Indian J. mycol. Res. 17 (1), 1—12 (1979)

PRESIDENTIAL ADDRESS

MICROBIAL METABOLITES

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Microorganisms secrete various substances during their growth phase which are products of the biosynthetic mechanism involved in metabolism essential for growth and nutrition. With many microorganisms such essential substances are often produced in excess and accumulate in the substratum. Microbiological studies with fungi and bacteria revealed the presence of pigments, acids and enzymes in synthetic media upon which they were induced to grow by some early workers of the nineteenth century. The discovery of lactic acid fermentation by Pasteur in 1957 could be cited as one of the earliest evidences to prove that lactic acid fermentation is caused by the microorganism *Lactobacillus*. However, the alcoholic fermentation of ethyl alcohol in which alcohol is produced from sugars by yeast (*Saccharomyces cerevisiae*) has been know from long since (Frey, 1930). Among the fungi, species belonging to Phycomycetes and Basidiomycetes are known to be producers of citric acid, itaconic acid, gluconic acid, vitamins, amino acids, pigments, enzymes, alkaloids, antibiotics and many other substances (Peppler, 1967; Thoma, 1977). Species of *Penicillium* and *Aspergillus* are potential sources of many secretary products including steroids (Peterson *et al*, 1952),gallic acid (Van Tigham, 1867, 1887), fat (Lockwood *et al*., 1934), gentisyl alcohol (Birkinshaw *et al*., 1942), citric acid (Currie, 1917 ; Shu and Johnson, 1948 ; Chaudhuri, 1974 ; Abraham and Chaudhuri, 1977 ; Das, 1973) etc.

Although a large number of bacteria have been exploited as sources of various organic products, the potentialities of fungal organisms which abound in plenty on the surface of the earth and its atmosphere can hardly be ruled out as only a few of them have been biochemically studied and fewer still exploited commercially in the fermentation industries (Foster, 1949 ; Turner, 1971). Some important metabolites secreted by fungi are listed in Table 1.

Earlier methods adopted in the fermentation of useful biochemical products of known microorganisms were base on simple techniques without reference to the genetic control of such substances involving metabolic activities within the organism. Precise studies on the steps of biosynthesis of accumulatory products using genetic and biochemical techniques have in due course replaced the earlier methods in consequence of the discovery of gene enzyme relationship in the biochemical control of essential metabolites by Beadle and Tatum (1941, 1945). It was shown that mutation of a particular gene causes the lack of capacity for production of a particular enzyme which is responsible for the elaboration of a product. In other words mutation blocks the reaction that brings about the formation of an essential substance necessary for the metabolic existence of a cell and such a mutation may cause lethality to the cell or the organism unless the substance is supplemented as a nutritional requirement. Nutritional mutants have provided clues much valuable in the understanding of metabolic patterns in microorganisms.

Metabolites may be classified according to their involvement in the synthetic processes. The interrelated series of enzyme catalysed chemical reactions result in the accumulation of the primary metabolites which are utilized by the organism as intermediates in the synthetic process. The key macromolecules like protein, RNA and DNA are also formed in the system in this way and the chemical reactions associated with the synthetic process provide the microorganism with its required energy. By and large secondary metabolism is concerned with the syntheses that lead to the end products which generally do not have any specific role in the economy of the organism. Thus primary metabolism is essential to the sustenance of any living organism while secondary metabolism is restricted to the lowly organised living beings particularly the microorganisms.

Table 1. *Fungi and their metabolites*

Class	*Name of the Organism*	*Metabolites Produced*
Phycomycetes	*Phycomyces blakeslaeanus*	ergosta 5,7,24(28)-trienzol.
	Mucor mucedo	Trisporic acid B
		Trisporic acid C
	Achlya bisexualis	antheridiol
	Blakeslea trispora	Trisporone
Ascomycetes	*Gibberella fugikuroi*	13-epi-(-) monoyl oxide Gibberellins
	Penicillium funiculosum	Cholesterol
	P, charlesic	Itaconic acid
	P. canadense	Canadensic acid
	P. glaucum	Gluconic acid
	P. rubrum	Rubnaloxin A and B
	P. citro-viride	Depicolinic acid
	P. nigricans	L-pheny calanie anlaydride
	P. patulum	Patulin, m-hydroxybenzyl alcohol
	Aspergillus terreus	Itaconic acid
	A. ochraceus	Ochlatoxin A, B, C
	A. flavus	Aflatoxin B, B2, M and M2
	A. itaconicus	Itaconic acid, Itaconitin
	A. niger	14-dehydroergosterol, Glutaconic acid, Citric acid
	A. fumigatus	Fumigatin, 3 hydroxytoluquinone, Orcinol
	Claviceps purpurea	5,6-dihydroergosterol
Basidiomycetes	*Polyporus officinalis*	Agaric acid
	P. pinicola	5,6 - dihydro ergosterol
	Agaricus campestris	Indigo
	Coprinus	4 - methoxytoluquinone
	Lentinus dege	4 - methoxy toluquinone, 6-hydroxy-4 methoxytoluquinone
	Amanita muscaria	Muscarine, Muscarufin.

Biochemical studies in relation to nature and function of enzymes revealed information on single reactions as well as series of reactions occurring in cells and tissues. Such reactions could be found in the pattern of citric acid and sugar metabolism (Wagner and Mitchell, 1965). By using inhibitors to stop specific reactions the sequence of biosynthesis could have been possible to be followed.

The media for growing fungi contain a carbon source usually glucose, a nitrogen source, usually ammonia or nitrate but often an amino acid, phosphate sulphate, magnesium, potassium and the trace elements such as iron, manganese, zinc, molybdenum and copper. In addition to and sometimes in place of these chemically defined constituents complex natural materials are often added to the medium. These include corn steep liquor, yeast extract, vegetable juices and protein hydrolysates. Often several different media will support the growth of an organism but not all lead to the production of a desired compound which depend not only on the composition of the medium, but also on methods of culturing in relation to pH of medium, temperature, light and certain other conditions which demand continuous stirring of the fermentation flask.

The difference in secondary metabolism observed on media containing different nitrogen sources may result from an indirect effect. For example the change in pH of the medium during the course of a fermentation is partly dependent upon the nitrogen source, and pH is known in some cases to have a marked effect on secondary metabolism. It is not only rhe qualitative composition of the medium which is important, but also the ratio of the constituents determines the order in which each nutrient becomes exhausted and this in turn influences the course of fermentation.

The significance of the phenomena of formation and accumulation of various chemical substances within or outside the microorganism, into its environment could be envisaged with the growing knowledge of biologlcal and biochemical characteristics of various systems of fungi and bacteria which were found to be capable of chemical transformation of substances to products necessarily in demand by the human society. Researches in the physiology and biochemistry of microorganisms therefore opened up possibilities of introducing operations which gradually developed into the field of industrial microbiology. Biochemical

activities in the production of metabolites by microorganisms and the impact of biochemical engineering involved in the industrial production of various substances have been given due attention and discussed in details by several authors (Foster, 1949 ; Prescott and Dunn, 1959; Wagner and Mitchell, 1965 ; Peppler, 1967 ; Turner, 1971 ; Ghosh and Fiechter, 1971 ; Demain, 1972).

Borrow *et. al.* (1961) and Bu'lock (1967) have shown that growth and metabolism of a fungus in submerged conditions could be divided into distinct phases. The organism grows in an exponential manner with uptake of essential nutrients in the initial phase which is termed balanced phase or 'tropophase' in which nitrogen or phosphorus is usually exhausted in the medium as a result replication ceases. At this point metabolic changes take place and secondary metabolites are produced when the organism enters into its 'idiophase' stage that lasts till the carbon source is exhausted and autolysis sets in. In the nitrogen limited fermentations the 'idiophase' has been further divided into 'storage phase' and 'maintenance phase' by Borrow *et al.* (1961). In the storage phase cell weight increases due to accumulation of fat and carbohydrate when there is production of secondary metabolites. In the maintenance phase dry weight remains constant but simultaneously uptake of glucose and production of secondary metabolite continues. In those cases where nutrients other than nitrogen are depleted a 'transition phase, between the balanced phase and the storage phase has been recognised. In this phase cell proliferation continues at a slow rate.

According to Bu' Lock idiophase is marked by several phases : (i) exhaustion of an essntial nutrient leading to termination of cell replication; (2) accumulation of primary intermediates and (3) inductien of the enzymes necessary for secondary biosynthesis or activation of enzymes formed during the tropophase. Secondary metabolites may also induce the synthesis of enzymes. An interesting case has been cited by Bu'Lock in which he could distinguish between induction and activation of enzymes mediating secondary biosynthesis. 6- methylsalicylic

acid produced by *Penicillium urticae* is converted to genetical derivatives and later patulin. It was shown that 6 MS synthetase is a metabolically stable enzyme produced during tropophase and activated during the idiophase. However, the conversion of 6 MS gentisyl derivatives to patulin was shown to be mediated by labile enzymes which are formed during the idiophase.

Amino acids, nucleotides and vitamins are primary metabolites. These constitute the small molecules which in course of metabolism are converted into co-enzymes. The industrial use of microorganisms for fermentation of primary metabolites has been possible due to certain devices which bypass feedback regulation so as to decrease the concentration of the inhibitory end product. Accumulation of inhibitory product can be checked by inducing a mutant which lacks an enzyme at an intermediate step of the pathway and produces the intermediate compounds in sufficient quantity. It is necessary in such cases to feed the organism with the end product at a low level in order to avoid accumulation of the repressive compound. This principle has been adopted in the manufacture of citrullin in which an auxotroph has been made use of (Okumura *et al.*, 1964) and also in the production of ornithine by using an argininеless *Corynebacterium glutamicum* (Kinoshita *et al.*, 1963). End products of organisms having branched pathways can also be regulated by a suitable auxotroph which is unable to produce one of the products and requires limited amount of that product to keep the process of synthesis going practically without any feedback effects so that the precursor of that mutant is accumulated in profuse quantity. Inosine 5'-monophosphate which is an important flavour potentiator is produced in large quantities (13 g/1) by the feedback inhibition technique with suitable mutants (Demain *et al.*, 1966b ; Furuya *et. al.*, 1968).

Enzymes associated with biosynthetic cycles are generally controlled by feedback regulation. There may be two types of regulation, (1) feedback inhibition and (2) feedback repression. When the ultimate product of a pathway inhibits the action of any of the earlier enzymes of the same pathway the regulation is said to caused by Feed back inhibition. On the other hand when inhibition of formation of one or more enzymes in a pathway is caused by a derivative of the

end product the regulation is said to be caused by feedback repression. Regulatory inhibition which commonly occurs in microorganisms is involved in the mechanism of synthesis of molecules to be incorporated into macromolecules such as amino acids, purines and pyrimidines (Sasaki 1965; Newell and Tucker. 1966 ; Papiska and Lichstein, 1968).

It has been shown by several workers that synthesis of vitamin is controlled by feedback repression as well as by catabolic repression obviously to check overproduction. From a comparative study of the number of molecules per cell required for the regulation of vitamin synthesis and amino acid synthesis it has been found that about 1000 vitamin molecules are required for the former and 50 million molecules for the latter. This is an instance that shows how nature checks overproduction of vitamins by minimising the number of enzymes for vitamin synthesis.

Generally the biosynthetic pathways are branched so that more than one end products are formed. Feedback regulation in such cases could hamper the production of one or the other product of metabolism and cause starvation of the cell. However, there are preventive measures to by pass such a situation. Mechanism by which the cell avoids the danger of starvation may be by the interference of isoenzymes, concerted feedback regulation and cumulative feedback regulation. Control of synthesis by isoenzymes is found in the *E. coli* system in which aspartokinases are regulated by lysin, threonine and methionine in the metabolism of aspartic acid and allied metabolities (Standtman, 1968).

In multivalent or concerted feedback regulation only one enzyme is involved but more than one end product should necessarily be present in excess to inhibit or repress. The branched chain amino acid pathway of *Salmonella typhimurium* is controlled by this type of regulation (Freundlich, *et al.*, 1963).

Compounds which are of medical, industrial and nutritional importance are commonly found in microorganisms. Some microorganisms have been exploited in the production of such compounds. The strain selected for fermentation of products must be capable of producing the desired substance in excess. It has been found that such excess production of metabolites is due to some abnormality or aberration in the regulatory mechanism of the organism. The aim of the

microbiologist is to select such improved strains which might be better utilized in industry for higher production. Application of genetic and fermentation techniques has made it possible to increase the rate of production of metabolites many folds. It is important to find the right precursor which fits into the biosynthetic pathway and proves effective to increase the desired product. Some precursors may have both stimulating and inhibitory effects. Directed biosynthesis has been achieved in the case of actinomycins and Tyrocidines in the biosynthesis of amino acids.

Overproduction of metabolites by microorganisms is not the general rule as it is uneconomic to the system. In the process of evolution therefore there has been a tendency to prevent overproduction of substances formed within the tissues, However, the aim of the biologist is to utilize the efficiency of the system with regard to production by introducing favourable fermentation processes with the highest possible conversion of nutrient into the desired product. Regulatory mechanism in microorganisms is a safeguard against non-functioning of the cell. Organisms which could be applied to the fermentation technique are rigidly controlled by their regulatory mechanism. Demain (1966) has given an excellent account of the principles underlying the genetic and environmental implication in fermentation processes. It is the property of a living cell to hydrolyze the carbon source in a medium containing starch, ammonia and minerals to convert it to glucose. degrade it to 3-carbon compounds by Emden- Meyerhof pathway or the hexose monophosphate pathway, energy being provided by the tricarboxylic acid cycle. Quite a number of enzymatic steps have been found to be associated with the process to convert the intermediate products to amino acids, ribonucleotides, deoxyribonucleotides, vitamins. fatty acids etc. It is estimated that each biosynthetic process requires about 10 enzymes. The building blocks are then polymerized into a large number of proteins, ribonucleic acid, deoxyribonucleic acids, mucopeptides polysaccharides and lipids involving many steps so that the whole process is regulated in a precise manner.

A typical bacterial cell is endowed with the capacity of producing only the necessary enzymes at the requisite amount at any time of requirement. The activities of the enzyme are regulated by activation and inhibition. It is to be noted

that the genetic constitution or the genotype of a microorganism remaining unaltered itself can change its composition and metabolic activity in accordance with the changes in the environment and thus the phenotypic expression can also change. The regulatory mechanism which controls the production of a metabolite as the end product of biosynthesis and also the intermediates is involved in the process of induction, catabolite regulation and feedback regulation.

Constitutive enzymes are produced under all growth conditions such as the enzymes of the hexose monophosphate pathway. The inducible enzymes are formed only in the presence of their substrates or analogs in the medium. In presence of compounds like polysaccharide, oligosaccharide, or amino acid inducible enzymes are formed by the process of induction. In many cases addition of inducer to the medium may be necessary but the cellular system itself can have internal formation of inducer to activate metabolism.

Any organism can have more than one usable substances as growth substrate. But it should be uneconomical to induce formation of so many enzymes so as to catabolize all the substrates. When the primary substrate is glucose it is readily utilized with the help of the suitable enzyme. Other carbon sources are catabolized gradually by the corresponding enzymes. Catabolic repession can be cited by the example of repression of β-galactosidase in *Escherichia coli*. In certain cases acetate and citrate sources may act as repressor repressing glucose metabolism (Romano and Kornberg, 1968; Clarke and Lilly, 1969).

Inhibition of cyclic 3',5- adenosine monophosphate formation has been ascribed as the cause of catabolic repression by Perlman and Pastan (1969). With some enzymes in *E. coli* catabolic repression in reversed by cyclic 3', 5'-adenosine monophosphate.

Secondary metabolites are produced as related chemicals and, as mentioned earlier, have no special function to perform in the life process of an organism. Antibiotics are produced as secondary metabolites and so also mitomycins and aflatoxins. Secondary metabolites are not produced during the idiophase

(Bu'Lock, 1967) as was originally observed in penicillin fermentation. Five mitomycins,eight aflatoxins, ten polymixins have been found as products formed as secondary metabolites. Biosynthetic studies with penicillins and actinomycins have revealed that a large number of these antibiotics are produced as mixtures of secondary metabolites (Weinberg, 1970).

Feedback inhibition in fermentation may be overcome by inducing mutants, having enzymes resistant to such inhibition or those having enzyme forming system resistant to feedback repression. This could be achieved by using toxic analogs or antimetabolites of the fermentation product. Antimetabolite resistant mutants can be scored by plating the microorganism in sufficient numbers and pick up the surviving cells or colonies which when used will give a high production of the metabolite. Various antimetabolites have been used in producing mutants with altered controls and those havs been successfully used for enhanced production of amino acids. Thus antimetabolites such as canavanine, p-fluorophenylalanine, D-tyrosine, valine, norleucine, 2-thiazolealanine, 1,2,3-triazole-3-alanine, 3-4-dehydroproline, 2,6-diamino purine, 5-fluorouracil have been used for the production of arginine, phenylalanine, tyrosine, isoleucine,methionine, histidine, proline, adenine, uracil (Maas, 1961 ; Volkova *et. al.*, 1965 ; Cohen and Adelberg, 1958 ; Ramakrishnan and Adelberg, 1964 ; Rowbury, 1965 ; Moyed, 1961a; Sheppared; 1964; Jensen,1969; Baich and Pierson, 1965; Kalle and Gots, 1962 ; Kaplan *et. al*,1969) respectively.

The future of fermentation industry will obviously need a fresh outlook into the biochemical and genetic implications of the regulatory mechanisms of the production process. Importance of inducers, repressors and inhibitors will have to be taken into account in the designing of fermentation equipments. The spectacular advances in the study of genetics of microorganisms and their development in relation to environment at nutritional and biochemical level, will surely lead to more and more comprehensive and easily applicable technology ensuring high production of microbial metabolites so essential to human needs particularly in the developing countries. India in spite of her progress in the industrial field during

the last 24 years could do very little yet in the field of fermentation. It is perhaps not too late to put together the talents of the mycologists, microbiologists, biochemists and fermentation technologists to exploit the possibilities of microorganisms in the production of essential chemicals that are needed in medicine, food industries and multiple other small and large scale industries.

REFERENCES

Abraham, T. K. and Chaudhuri, K. L. (1977). *Ind. J. Microbiol.*, **17**; 93.

Baich, A. and Pierson, D. J. (1965). *Biochim. Biophys. Acta*, **140**; 379.

Beadle, G. W. and Tatum, E. L. (1941). *Proc. Nat. Acad. Sci* (U.S. A.) **27**; 499.

Beadle, G. W. and Tatum, E. L. (1954). *Physiol. Rev.* **25**; 643.

Birkenshaw, J. H., Raistrick, H. and Smith, G. (1942). *Biochem. J.* **36**; 829.

Borrow, A., Jefferys, E. G., Kessell, R. H. J., Lloyd, E. C., Lloyd, P. B., and Nixon, I. S. (1961). *Can. J. Bot.*, **7**, 227.

Bu'Lock, J. D. (1967), *Essays in Biosynthesis and Microbial Development*, John Willey and Sons, Inc, London,

Clarke, P, H, and Lilly, M, D, (1969), *Symp, Soc, Gen, Microbiol* **19**; 113,

Cohen, G, N, and Adelberg, E, A, (1958), *J, Bacteriol*, **76**; 328

Demain, A, L, (1966a), *Advan, Appl, Microbiol,*, **8**; 1

Demain, A. L., Abraham E. P. and Newton, G. G. F. (1966b). In "Antibiotics, Volume II. Biosyntheses" (eds by D. Gotllieb and P. D. Shaw). Springer-Verlag, New york.

Demain, A, L, (1972), *J, Appl, Chem, Biotechnol*, **22**; 345

Foster, J, W, (1949), *Chemical activities of fungi*, Academic Press Inc, New york N, Y,

Frey, C. N,, (1930), *Ind, Eng, Chem,*, **22**; 1154

Jensen, R, A, (1969), *J, Biol. Chem,*, **244**; 2816

Kalle, G, P, and Gots, J, S, (1962). *Proc, Soc, Exp, Biol, Med,*, **109**; 277

Mass, W, K, (1961), *Cold Spring Harb, symp, Quant Biol,*, **26**; 183

Moyed, H, A, (1961), *Cold Spring Harb Symp, Quant Biol,*, **26**; 333

Newell, P, C, and Tucker, R, G, (1966), *Biochem, J*, **98**; 39

Papiska, H, R, and Lichstein, H, C, (1968), *J, Bacteriol*, **95**; 1173

Peppler, H, J, (1967), *Microbial Technology*, Reinhold Publishing Co.

Perlman, R, and Pastan, J, (1969), *Biochem, Biophys Res, Commun*, **37**; 151

Prescott, S, C. and Dunn, C, G, (1959), *Industrial Microbiology*, Mc Graw, Hill Book Co, Inc. New York, Kogakusha Co Ltd, Tokyo, 3rd Ed,

Ramakrishnan, T, and Adelberg, E, A, (1964). *J. Bacteriol* **87**; 566

Romano, A. H. and Kornberg, H. L. (1968). *Biochim, Biophys Acta* **158**; 491

Rowbury, R. J. (1965). *Nature* **206**; 962

Sasaki, T. (1965). *J. Gen Appl, Microbiol* Tokyo, **11**; 203

Sheppard, D, E, (1964), *Genetics* **50**; 611

Stadtmann, E, R, (1966), *Advn, Enzymol* **28**; 41

Turner, W, B, (1971). *Fungal Metabolites*, Academic Press, London, New York

Wagner, R, P, and Mitchel, H, K, (1965), *Genetics and Metabolism*, John Willey and Sons Inc, New York

Weinberg, E, D, (1970), *Advan, Microbiol, Physiol* **4**: 1

Van Tigham, P, (1867), *Ann, Sci Nat, Botan*, S, **5**: 240

Van Tigham, P, (1887), *Comut rend* **65**: 1091

Chaudhuri, K, L, (1974), Characterization of useful mutation in fungi induced by radiation and radioisotopes and the study of their biosynthetic products: I, A, E, A, Research contracts, Fourteenth Annual Report, International Atomic Energy Agency, Vienna, pp, 133-135

Chaudhuri, K, L, (1976), *Studies on a few species of Ascomycetous fungi* D, Sc, Thesis, Calcutta University,

Currie, J, N, (1971), *J, Biol, Chem*, **31**: 15

Das, A, (1973), *Micopathologia et Mycologia applicata*, **49**: 205

Shu, P, and Johnson, M, J, (1948), *Ing, Eng, Chem*, **40**: 1208

Thoma, R, W. (1977), *Industrial Microbiology*, Dowden, Hutchinson and Ross Inc, Pennsylvania