined market of $4.4 billion, many semisynthetic penicillins, and the semisynthetic cephalosporins which have a market of $11 billion. Twenty-six thousand tons of penicillin G and 10 000 tons of penicillin V are made per year (van Nistelrooij et al., 1998). The precursor of the semisynthetic penicillins, 6-aminopenicillanic acid (6APA), is made at a level of 9000 tons per year (Lalonde, 1997). In 1992, the 16 900 tons of penicillin G produced were distributed as follows:

1. direct medical use: 12%
2. conversion to 6-APA: 65%
3. conversion to 7-ADCA and other intermediates: 20%
4. feed use: 2%.

Of the 7500 tons of penicillin V produced, the following distributions occurred:

1. direct medical use: 19%
2. conversion to 6-APA: 59%
3. conversion to 7-ADCA and other intermediates: 23%.

Of the 7350 tons of 6-APA produced from penicillins G and V, 48% was used for ampicillin and 27% for amoxicillin. A single semisynthetic cephalosporin, ceftriaxone (=rocephin) has a market of $1 billion. The market for tetracyclines is $1.4 billion, for erythromycins $3.5 billion, and for the glycopeptides vancomycin plus teicoplanin, $1 billion (McDaniel et al., 1999; Williams and Bardsley, 1999).

For years, the major pharmaceuticals (such as hypertensive and anti-inflammatory agents) used for non-infectious diseases were strictly synthetic products. Similarly, major therapeutics for non-microbial parasitic diseases in animals (e.g. coccidiostats and anthelmintics) came from the screening of synthesized compounds followed by molecular modification. Despite the testing of thousands of synthetic compounds, only a few promising structures were uncovered. As new lead compounds became more and more difficult to find, microbial broths filled the void and microbial products increased in importance in therapy of non-microbial diseases. Today, microbially produced polyethers (Westley, 1977) such as monensin, lasalocid and salinomycin dominate the coccidiostat market and are also the chief growth promotants in use for ruminant animals. The avermectins, another group of streptomycete products with a market of nearly 1 billion dollars per year, have high activity against helminths and arthropods (Campbell et al., 1983). Indeed, their activity is an order of magnitude greater than previously discovered anthelmintic agents, the vast majority of which were synthetic. Many microbial products with important pharmacological activities were discovered by screening for inhibitors using simple enzymatic assays (Umezawa, 1982). One huge success has been the statins, including lovastatin (mevinolin), pravastatin and others produced by fungi (Endo, 1985) which act as cholesterol-lowering agents. Lovastatin is produced by Aspergillus terreus. The statins are potent competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase from liver. They have a very large market of $8.4 billion (Stinson, 1998). Another important enzyme inhibitor is clavulanic acid, an actinomycete β-lactam, which acts as an inhibitor of β-lactamases and is sold in combination with penicillins. A third type of enzyme inhibitor on the market is acarbose, a natural inhibitor of intestinal glucosidase (Müller, 1986), which is produced by an actinomycete of the genus Actinoplanes. It decreases hyperglycemia and triglyceride synthesis in adipose tissue, liver, and the intestinal wall of patients suffering from diabetes, obesity, and type IV hyperlipidemia.

Also in commercial use are biopesticides including fungicides (e.g. kasugamycin, polyoxins), bioinsecticides (Bacillus thuringiensis crystals, nikkomycin, spinosyns), bioherbicides (bialaphos), antiherbimectins and coccidiostats as mentioned above, ruminant growth promoters (monensin, lasalocid, salinomycin), plant growth regulators (gibberellins), immunosuppressants for organ transplants [cyclosporin A, FK-506, rapamycin], anabolic agents in farm animals (zearelanone), uterocaractants (ergot alkaloids), and antitumor agents (doxorubicin, daunorubicin, mitomycin, bleomycin) (Demain, 1983; Chadwick and Whelan, 1992). Cyclosporin A has a market of $1.5 billion. The plant secondary metabolite, taxol, which is also made by fungi, has a $1 billion market (Mitscher et al., 1998); pine needles of the yew plant are its major commercial source. Many of the above compounds were first isolated as poor or toxic antibiotics (e.g. monensin, cyclosporin, rapamycin) or as mycotoxins (ergot al-
kaloids, gibberellins, zearelanone) before they were put to work for our benefit. The gibberellins are isoprenoid growth regulators controlling flowering, seed germination and stem elongation (Tudzinski, 1999). They are produced at a level of over 25 tons per year and have a market of $100 million. The protein crystal of *Bacillus thuringiensis* has a bioinsecticide market of $125 million (Stabb et al., 1994) but its major importance lies in its gene which is used to make recombinant plants insect resistant.

Genetics has had a long history of contributing to the production of microbial secondary metabolites, such as antibiotics (Holt and Saunders, 1985). The tremendous increases in fermentation productivity and the resulting decreases in costs have come about mainly by mutagenesis and screening for higher producing microbial strains. Mutation has also served to: (1) shift the proportion of metabolites produced in a fermentation broth to a more favorable distribution, (2) elucidate the pathways of secondary metabolism, and (3) yield new compounds. The medically useful products demethyltetracycline and doxorubicin (adriamycin) were discovered by simple mutation of the cultures producing tetracycline and daunorubicin (daunomycin), respectively. The technique of mutational biosynthesis has been used for the discovery of many new aminoglycoside, macrolide, and anthracycline antibiotics. It has recently been successfully employed in producing a new commercial avermectin, called doramectin (Denoya et al., 1995).

5. Recombinant DNA

The modern biotechnology industry has made a major impact in the business world. Biopharmaceuticals (recombinant protein drugs, vaccines and monoclonal antibodies) have a market of $15 billion. In the U.S. alone, there are about 1600 biotechnology companies, employing 153 000 people, with total revenues of $19.6 billion and sales of $13.4 billion (Ernst & Young, 1999). The U.S. biotechnology companies had 14 biopharmaceuticals approved in 1998 and 300 more were in phase II and III clinical trials. The industry was valued at $93 billion in 1997 (Reisman, 1999). The contract manufacturing market is $350–450 million per year. Over 80 biotechnology drugs are now on the market and over 1200 are in clinical trials. R&D spending in the US biotechnology industry approached $10 billion in 1998, an increase of 16% over 1997 (Ernst & Young, 1999). Canada has 280 companies, 10 000 employees and annual revenues of $1 billion (Yanchiski, 1999). Europe has almost 1200 companies, 45 000 employees and revenues of $3.7 billion; the UK and Germany are the major players. Japanese biotechnology has been conducted predominantly in the major pharmaceutical, food and beverage companies with only a few small biotechnology companies in existence. However, changes are occurring in Japan and small startups are being encouraged and formed.

The most well-known products of the modern biotechnology industry are the mammalian polypeptides such as erythropoietin (EPO) with a $2.9 billion market; interferon, $1.6 billion; human growth hormone (HGH; human somatotropin), $1.1 billion; human insulin, $1 billion; granulocyte colony stimulating factor (G-CSF), $720 million; and tissue plasminogen activator (tPA), $290 million, among others (Swartz, 1996; Pramik, 1999). Another important product is recombinant hepatitis B vaccine ($725 million). These polypeptides are mainly made in bacteria such as *E. coli* ($2.9 billion) and mammalian cell culture ($3.3 billion) (Langer, 1999) but yeasts, filamentous fungi and insect cells are also important. The de-
velopment of animal cell culture was facilitated by prior developments in microbial fermentation technology. In the very near future, it is expected that many products will be made in transgenic animals and plants. One reason is the high cost of mammalian cell products (e.g. 1992 selling prices for tPA, HGH, G-CSF and EPO were $23 000, $35 000, $450 000 and $840 000 per gram, respectively).

Recombinant DNA technology has also produced a revolution in agriculture where in 1998, the farm market included insect-resistant corn, potato, soybean; herbicide-resistant canola, cotton, soybean and corn; virus-resistant squash; and canola containing specialty oils and tomato with increased pectin. In 1999, transgenic plants composed 50% of the cotton crop, 30% of the soybean crop and 15% of the corn crop in the United States. Unfortunately, these amazing developments are currently being stymied by political pressure from anti-genetics groups. Little do they realize that the green revolution, which was so important in feeding the world in the last 50 years, came about through genetics in the form of plant breeding. The new genetics of biotechnology will be necessary to feed the ever-expanding world population.

Experiencing immediate impact from the developments in recombinant DNA technology was the enzyme industry which had been supplying industrial enzymes with a market of about $300 million in the 1980s. Enzyme companies, realizing that their products were encoded by single genes, rapidly adopted recombinant DNA techniques to increase enzyme production and to make new enzymes. As a result, today’s industrial enzyme market has annual sales of $1.6 billion with applications in food and starch processing (45%), detergents (34%), textiles (11%), leather (3%) and pulp and paper (1.2%) (Stroh, 1998). The protease subtilisin, which is used in washing powders, accounts for $200 million of this market (Wackett, 1997). Over 60% of these enzymes are recombinant products (Cowan, 1996). The world markets for some products of enzymatic reactions are as follows (Rozell, 1999):

1. High fructose corn syrup: $1 billion
2. Aspartame: $800 million
3. Acrylamide: $300 million.

Significant markets exist for specialty enzymes such as recombinant chymosin for cheese making ($140 million) (Stroh, 1994), restriction enzymes for molecular techniques ($100 million) (Wrotnowski, 1996) and Taq polymerase for PCR applications ($80 million) (Persidis, 1998). A huge market ($2.3 billion) exists for therapeutic enzymes (Barber, 1996), many of which are the same polypeptides mentioned above.

Recombinant DNA technology has been applied to the production of antibiotics (Chater, 1990). Many genes encoding individual enzymes of antibiotic biosynthesis have been cloned and expressed at high levels in heterologous microorganisms. Continued efforts in the application of recombinant DNA technology to fermentations have led to overproduction of limiting enzymes of important biosynthetic pathways, thereby increasing production of the final products (Skatrud et al., 1989). Antibiotic biosynthetic pathways are often encoded by clustered chromosomal genes, especially in bacteria, which facilitates transfer of an entire pathway in a single manipulation. Even in fungi, pathway genes are sometimes clustered, such as the penicillin biosynthesis genes in Penicillium.

For the discovery of new or modified products, recombinant DNA techniques are being used to introduce genes coding for antibiotic synthetases into producers of other antibiotics.
or into nonproducing strains to obtain modified or hybrid antibiotics (Epp et al., 1989; Strohl et al., 1989; Khosla et al., 1996).

6. Final comments

Today, microbiology is a major participant in global industry. The best is yet to come as microbes move into the environmental and energy sectors. As stated many years ago by Jackson W. Foster “Never underestimate the power of the microbe,” and by David Perlman “If you take care of your microbial friends, they will take care of your future” and you will live happily ever after.

Acknowledgments

I thank Aiqi Fang for advice and Fang Fang Traves for help in preparation of the manuscript.

References


Epp JK, Huber MLB, Goodson T, Schoner BE. Production of a hybrid macrolide antibiotic in *Streptomyces am-

Ernst & Young. The Ernst & Young Fourteenth Annual Report on the Biotechnology Industry. Ernst & Young LLP, 1999.


