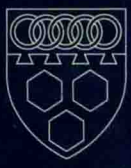
Ciba Foundation Symposium 171



SECONDARY METABOLITES: THEIR FUNCTION AND EVOLUTION

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Contents

Symposium on Secondary Metabolites: their Function and Evolution, held at the Ciba Foundation, London, 18–20 February 1992

The topic of the symposium was proposed by Professor Julian Davies and Dr Dudley Williams

Editors: Derek J. Chadwick (Organizer) and Julie Whelan

Introduction

Julian Davies

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The number and the diversity of secondary metabolites are subjects that have intrigued scientists for many years. I mean diversity in many senses: there is the diversity of chemical molecules, and the diversity of sources of these metabolites. One can argue that every living organism on earth either makes secondary metabolites, or, at the very least, participates in some form of secondary metabolism. It is important also to recognize the diversity of the potential functions of these molecules.

I would suggest that at least ten biological functions of secondary metabolites can be proposed (Table 1). I am not implying that these are the only functions, or that known secondary metabolites necessarily have these particular functions. The point of this list is to illustrate the wide diversity of functions that have been proposed for secondary metabolites, and the fact that we will, I hope, probably have additional functions suggested at this meeting. Some secondary metabolites are likely to have more than one biological role.

An interesting point about secondary metabolism is that whereas primary metabolism (intermediate metabolism) is *linear*, in the sense that its products stay with the organism and it is responsible for guaranteeing that an organism has sufficient nutrients and all the means it needs to produce the next generation,

TABLE 1 Some suggested biological functions for secondary metabolites

1. Competitive weapons against other bacteria, fungi, plants, amoebae, insects, etc. (Self-protection/exclusion)
2. Metal-transporting agents
3. Involved in plant–microbe symbiosis
4. Nematode–microbe symbiosis
5. Insect–microbe symbiosis
6. Sexual hormones (pheromones)
7. Differentiation effectors, between and within cells
8. Excretion of unwanted products
9. Products of 'selfish' DNA
10. Reserve pool of new pathways

secondary metabolism is a kind of 'lateral thinking' of microorganisms, or of any multicellular organism that produces a secondary metabolite. It is responsible for interactions between the organism and its environment. A good example is a class of compounds that one doesn't often think of as secondary metabolites, but which are in fact representative of secondary metabolism. This concerns pathogenic organisms. It is interesting to note that in microbial pathogens such as *Listeria*, the enzymes and toxins required for pathogenicity (that is, the interactions of the organisms with their mammalian host cells) are produced in a phase when the organism is not growing. It is a late phase of of development of the particular organism. So one can think of many substances associated with pathogenicity as being representative of secondary metabolism. I don't want to extend this analogy too far, but want to emphasize the point that the concept of secondary metabolism and the production of secondary metabolites seems to be concerned with what is going on outside the producing organism, rather than events going on inside. I think this is an important distinction.

There is much controversy about secondary metabolites in Nature, and in particular the question of what a secondary metabolite may actually do for the organism producing it. Many views have been expressed on this subject. Some people believe that we don't really know what secondary metabolites do, or at least that we can't establish what secondary metabolites may do for the organism concerned. Others take a different view. Dudley Williams, the co-proposer of this symposium, believes that secondary metabolites do play an important role in the life of the producing organism. This is something which clearly is open to discussion. I would like to have your suggestions on the functions of secondary metabolites in the organisms that produce them. I would like also to encourage you to discuss the whole gamut of possibilities with respect to the origins of secondary metabolites, and the production of secondary metabolites, in addition to their functions. We will never be able to comprehend the enormous diversity of these products, and the general considerations of what this kind of diversity means. This is what is interesting about this topic; it's why we are here, and why I am looking forward to a very interesting three days talking about this subject. Secondary metabolism has been ignored; there is nothing 'secondary' about its importance in biology!

Microbial secondary metabolism: a new theoretical frontier for academia, a new opportunity for industry*

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Abstract. Microbial secondary metabolites are the low molecular mass products of secondary metabolism. They include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, receptor antagonists and agonists, pesticides, antitumour agents and growth promoters of animals and plants. They have a major effect on the health, nutrition and economics of our society. They have unusual structures and their formation is regulated by nutrients, growth rate, feedback control, enzyme inactivation and induction. Regulation is influenced by unique low molecular mass compounds, transfer RNA, σ factors and gene products formed during post-exponential development. The synthases of secondary metabolism are often coded by clustered genes on chromosomal DNA and infrequently on plasmid DNA. The pathways of secondary metabolism are still not understood to a great degree and thus provide a new frontier for basic investigations of enzymology, control and differentiation. Cloning and expression of genes in industrial microorganisms offer new opportunities for strain improvement and discovery. Microbial metabolites have already established themselves as coccidiostats, immunosuppressants, antihelminthic agents, herbicides and cholesterol-reducing drugs. Great potential exists for the discovery of antiviral, antiparasitic, antitumour and pharmacological compounds and new agricultural products. The future for natural products is bright indeed.

1992 Secondary metabolites: their function and evolution. Wiley, Chichester (Ciba Foundation Symposium 171) p 3–23

Secondary metabolites, also known as idiolites, are special compounds, often possessing chemical structures quite different from the primary metabolites (such as sugars, amino acids and organic acids) from which they are produced.

*Because of space limitations I have had to eliminate all citations prior to 1987, as well as citations to the work of my group. I apologize to all whose citations have been omitted and to my own students and associates: specific citations will be supplied upon request.

Idiolites from microorganisms are not essential for the growth of the producing culture but serve diverse survival functions in Nature. These special metabolites, in contrast to the general nature of primary metabolites, are produced only by some species of a genus, and by some strains of a species. Their unusual chemical structures include β-lactam rings, cyclic peptides containing 'unnatural' and non-protein amino acids, unusual sugars and nucleosides, unsaturated bonds of polyacetylenes and polyenes, and large macrolide rings. Idiolites are typically produced as slightly differing components of a particular chemical family, as a result of the low specificity of some enzymes of secondary metabolism. The main types of biosynthetic pathways involved are those forming peptides, polyketides, isoprenes, oligosaccharides, aromatic compounds and β-lactam rings. Knowledge of the pathways varies from cases in which the amino acid sequences of the enzymes and nucleotide sequences of the genes are known (for example, for penicillins and cephalosporins), to those in which even the enzymic steps are still unknown. Although most secondary metabolites are small (less than 1500 Da) and are produced by non-ribosomal systems, there does exist a family of ribosomally derived antibiotics of higher molecular weight (3000–4000 Da, 32–34 residues) known as lantobiotics (Bannerjee & Hansen 1988, Schnell et al 1988). These include nisin (produced by *Streptococcus lactis*), subtilin (*Bacillus subtilis*) and epidermin (*Staphylococcus epidermidis*).

Regulation of secondary metabolism

The intensity of secondary metabolism can often be increased by the addition of limiting precursors. Examples are shown in Table 1. Secondary metabolism occurs best at submaximal growth rates after growth has slowed down. The distinction between the growth phase (trophophase) and production phase (idiophase) is sometimes very clear, but in many cases idiophase overlaps trophophase. The timing between the two phases can be manipulated—the two phases are often distinctly separated in a complex medium favouring rapid growth, but overlap partially or even completely in a chemically defined

TABLE 1 Increase in intensity of secondary metabolism resulting from the addition of limiting precursors

Group	*Species*	*Secondary metabolite*	*Precursor*
Unicellular bacteria	*Bacillus polymyxa*	Colistin	Diamino-butyric acid
	Bacillus brevis	Gramicidin S	L- or D-Phenylalanine
Filamentous bacteria	*Streptomyces clavuligerus*	Cephamycin C	Lysine
Fungi	*Penicillium chrysogenum*	Penicillin G	Phenylacetic acid

medium supporting slower growth. A secondary metabolite is not 'secondary' because it is produced after growth, but because it is not involved in the growth of the producing culture. Thus, elimination of the production of a secondary metabolite by mutation will not stop or slow down growth; indeed, it may increase the growth rate.

The factors controlling the onset of secondary metabolism are complex and not well understood. Growth rate is important, but we do not know the mechanism(s) involved. Deficiencies in certain nutritional factors are also important, but again we are ignorant of the basic mechanisms.

The delay often seen before the onset of secondary metabolism was probably established by evolutionary pressures. Many secondary metabolites have antibiotic activity and could kill the producing culture if produced too early. Of course, the resistance of antibiotic producers to their own metabolites is well known (Cundliffe 1989 and this volume: 1992). Antibiotic-producing species possess suicide-avoiding mechanisms which are often inducible, but in some cases are constitutive. In the case of inducible resistance, death could result if the antibiotic is produced too early and induction is slow. Delay in secondary metabolite production until the starvation phase makes sense if the product is being used as a competitive weapon or endogenously as an effector of differentiation. In nutritionally rich habitats such as the intestines of mammals, where enteric bacteria thrive, secondary metabolite production is not as important as in soil and water, where nutrients limit microbial growth. Thus, secondary metabolites tend not to be produced by enteric bacteria such as *Escherichia coli* but by soil and water inhabitants such as bacilli, actinomycetes and fungi. Nutrient deficiency in Nature often induces morphological and chemical differentiation—that is, sporulation and secondary metabolism, respectively; both are beneficial for survival in the wild. Thus the regulation of the two types of differentiation is often related.

Most secondary metabolites are formed via enzymic pathways. The enzymes occur as individual proteins, free or complexed, or as parts of large multi-functional polypeptides carrying out a multitude of enzymic steps, as in polyketide synthases and peptide synthetases. The genes encoding the enzymes of secondary metabolism are usually chromosomal, but a few have been shown to be plasmid-borne, such as methylenomycin A of *Streptomyces coelicolor*. Whether chromosomal or plasmid-borne, the genes are usually clustered, especially in prokaryotes, but not necessarily as single operons. Expression of these genes is under strong control by nutrients, inducers, products, metals and growth rate. In most cases, regulation is at the level of transcription, as revealed by the absence of mRNA encoding idiolite synthases until growth rate has decreased.

Regulation by the carbon source

Glucose, usually an excellent carbon source for growth, interferes with the formation of many secondary metabolites. Polysaccharides (e.g. starch),

TABLE 2 Carbon sources interfering with secondary metabolism

Idiolite	*Interfering carbon source*	*Non-interfering carbon source*
Actinomycin	Glucose, glycerol	Galactose, fructose
Bacilysin	Glucosamine, starch, maltose, glycerol, ribose, xylose	Glucose
Benzodiazepine alkaloids	Glucose	Sorbitol, mannitol
Cephalosporin	Glucose, glycerol, maltose	Sucrose, galactose
Chlortetracycline	Glucose	Sucrose
Cycloserine	Glycerol	
Enniatin	Glucose	Lactose
Ergot alkaloids	Glucose	Polyols, organic acids
Erythromycin	Glucose, sucrose, glycerol, mannose, 2-deoxyglucose	Lactose, sorbose
Kanamycin	Glucose	
Oleandomycin	Glucose	Sucrose
Penicillin	Glucose, fructose, galactose, sucrose	Lactose
Peptide K-582	Glycerol	Glucose, sucrose, fructose, sorbitol
Puromycin	Glucose	
Rebecamycin	Sugars	Trisaccharides, polysaccharides
Tetracycline	Glucose	
Tylosin	Glucose, 2-deoxyglucose	Fatty acids

oligosaccharides (e.g. lactose) and oils (e.g. soybean oil, methyloleate) are often preferable for fermentations where secondary metabolism is desired. Examples of interfering carbon sources are given in Table 2. It should be noted that in certain cases (e.g. bacilysin) glucose is not an interfering carbon source, but other carbon compounds are.

In many secondary metabolite pathways, the enzymes subject to control by the carbon source are known. One is phenoxazinone synthase, an enzyme of the actinomycin pathway in *Streptomyces antibioticus*. Repression by glucose is exerted at the level of transcription; specific mRNA is low in trophophase, high in idiophase, and much lower in a glucose than in a galactose medium.

Regulation by the nitrogen source

Many secondary metabolic pathways are negatively affected by nitrogen sources favourable for growth—for example, ammonium salts. As a result, complex fermentation media often include a protein source (such as soybean

TABLE 3 Nitrogen sources interfering with secondary metabolism

Idiolite	*Interfering nitrogen source*	*Non-interfering nitrogen source*
Actinomycin	L-Glutamate, L-alanine, L-phenylalanine, D-valine	L-Isoleucine
Aflatoxin	Nitrate	NH_4^+
Alternariol	Nitrate, L-glutamate, urea	
Bikaverin	Glycine	
Candicidin	L-Tryptophan, L-tyrosine, L-phenylalanine, *p*-amino-benzoate	
Cephalosporin	NH_4^+, L-lysine	L-Asparagine, L-arginine
Chloramphenicol	NH_4^+	D-Serine, L-proline, DL-phenylalanine, DL-leucine, L-isoleucine
Erythromycin	NH_4^+	
Leucomycin	NH_4^+	Uric acid
Macbecin	L-Tryptophan, *p*-amino-benzoate, anthranilate	
Penicillin	NH_4^+, L-lysine	L-Glutamate
Rifamycin	NH_4^+, L-tryptophan, *p*-amino-benzoate	Nitrate, L-phenylalanine
Streptomycin	NH_4^+	Proline
Streptothricin	NH_4^+	DL-Aspartate, L-glutamate, DL-alanine, glycine
Tetracycline	NH_4^+	
Trihydroxytoluene	NH_4^+	
Tylosin	NH_4^+	Valine, L-isoleucine, L-leucine, L-threonine

meal) and defined media a slowly assimilated amino acid (such as proline) as the nitrogen source to encourage high production of secondary metabolites. Processes subject to regulation by the nitrogen source are shown in Table 3. Little information is available on the mechanisms underlying the negative effects of NH_4^+ and certain amino acids. In the production of tylosin, the sensitive enzyme appears to be valine dehydrogenase, which is repressed and inhibited by NH_4^+. Because valine is the best source of the acetate, propionate and butyrate precursors supplying the carbon atoms of the macrolide ring system, protylonolide, interference in valine degradation suppresses tylosin synthesis. In *Cephalosporium acremonium* (syn. *Acremonium chrysogenum*; *A. stricta*), at least two enzymes of the cephalosporin biosynthetic pathway, ACV synthetase and expandase, are repressed.