STADLER SYMP. Vol. 8 (1976) University of Missouri, Columbia
pp. 41-55

GENETIC REGULATION OF FERMENTATION ORGANISMS

(Fermentation, Regulation, Antibiotics)

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SUMMARY

An effective fermentation organism is a wasteful creature that overproduces and excretes its metabolic intermediates and end products. Cultures obtained from screening programs usually possess subnormal regulatory controls. Development programs to increase product formation modify the residual control mechanisms so that the culture's "inefficiency" is increased.

For production of primary metabolites, feedback inhibition and repression must be bypassed. This is usually accomplished by limiting the intracellular concentration of feedback inhibitors and repressors. Auxotrophic mutants and analogue-resistant mutants are most often used for this purpose. Development of fermentations for secondary metabolites, such as antibiotics, is less rational because of our ignorance of the biosynthetic pathways and regulatory controls involved. However, evidence is accumulating that such fermentations are subject to (a) feedback regulation by the idiolite itself, (b) feedback regulation by primary metabolites that share a branched pathway with the secondary metabolite, (c) feedback regulation by inorganic phosphate, (d) catabolite regulation by rapidly utilized carbon sources, (e) induction by primary metabolites, and (f) ATP regulation. Secondary metabolites are not usually formed during growth because the enzymes of secondary metabolism are repressed during the trophophase. We have no clear idea about the type of repression control, but it probably involves growth rate as well as the factors mentioned above.

Since the controls discussed above are genetically determined, mutations to increase productivity have been useful to the fermentation industry for over 30 years. Although such strain improvement programs usually involve random screening of survivors of mutagenesis, some recent progress has been made in the application of more rational screening procedures. Mutants are also used to change the spectrum of metabolites, to

produce new antibiotics, and to elucidate the pathways of secondary metabolism.

Extensive research is now taking place on the genetic mapping of antibiotic-producing microorganisms, especially actinomycetes. The model for this work is the genetic map of Streptomyces coelicolor, and the maps of more recently examined actinomycetes, including Nocardia, appear to be similar. At least four of the genes of methylenomycin A production in S. coelicolor are plasmid-bound.

INTRODUCTION

A growing microorganism breaks down high molecular weight carbon and energy sources, brings the smaller derivatives into the cell, degrades them to smaller molecules, converts these to amino acids, nucleotides, vitamins, carbohydrates, and fatty acids, and finally builds these basic materials into proteins, coenzymes, nucleic acids, mucopeptides, polysaccharides, and lipids. Hundreds of enzymes must be made and must act in an integrated manner to avoid total chaos. To do this, regulatory mechanisms have evolved that enable a species to compete efficiently with other forms of life and to survive in nature. Thus, the ideal cell does not overproduce metabolites, regardless of its environment. Some of the important control mechanisms are substrate induction, feedback regulation, catabolite regulation, and energy charge regulation.

Let us now shift our attention from the viewpoint of the microorganism to that of the fermentation microbiologist. What we desire is a wasteful strain, which will overproduce and excrete a particular compound that can then be isolated (DEMAIN, 1972). Usually, organisms from culture collections or from nature are first screened for their ability to overproduce the desired product. Without necessarily realizing it, the microbiologist is searching for the organism with the weakest regulatory mechanisms. Once the desired strain is found, a development program is begun to improve yields by modification of culture conditions and by mutation. The microbiologist is actually modifying the regulatory controls remaining in the original culture so that its inefficiency can be further increased. Until very recently, these manipulations were done in total ignorance of the basic factors involved. Due to the increase in our knowledge of microbial biochemistry and genetics, we now have some idea of these factors, and new fermentation processes for primary products, such as amino acids, vitamins, and purine nucleotides, are being developed on a more rational basis. On the other hand, the development of fermentations for secondary metabolites (those with no function in growth) still relies mainly on the empirical approach because of our ignorance of the pathways and the regulatory circuits involved in secondary metabolism.

Only within the last few years has evidence begun to accumulate to support the thesis that the organisms selected by our screening procedures are, in fact, subnormally regulated.

For example, *Corynebacterium glutamicum*, a species used for the commercial production of L-lysine, has been found to possess an initial enzyme of the lysine branch (dihydrodipicolinate synthetase) that is resistant to feedback inhibition by lysine (NAKAYAMA et al., 1966). In *Claviceps paspali*, the production of alkaloids involves a precursor, tryptophan. In this organism, the first enzyme of the tryptophan biosynthetic branch (anthranilate synthetase) is resistant to feedback inhibition by tryptophan (LINGENS et al., 1967), thus allowing for tryptophan overproduction. Chloramphenicol production by *Streptomyces* sp. occurs by a shunt pathway from shikimic acid, a normal intermediate in aromatic amino acid biosynthesis. In the producing organism, the first enzyme of aromatic biosynthesis [3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthetase] resists feedback inhibition and repression by phenylalanine, tryptophan, and tyrosine (LOWE and WESTLAKE, 1971).

OVERPRODUCTION OF PRIMARY METABOLITES

In biosynthetic pathways leading to primary metabolites, the main regulatory control mechanism is feedback, which involves inhibition of an early biosynthetic enzyme and/or repression of one or more of the biosynthetic enzymes by the final product or its derivative. Most processes designed to produce primary metabolites work by limiting the intracellular concentration of feedback inhibitors or repressors. Usually, one employs an auxotrophic mutant. Since such an organism requires the end product for growth, one can limit the intracellular concentration of this inhibitory or repressive end product by feeding it to the culture at growth-limiting levels; feedback regulation is then bypassed, and high levels of the desired intermediate accumulate. Some examples are given in Table 1.

TABLE 1

Overproduction of Primary Metabolites

A. By partial starvation of auxotrophic mutants

Product	Auxotrophic requirement
L-Glutamic acid	Glycerol
L-Lysine	Homoserine
L-Threonine	Lysine, methionine, isoleucine

B. By selecting antimetabolite-resistant mutants

Product	Antimetabolite resistance
L-Threonine	α-Amino-β-hydroxyvaleric acid
L-Methionine	Ethionine
L-Tryptophan	5-Fluorotryptophan

A second way to eliminate feedback regulation and accumulate primary metabolites is to alter the structure of the enzyme subject to inhibition or to modify the regulatory genes so that the system is no longer repressible. This modification is done by selecting mutants that resist the toxic effects of an analogue of the desired product. Many of the resistant mutants overproduce and excrete the natural end product (Table 1). Certain feedback-resistant overproducing mutants possess desensitized enzymes, whereas others have derepressed enzyme-forming systems. Strains with both types of mutation usually show synergistic excretion of the natural metabolite.

FEEDBACK REGULATION OF SECONDARY METABOLISM

The major obstacle in applying the above clear-cut principles to the rational development of secondary metabolite fermentations has been our ignorance; we knew little of the pathway intermediates, less about the enzymes, and almost nothing about the feedback regulation of secondary metabolism. However, the situation is changing rapidly as we learn more and more about secondary biosynthesis and its control (DREW and DEMAIN, 1975a).

Considerable evidence is accumulating that secondary metabolites (or idiolites) exert feedback regulation on their own formation. For example, antibiotics such as chloramphenicol (JONES and WESTLAKE, 1974), ristomycin (EGOROV et al., 1971), virginiamycin (YANAGIMOTO and TERUI, 1971a), aurodox (LIU et al., 1975b), cycloheximide (KOMINEK, 1975), penicillin (GORDEE and DAY, 1972), and mycophenolic acid (MUTH and NASH, 1975) limit their own synthesis. With the exceptions of chloramphenicol and mycophenolic acid, the feedback mechanism(s) is unknown. Chloramphenicol feedback acts by repressing arylamine synthetase (JONES and WESTLAKE, 1974). This newly discovered enzyme is the first enzyme of the chloramphenicol branch of the aromatic amino acid biosynthetic pathway, which converts chorismic acid to an identified aromatic amine. In the producing culture, this key enzyme is produced just before chloramphenicol formation and then rapidly disappears. Maximum repression occurs at 100 mg/liter, a concentration of the antibiotic that does not affect growth or markedly repress chorismate mutase, prephenate dehydratase, or anthranilate synthetase. Mycophenolic acid inhibits its own formation by inhibiting the final enzyme, an O-methyltransferase, in *Penicillium stoloniferum* (MUTH and NASH, 1975).

Feedback regulation is also observed in nonantibiotic idiolites. In *Claviceps* strain SD58, an alkaloid end product, elymoclavine, inhibits DAHP synthetase (the first enzyme of aromatic amino acid synthesis, which produces the precursor tryptophan) (SCHMAUDER and GRÖGER, 1973). In addition, the first enzyme unique to alkaloid synthesis, dimethylallyl transferase, is inhibited by agroclavine and elymoclavine (HEINSTEIN et al., 1971).

A second type of feedback regulation is involved in the case of branched pathways leading to primary and secondary me-

tabolites. In such cases, negative feedback regulation of an early common enzyme by the primary end product might be expected to diminish production of the idiolite. The original finding of the depression of penicillin formation by L-lysine (DEMAIN, 1957) was puzzling until it was realized that α-aminoadipate is an intermediate in the biosynthesis of both lysine and penicillins. L-lysine also decreases production of the related antibiotic cephalosporin C (D'AMATO and PISANO, 1975).

Lysine diminution of penicillin formation is caused by a feedback effect on its own biosynthesis. Although penicillin production media do not contain the high levels of free lysine required to inhibit penicillin production, the internal concentration of lysine is the important factor in the practical sense. In a series of regulatory mutants that overproduce lysine, an inverse correlation was found between lysine production and penicillin formation (MASUREKAR and DEMAIN, 1974).

To determine whether lysine reduces penicillin production by feedback repression or inhibition, MASUREKAR and DEMAIN (1972) studied the effect of lysine on [^{14}C]-valine incorporation into penicillin in short-time experiments. Because the presence of lysine during growth did not inhibit subsequent valine incorporation by washed mycelial suspensions, repression could not have been the inhibitory mechanism. However, marked inhibition was observed when lysine was added to the incorporation mixture. These results indicated that the depressive effect of lysine was caused by inhibition of an enzyme involved in penicillin biosynthesis. Later studies (DEMAIN and MASUREKAR, 1974) showed that the first enzyme of lysine biosynthesis, homocitrate synthase, is susceptible to lysine inhibition in cells of *P. chrysogenum*. The specific activity of this enzyme is high during the trophophase (growth phase) but drops at the onset of the idiophase (production stage); the residual activity is markedly inhibited by lysine. The resulting decrease in the intracellular concentration of L-α-aminoadipate apparently is the cause of the reduction in penicillin formation.

Similarly, a mixture of the three aromatic amino acids reportedly depresses production of candicidin in *Streptomyces griseus* (LIU et al., 1972). [The active amino acid in the mixture is L-tryptophan (MARTIN and DEMAIN, unpublished results).] The apparent reason for this depression is that the p-aminobenzoic acid moiety of candicidin is made via a branch of the aromatic amino acid biosynthetic pathway.

A third type of feedback regulation involves inorganic phosphate. Many fermentations must be conducted in the presence of levels of inorganic phosphate that are suboptimal for growth. In some cases, the diminution in product formation caused by phosphate probably involves the well-known feedback regulation of phosphatases by inorganic phosphate. Phosphatases must participate in biosynthesis because biosynthetic intermediates of many secondary pathways are phosphorylated, although the ultimate products are not. For example, streptomycin biosynthesis, which is markedly inhibited by phosphate

(DEMAIN and INAMINE, 1970), includes at least three phosphate-cleaving steps in the formation of the streptidine moiety alone (WALKER, 1971). The final enzymatic step in streptomycin production appears to be the cleavage of phosphate from streptomycin phosphate. The enzyme catalyzing this reaction is inhibited by inorganic phosphate (WALKER and WALKER, 1971). Thus when a streptomycin fermentation is conducted in a complex medium containing a 10 mM phosphate supplement, streptomycin production decreases, while the biologically inactive streptomycin phosphate accumulates extracellularly (MILLER and WALKER, 1970).

CARBON CATABOLITE REGULATION OF SECONDARY METABOLISM

The inhibition or repression of enzymes by catabolism of a rapidly used carbon source, usually glucose, affects secondary metabolism. After years of empirical development, most fermentations are now conducted with sources of carbon and energy other than glucose. If glucose is used, it is usually fed at a slow, continuous rate so that catabolites do not accumulate.

Glucose markedly decreases alkaloid production by *C. paspali*, so, in this case, polyols and organic acids are the preferred carbon and energy sources. Glucose also suppresses the production of actinomycin, penicillin, cephalosporin, violacein, indolmycin, prodigiosin, siomycin, mitomycin, bacitracin, neomycin, and coumermycin. When the enniatin fermentation is carried out with glucose (which is rapidly utilized), production occurs only after growth. If the slowly metabolized lactose is used, antibiotic production accompanies growth (ANDHYA and RUSSELL, 1975). Although these effects may be indirectly caused by acid production or oxygen depletion, some enzymes of secondary biosynthesis are known to be repressed by glucose. One is phenoxazinone synthetase (GALLO and KATZ, 1972), an enzyme of actinomycin biosynthesis. Galactose is not repressive and is preferred as a carbon source for actinomycin formation in a chemically defined medium. O-Demethylpuromycin methylase, the last enzyme of puromycin biosynthesis, is also repressed by glucose (SANKARAN and POGELL, 1973). In *Streptomyces niveus*, the producer of novobiocin, citrate causes catabolite repression and glucose does not (KOMINEK, 1972). In a medium containing citrate and glucose, citrate is used first. Only after citrate exhaustion and a diauxic growth lag is glucose used and novobiocin formed.

INDUCTION OF SECONDARY PATHWAYS

In certain fermentations for secondary metabolites, the response to stimulatory additives resembles the phenomenon of enzyme induction. One example involves tryptophan, a stimulatory precursor of ergoline alkaloids in *Claviceps*. Although the stimulatory action of tryptophan was once thought to result solely from its precursor activity, the following observations indicate that it also acts as an inducer of one or more idiophase enzymes: (a) tryptophan analogues, such as thiotrypto-

phan, are not incorporated into alkaloids but still stimulate alkaloid production (KRUPINSKI et al., 1976); (b) stimulation of alkaloid biosynthesis requires tryptophan addition during the trophophase; a later addition has little or no effect (VINING, 1970); (c) added tryptophan is removed from the medium during growth and reaches two- to threefold the normal intracellular concentration just before alkaloid production (ROBBERS et al., 1972). Very recently, KRUPINSKI et al. (1976) showed that the addition of tryptophan or thiotryptophan induces the first enzyme of alkaloid biosynthesis, dimethylallyl tryptophan synthetase.

A similar induction-like effect is seen in the stimulation by methionine of cephalosporin C biosynthesis in *Cephalosporium acremonium* (DEMAIN, 1974a; DREW and DEMAIN, 1975b). The amino acid is not required for growth, although it can be used as a sole source of nitrogen or sulfur. The mechanism of methionine stimulation has been a controversial matter. It is clear that methionine sulfur is an efficient precursor of the sulfur atom of cephalosporin C; [^{35}S]-methionine is incorporated into the antibiotic in an undiluted state, presumably by the reverse transsulfuration pathway (Fig. 1). Despite this evidence of methionine sulfur incorporation into antibiotic, it is certain that this is not the mechanism responsible for the stimulation produced by methionine.

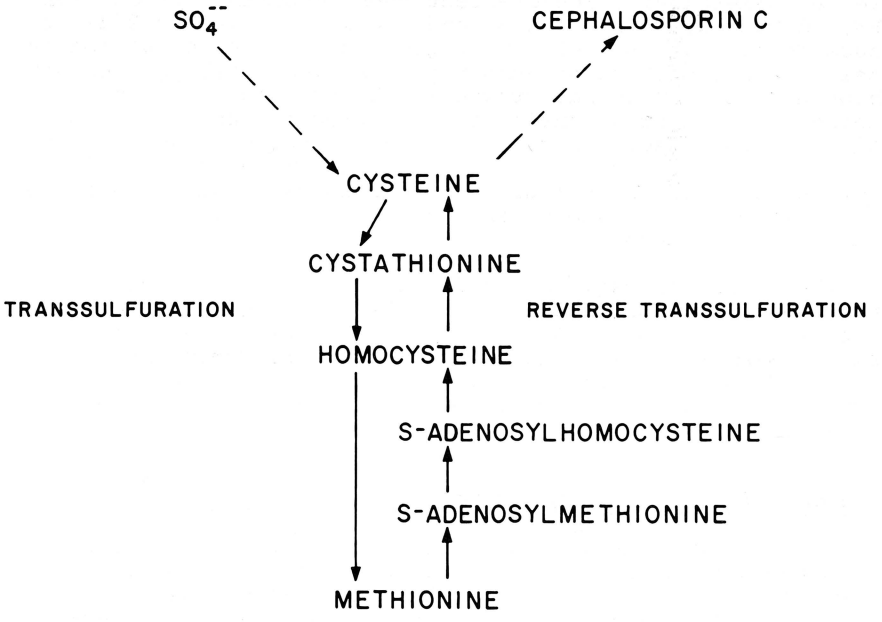

Figure 1. Fungal pathway of sulfur metabolism.

One reason for suspecting a role for methionine other than

that of a sulfur precursor was the finding that the amino acid exerts its major effect on cephalosporin C synthesis when added during growth, i.e., before antibiotic synthesis commences. This phenomenon suggested some sort of a regulatory action. Another reason was the inability of other sulfur compounds to optimize cephalosporin C biosynthesis. Of particular importance was the inactivity of intermediates between methionine and cephalosporin C, e.g., cystathionine and cysteine. Studies of an early blocked sulfur auxotroph (274-1), which grows on cysteine, cystathionine, and methionine but not on sulfate, demonstrated the utility of such organic sulfur sources in antibiotic production. It was reasoned that if methionine were acting solely as a sulfur precursor for the antibiotic, the sulfur amino acids should stimulate cephalosporin C production in the order cysteine > cystathionine > methionine. The order actually observed (DREW and DEMAIN, 1975b) was the opposite of the above, i.e., methionine > cystathionine > cysteine. This observation suggested that cysteine and cystathionine must be first converted into methionine by transsulfuration before they can stimulate antibiotic formation, even though their activity as sulfur precursors should not involve methionine. To examine this point, various types of sulfur auxotrophs were utilized. For example, it was known that the early blocked auxotroph 274-1 could produce cephalosporin C when growing on cysteine. The pathway from cysteine to cephalosporin C should not involve methionine as an intermediate. An experiment was done to determine whether genetically blocking transsulfuration from cysteine to methionine would affect cephalosporin C production (DREW and DEMAIN, 1975b). A second block was added to 274-1 to produce double mutant 11-8, which grew on methionine but not on cysteine. This methionine auxotroph was grown with just enough methionine to allow normal growth in the presence of excess cysteine. Little or no antibiotic was produced unless excess methionine was added. Of crucial significance was the finding that excess norleucine could replace excess methionine--and norleucine contains no sulfur! It thus appears that methionine is a regulator of cephalosporin C production. However, we still do not know the mechanism.

Other possible examples of induction include the stimulation of (a) fosfomycin synthesis by the methyl group precursor methionine (ROGERS and BIRNBAUM, 1974), (b) virginiamycin synthesis by a molecule excreted by the producer, *Streptomyces virginiae* (YANAGIMOTO and TERUI, 1971b), and (c) rifamycin formation in *Nocardia mediterranei* by diethylbarbiturate (LANCINI and WHITE, 1973).

ATP REGULATION IN SECONDARY METABOLISM

Chlortetracycline formation is markedly reduced by inorganic phosphate; in batch fermentations, the idiophase begins when phosphate in the medium is exhausted. Since chlortetracycline biosynthesis involves no known phosphorylated intermediates, the detrimental effect of phosphate probably does not involve feedback regulation of phosphates. There is a distinct possibility that the mechanism of phosphate inhibition of chlor-

tetracycline biosynthesis involves regulation by ATP or energy charge. Janglova et al. (1969) examined the ATP content of two strains of *Streptomyces aureofaciens*, one a low producer (200 µg/ml) and the other a high producer (2000 µg/ml) of chlortetracycline. In both strains, ATP concentration increased during growth, then rapidly decreased and remained at a low level for the rest of the fermentation cycle. Throughout the experiment, the low producer was found to have two to four times as much ATP as the high producer.

Like chlortetracycline, candicidin is best produced at a phosphate concentration that limits growth (LIU et al., 1975a). The addition of phosphate at any time inhibits candicidin production and stimulates growth, glucose utilization, and oxygen uptake. In the normal fermentation, the internal concentration of ATP drops sharply at the end of DNA replication but before antibiotic synthesis (MARTIN and DEMAIN, unpublished). This phenomenon suggests that the addition of phosphate inhibits antibiotic formation by increasing intracellular ATP. Indeed, the addition of inorganic phosphate leads to an immediate threefold increase in the intracellular level of ATP (MARTIN and DEMAIN, unpublished). Phosphate-resistant mutants are now under study.

Inorganic phosphate, which at high levels totally inhibits alkaloid formation, has been shown to repress the first enzyme unique to alkaloid biosynthesis (dimethylallyl tryptophan synthetase) (KRUPINSKI et al., 1976) as well as chanoclavine-I-cyclase in *Claviceps* SD58 (ERGE et al., 1973). The addition of tryptophan, thiotryptophan, or other analogues that reverse phosphate inhibition of alkaloid synthesis derepresses these enzymes (KRUPINSKI et al., 1976). The mechanism that links phosphate inhibition and tryptophan induction of alkaloid synthesis is still unknown.

THE TROPHOPHASE - IDIOPHASE RELATIONSHIP IN SECONDARY METABOLISM

An interesting problem of secondary metabolism centers on the mechanism by which the formation of secondary products is usually retarded until the trophophase approaches completion. Secondary metabolites fail to appear during growth because the enzymes responsible for their formation are repressed during the trophophase (DEMAIN, 1974b). Unfortunately, we know very little about the repression-depression mechanisms involved, but they probably include some of the mechanisms mentioned above. For example, carbon catabolite repression of phenoxazinone synthetase probably is important in the actinomycin fermentation.

During our studies on the biosynthesis of gramicidin S (GS) (DEMAIN et al., 1975), we obtained data suggesting that, in certain fermentations, the growth rate regulates the formation of antibiotic synthetases. As with many secondary metabolites, GS is not produced until late in the cell cycle because the GS synthetases are not formed until the latter part of the logarithmic growth phase. After reaching the peak of their

specific activities, the enzymes rapidly disappear as the cells proceed into the stationary growth phase. We wondered whether the onset of synthetase formation was due to an exhaustion of a specific compound or to a decrease in growth rate. We turned to the chemostat to answer this question, since continuous culture allows for an examination of nutritional deficiency independent of growth rate. The basic medium contained glycerol, ammonium, sulfate, and phosphate as single sources of C, N, S, and P. Successful production of GS synthetases I and II was achieved by limiting carbon, nitrogen, sulfur, or phosphorus. Synthetase production was low at high dilution rates (0.45 to 0.50 hr^{-1}) and increased as the dilution rate was lowered. It thus appears that a high growth rate is incompatible with GS synthetase production. Although the mechanism is unknown, it is clear that such a relationship precludes synthetase formation during exponential growth in batch culture and allows synthetase formation as the cells leave the logarithmic growth phase.

As the dilution rate was further decreased in the chemostat, enzyme specific activity reached a peak and then dropped. The peak occurred at different growth rates for each type of limitation, i.e., at 0.38 hr^{-1} for nitrogen, 0.32 hr^{-1} for carbon, 0.22 hr^{-1} for sulfur, and 0.18 hr^{-1} for phosphorus limitation. The highest specific activity was obtained under a phosphorus or sulfur limitation. The reason for the decline in enzyme activity at very low dilution rates is not known but could be due to the activity of an "inactivase" formed at low growth rates, especially under carbon or nitrogen deficiency.

GENETIC IMPROVEMENT OF IDIOLITE PRODUCTION

Many of the metabolic controls described above can be bypassed or exploited by environmental manipulation. Thus, tryptophan can be added to an ergot alkaloid fermentation to induce the secondary process; phosphate is restricted in streptomycin fermentations to avoid feedback inhibition of phosphatases; and rapidly metabolized sugars, such as glucose, are either avoided or fed slowly to penicillin fermentations to prevent catabolite repression. However, since each of the regulatory mechanisms is genetically determined, mutation has had a major effect on the production of secondary metabolites (DEMAIN, 1973). Strain improvement programs, which have utilized various mutagens, are chiefly responsible for the dramatic increases in the production of antibiotics over the years. Penicillin titers, for example, have increased from 5 mg/liter to 20,000 to 30,000 mg/liter in 30 years of development. Most industrial mutation programs are conducted by randomly testing surviving clones in shaken flasks. In addition, colonies with genetically altered morphology or color are often selected for testing in liquid cultures. Unfortunately, we know little or nothing of the mechanisms responsible for superior production by such mutants.

Some success has been experienced in applying concepts derived from studies dealing with the effect of mutation on regulatory control of primary biosynthesis. For example, the re-

moval of a primary biosynthetic enzyme activity by mutation and its suppression by a second mutation often yields feedback-resistant mutants. With regard to secondary metabolism, it has been found that suppression of an *ilv* mutation in *Streptomyces antibioticus*, a *met* mutation in *Streptomyces viridifaciens*, and a *cys* mutation in *Streptomyces lipmanii* improved production of actinomycin (POLSINELLI et al., 1965), chlortetracyline (DULANEY and DULANEY, 1967), and cephamycin C (GODFREY, 1973), respectively. The development of mutants resistant to toxic analogues of precursors has also led to improved idiolite-producing mutants. For example, certain mutants of *Pseudomonas aureofaciens* resistant to tryptophan analogues turned out to be improved pyrrolnitrin producers (ELANDER et al., 1971), and a trifluoroleucine-resistant mutant of *S. lipmanii* produced three times more cephamycin C than its parent (GODFREY, 1973). Since secondary metabolites often inhibit the growth of the producing culture, the antibiotics themselves have been used to select for higher-producing mutants.

Mutation has also been employed to eliminate undesirable or less active products when the microbial broth contains a mixture of such metabolites (DEMAIN, 1973). Another useful genetic application has been the production of new, active secondary metabolites either by simple blockage of the secondary pathway (McCORMICK et al., 1957) or by genetically eliminating the ability to make one moiety of the product and feeding analogues of the missing moiety to the mutant ("mutational biosynthesis") (SHIER et al., 1969; NAGAOKA and DEMAIN, 1975). Such nonproducing mutants ("idiotrophs") have also been extremely useful in elucidating the pathways of secondary metabolism (DEMAIN, 1973).

GENETICS OF SECONDARY METABOLISM

Of great importance for the future development of secondary metabolite fermentations will be the mapping of production genes. Only recently have studies begun on the genetic maps of producing organisms such as actinomycetes. The model for such investigations is the genetic map of *Streptomyces coelicolor*, as developed by the techniques of HOPWOOD et al. (1973) and SERMONTI (1969). The map of *S. coelicolor* is very similar to those of other *Streptomyces* species, such as *S. bikiniensis* (COATS and ROESER, 1971), *S. olivaceus* (MASTELYUKH et al., 1973), and *S. glauescens* (BAUMANN et al., 1974). More important, genetic maps of commercial species, such as *S. rimosus* (oxytetracycline) (ALACEVIC et al., 1973) and *Nocardia mediterranei* (rifamycins) (SCHUPP et al., 1975), appear to be similar to that of *S. coelicolor*. Of recent interest is the finding that actinomycetes contain plasmids. In *S. coelicolor*, the plasmid is a sex factor (SCP1) that can be transferred to *Streptomyces lividans* along with chromosomal genes (HOPWOOD, 1974). Such plasmid transfer techniques should prove useful in amplifying production genes in industrial strains. KIRBY et al. (1975) have found at least four of the *S. coelicolor* genes coding for enzymes of antibiotic (methylenomycin A) formation to reside on the plasmid.

ACKNOWLEDGMENT

The preparation of this manuscript was supported by grants from the National Science Foundation and the Universal Foods Corporation.

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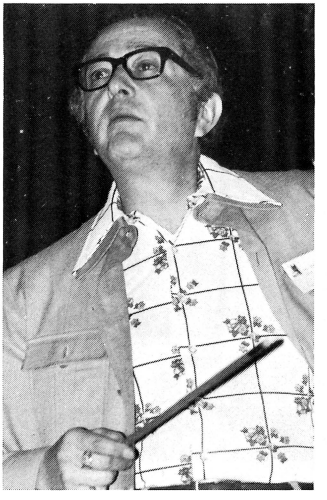
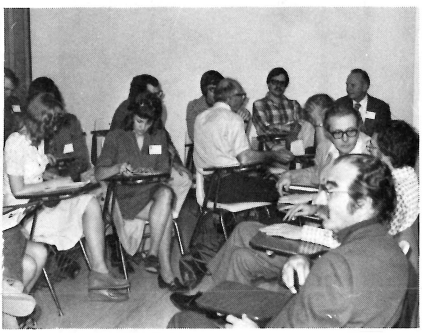


Demain

Arnold Demain at the Symposium

Joseph Cassiday with Demain



Discussion Groups