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Resting cell studies on formation of water-soluble red pigments by *Monascus* sp.

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SUMMARY

A resting cell system was developed for the biosynthesis of soluble red pigments by *Monascus*. The medium contains glucose, glycine, $ZnSO_4$ and $MnSO_4$ in pH 7.0 MOPS buffer containing cycloheximide to prevent protein synthesis. The linear production observed over a period of at least four h was due to de novo polyketide synthesis and biological methylation, as shown by inhibition with cerulenin, iodoacetamide and ethionine. Production was inhibited by carbonyl reagents and stimulated by pyridoxamine suggesting that the conversion of endogenous intracellular orange pigments to extracellular red pigments involves Schiff base intermediates and vitamin B_6 a cofactor. The resting cell system was used to study the mode of action of nutritional effectors previously pinpointed by experiments with growing cells. The negative effects of high concentrations of phosphate and Mg^{++} are due to inhibition of pigment synthase action, not to repression or inactivation of these enzymes. The positive effects of trace metals, especially Zn^{++}, are due to stimulation of growth and enzyme action, not to induction or stabilization of the synthases.

INTRODUCTION

Monascus spp. produce six related pigments [25] which can be divided into three groups: two are orange (rubropunctatin and monascorubrin), two are yellow (monascin and ankaflavin) and two are red (rubropunctamine and monascorubramine). These are used commercially in the Orient as non-toxic colorants for the food industry. The yellow pigments are reduced forms of the orange colors and the red pigments contain a nitrogen atom where the orange pigments contain an oxygen. The members of each pair differ in the length of the side chain attached to the β-ozolactone ring. We refer to these water-insoluble pigments as 'conventional' as compared to new water-soluble pigments which we have obtained by growing *Monascus* sp. in a chemically-defined medium containing glutamate (MSG) as a nitrogen source [13]. In this case, the nitrogen atom of the ring is replaced by the nitrogen of glutamate and the rest of the glutamate molecule remains as a side-chain. The new red pigments have improved properties over the conventional red pigments, rubropunctamine and monascorubramine, which make them promising for wide use in the food, beverage and cosmetic industries.

The biosynthesis of the orange and yellow pigments of *Monascus* spp. involves condensation of six molecules of acetate into the ring system [2,4,15,27], the incorporation of hexanoate or octanoate respectively as the two different aliphatic side-chains on the β-ozolactone ring [8] and the attachment of the methyl group at C-7 from *S*-adenosylmethionine. The basic pathway is one of polyketide biosynthesis [23,27]; virtually nothing is known about the regulation of this pathway in *Monascus*. Carels and Shepherd [3] proposed that the orange pigments are initially biosynthesized and the yellow and red pigments are derived from them.

Our earlier studies [12] on red pigment production by growing cells in chemically-defined medium revealed glucose and maltose as the best carbon sources and glutamate as a markedly stimulatory nitrogen source. High phosphate levels (>70 mM) inhibited growth and pigment formation, whereas high $MgSO_4$ (16 mM) stimulated growth but inhibited specific pigment formation. Trace metals, especially Zn^{++}, increased pigment formation.

Conflicting conclusions about nutrient effects on *Monascus* pigment production that appear in the literature are the results of the use of complex media and different strains of *Monascus* [see 11 for review]. It is important to gain understanding of the factors regulating biosynthesis of the soluble red pigments. Resting cell systems provide a useful tool for studying the regulation of complex biosynthetic pathways [6]. In the present contribution, we describe the development of a resting cell system and its use to examine the nature of the biosynthetic process as well as determine the mode of action of certain nutritional effectors of pigment biosynthesis.

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MATERIALS AND METHODS

Microorganism

Monascus sp. strain TTWMB 6093, which is a high pigment producer obtained from previous screening efforts [13], was used in this work.

Medium

A chemically-defined medium [12] was used for fermentation. It contains maltose 50 g; MSG, 12.6 g; K_2HPO_4, 2.4 g; KH_2PO_4, 2.4 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; KCl, 0.5 g; $ZnSO_4 \cdot 7H_2O$, 10 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; $MnSO_4 \cdot H_2O$, 3 mg per liter of distilled water. The initial pH of the medium was 5.5.

Cultivation methods

The stock culture was kept on YM agar containing yeast extract, 3 g; malt extract, 3 g; peptone, 5 g; glucose, 20 g; agar, 1.5 g; and distilled water, 1 L. Mycelial blocks were used for inoculating seed cultures. The seed medium was YM broth (which contains the same nutrients as YM agar but without agar) which was incubated at 30 °C, 250 rpm for 3 days. A 10% volume of seed culture was inoculated into fermentation medium and incubated at 30 °C, 250 rpm for 6 days or other specified time.

Chemicals

Six authentic pigments known to be produced by *Monascus* were supplied by Dr J.G. Sweeny of the Coca-Cola Company, Atlanta, GA, USA. Other chemicals were purchased commercially and were of the highest purity available, the major source being Sigma Chemical Company.

Preparation of resting cell suspension

After 36 h (or other indicated times) of incubation, the fermentation broth was filtered. The wet mycelial cake was resuspended in pH 7.0 buffer containing 134 mM MOPS and 134 μg ml^{-1} cycloheximide (MOPS–CH buffer) and washed with the same buffer twice to remove most of the extracellular pigment. One part of damp dry mycelia was mixed with nine parts of pH 7.0 MOPS–CH buffer and homogenized in a Waring Blender at the highest speed for 1 min to prepare a homogeneous cell suspension.

Preparation of resting cell incubation mixture

Five ml of resting cell suspension were used to inoculate 10 ml MOPS–CH buffer and 5 ml aqueous solution containing other specified components. The 20 ml inoculated mixture was incubated at 30 °C, 250 rpm for 1.5 h, or other specified time.

Estimation of fermentative production of pigments

At the end of the fermentation, pigment productivity was measured as described previously [12].

Estimation of resting cell production of pigments

At the end of incubation, the mycelia from a single flask were filtered and washed twice, each time with 10 ml distilled water. The combination of filtrate and washings was made to 40 ml. The OD_{500} reading of the combination was considered to be 'extracellular pigment'. The water-washed mycelia were soaked in 20 ml of pH 7.0 methanol for 12 h before filtration. Twenty ml of methanol were used to wash the mycelia retained on the filter. The combination of the extract and the washing was made to 40 ml and considered to be 'cell-bound pigment'. The OD_{500} reading was measured as described in the measurement of fermentative production but normalized to the original 20 ml of broth. For specific production by resting cells, the absorbance was divided by the mg dry cell weight in 1 ml of broth.

HPLC analysis of pigments

The apparatus was a Waters liquid chromatograph equipped with a model 481 LC spectrophotometer monitoring absorbance at 500 nm for red and orange pigments or at 400 nm for yellow pigments. A μBondapak C_{18} column was used under ambient conditions. Elution was carried out with an initial 15% acetonitrile aqueous solution linearly increasing to 80% acetonitrile in 18 min at a flow rate of 1.0 ml min^{-1} [13].

Dry cell weight (DCW)

The mycelia after extraction of cell-bound pigment were dried at 80 °C for 24 h and weighed.

Studies of effects of potential regulatory compounds

For the study of regulatory compounds, various potential effectors were added at different concentrations to the resting cell incubation mixture or fermentation medium for the examination of their positive or negative effects in comparison with controls.

RESULTS

Development of resting cell system

The ability to support production of water-soluble red pigments was first tested in the MOPS–CH buffer system with or without a carbon source (1.25% maltose) and a nitrogen source (0.16% MSG) (Fig. 1). In the absence of

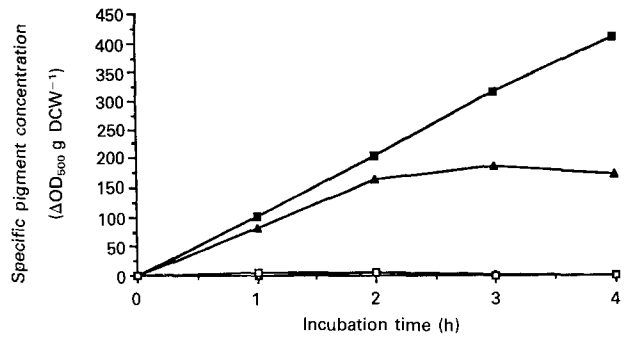

Fig. 1. Effect of incubation time and nutrients on red pigment formation by resting cells. Closed symbols, extracellular pigment. Open symbols, cell-bound pigment. ▲, △, absence of nutrients. ■, □, 1.25% maltose + 0.16% MSG.

nutrients, resting cells harvested from a 48-h fermentation produced extracellular pigments linearly for 2 h. The addition of a carbon source and an amino acid stimulated the rate of production of extracellular pigments and extended this linear production up to at least 4 h. The very low content of cell-bound pigment seemed not to change in the presence of nutrients. Thus, pigment production by resting cells was virtually all extracellular.

Cells harvested at different times from the fermentation were incubated as resting cells in MOPS–CH buffer containing 1.25% maltose and 0.16% MSG for 1 h (Fig. 2). The content of cell-bound pigments did not change during the 1-h incubation of the resting populations of any age (data not shown) but older populations contained more cell-bound pigments than did younger populations. The ability to produce extracellular pigments was best in cells harvested at 36–60 h of fermentation and decreased thereafter.

(a) Effect of amino acids. The effect of different amino acids on extracellular pigment production by resting cells was compared at a concentration of 20 mM in two separate experiments and the results are shown in Table 1. The differences in resting cell production were not great. Apparently each amino acid can act as side-chain precursor for the resting cell production of a soluble red pigment [13]. For example, the major pigments found in the glycine system are glycine derivatives of the well-known orange pigments, rubropunctatin and monascorubrin. Only four amino acids (glutamine, asparagine, glycine and arginine) supported better production than did MSG. Although glutamine and asparagine supported higher pigment production than glycine, they are basic amides containing two amino groups and are not easy to dissolve in water. Thus glycine was chosen as the amino acid source for further resting cell studies.

(b) Effect of sugar concentration. In our previous studies of the carbon source effect on red pigment production by growing cells, it was found that glucose and maltose were the two best carbon sources [12]. Different concentrations

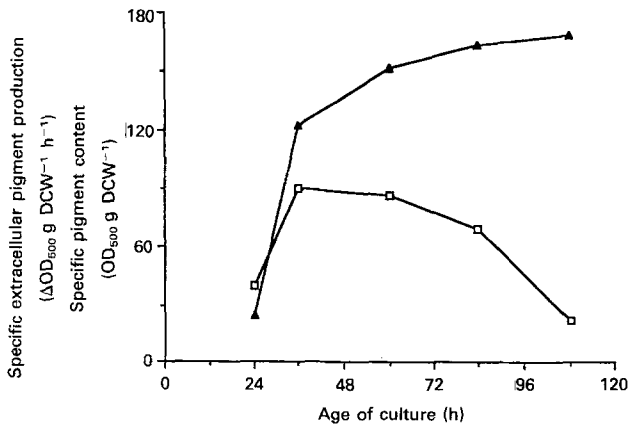

Fig. 2. Effect of culture age on the content of cell-bound pigments (▲) and the ability of cells to form extracellular red pigments under resting conditions (□).

TABLE 1

Effect of different amino acids on extracellular pigment production by resting cells

Amino acid (20 mM)	Pigment production relative to MSG	
	Expt 1	Expt 2
None	0.83	0.82
Gln	1.32	1.26
Asn	1.28	1.27
Gly	1.26	1.17
Arg	1.18	–
Thr	1.06	–
Ser	1.02	–
Glu (MSG)	1.00	1.00
Val	0.98	–
Ile	–	0.97
Leu	–	0.95
Ala	0.94	–
His	0.93	–
Trp	–	0.92
Phe	–	0.89
Lys	0.89	–
Met	0.87	–
Pro	0.80	–
Cys	0.75	–

of glucose and maltose were compared in the resting cell system within the range of 0.01% to 1.25% in the presence of 20 mM glycine as nitrogen source (Fig. 3). Glucose supported better pigment production by resting cells than maltose even though the cells had been grown in maltose. Glucose at a concentration of 0.25% was chosen for further studies to ensure that sufficient carbon source is present to support increases in pigment production brought about by other nutrients.

(c) Effect of pH. In growing cell studies, the pH value of the medium had markedly affected red pigment formation. In the range of 4.0 to 6.3, higher initial pH values favored the specific production of red pigment [12]. In the resting cell system, in the presence of 40 mM glycine, we found 7.0

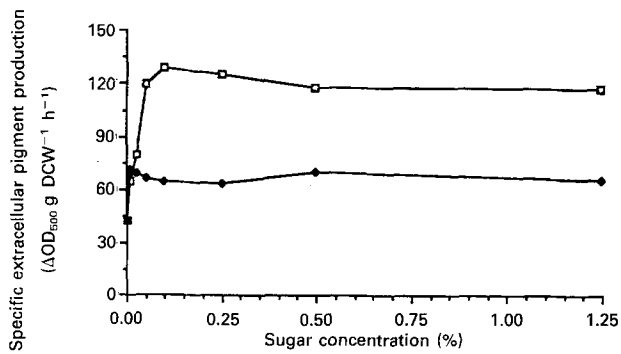

Fig. 3. Effect of sugars on resting cell production of red pigments. □, glucose; ◆, maltose.

to be the optimum pH for resting cell production of red pigments (data not shown).

(d) Effect of phosphate and magnesium. Studies of inorganic salt effects on pigment production by growing cells had shown that high concentrations of phosphate and $MgSO_4$ are inhibitory to pigment production [12]. In the resting cell system, phosphate was found to inhibit when added at concentrations above 2 mM, whereas $MgSO_4$ inhibited at concentrations higher than 0.5 mM. Thirty percent inhibition was observed at the highest concentrations tested, i.e. 50 mM phosphate and 2.5 mM $MgSO_4$ (data not shown).

(e) Effect of trace metals. In growing cell studies, three trace metals (Zn^{++}, Mn^{++} and Fe^{++}) were stimulatory for growth and pigment production [12]. The stimulatory effects of the three metals were examined in the resting cell system and they were again stimulatory to pigment production. Among the three metals, Zn^{++} had the strongest stimulatory effect and was optimal at 2 μM; Mn^{++} and Fe^{++} were optimal at 20 μM. A combination of 2 μM Zn^{++} and 20 μM Mn^{++} showed a stronger effect than Zn^{++} alone, but 20 μM Fe^{++} had no effect in the presence of 2 μM Zn^{++} (data not shown).

The above investigations of nutrient and pH effects led to a useful resting cell system that is suitable for comparing the specific red pigment productivity of cells grown under different growth conditions and the effects of potential inhibitors and stimulators of the biosynthetic process. This resting cell system contains 0.25% glucose, 40 mM glycine, 2 μM $ZnSO_4$, 20 μM $MnSO_4$ and 100 μg ml^{-1} CH in 100 mM MOPS buffer at pH 7.0.

Effects of inhibitors of polyketide biosynthesis and methylation

To determine whether red pigment formed during resting cell incubation is due to de novo polyketide synthesis [27] rather than merely leakage from the cells of pigment formed earlier during growth, we studied the effect of inhibitors of polyketide biosynthesis, i.e. cerulenin and iodoacetamide [16,17,21]. We were also interested in the effect of L-ethionine, a competitive inhibitor of methylation, since a biological methylation appears to be involved in pigment formation [8]. These three potential inhibitors were all found to inhibit production; cerulenin was the most potent of the three (Fig. 4). Thus, short-time red pigment production by resting cells probably occurs through a polyketide pathway involving a methylation step.

Effect of carbonyl reagents and vitamin B_6

Use of MSG, glycine and leucine as nitrogen sources leads to the resting cell production of water-soluble red pigments which have the same retention times as their corresponding derivatives of orange pigment obtained by chemical synthesis [13]. The conversion of intracellular orange pigments to extracellular red pigments is probably enzymatic and Schiff base intermediates [5,9] may be involved. If this is true, the enzyme reaction forming Schiff base intermediates should be inhibited by carbonyl reagents

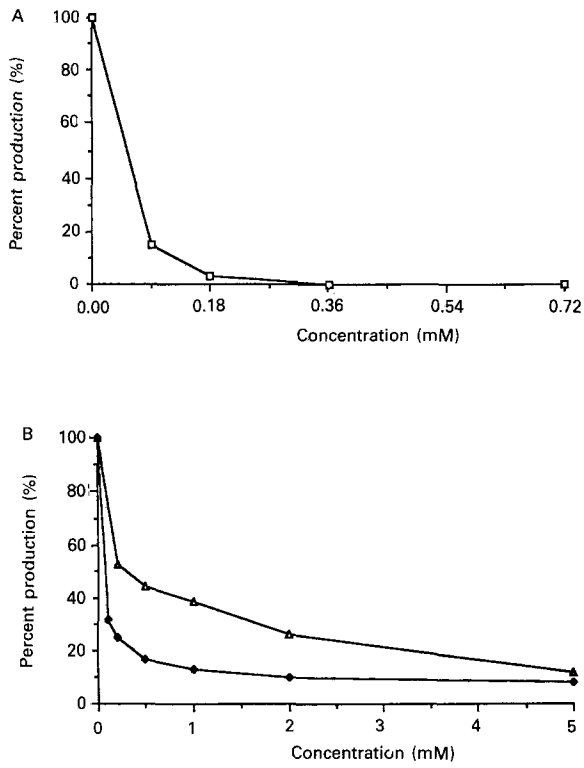

Fig. 4. Inhibition of resting cell production of red pigments by (A) cerulenin and (B) △, ethionine; ◆, iodoacetate.

[5,14,20,24]. Hydroxylamine (NH_2OH) and phenylhydrazine are carbonyl reagents which are well-known as effective inhibitors of vitamin B_6-dependent enzymes [24,28], many such reactions involving Schiff base formation. Their effect on resting cell production of red pigments was tested in the absence of glycine to avoid its possible competition with the carbonyl reagents. NH_2OH showed 85% inhibition of extracellular pigment production and completely blocked cell-bound production (Table 2). Phenylhydrazine inhibited extracellular production by 80% but did not inhibit cell-bound pigment production. Since extracellular pigment is virtually all of the soluble type, the inhibition effects strongly suggest the enzymatic involvement of Schiff base intermediates in the formation of soluble red pigments by resting cells.

Vitamin B_6 compounds act as coenzymes for the formation of Schiff base intermediates in many enzymatic reactions [22,24]. We thus examined the possible involvement of vitamin B_6 in the formation of soluble red pigments. Four types of vitamin B_6 (pyridoxal phosphate, pyridoxal, pyridoxine, pyridoxamine) were tested in the resting cell system and the three non-phosphorylated forms showed some stimulation (5–18%) of pigment production (data not shown). In order of decreasing activity, they were pyridoxamine, pyridoxine and pyridoxal. When the three compounds were tested on growing cells, the same order was observed; pyridoxamine showed 80% stimulation at 2.5 mM, pyridoxine 56% and pyridoxal 23%.

TABLE 2

Inhibition of resting cell production of red pigment by carbonyl reagents

Inhibitor (mM)	Specific pigment production rate (ΔOD_{500} g DCW^{-1} h^{-1})	
	Extracellular	Cell-bound
None (control)	159	64
Hydroxylamine		
2.5	47	−1
5	42	−1
10	33	−1
20	23	−1
Phenylhydrazine		
2.5	78	86
5	33	77
10	32	87
20	33	89

Modes of action of potential effectors of pigment production

(a) High concentrations of phosphate and $MgSO_4$. Studies of inorganic salt effects on growing cells showed that high concentrations of phosphate and $MgSO_4$ are inhibitory to specific pigment formation [12]. The resting cell data described above revealed that phosphate at concentrations higher than 2 mM and $MgSO_4$ at concentrations higher than 0.5 mM inhibit the action of pigment synthase(s). To determine whether high concentrations of phosphate and $MgSO_4$ also repress the formation of pigment-forming enzymes or inactivate them, increased concentrations of the two salts were compared with their usual concentrations in the fermentation medium. We found that increasing phosphate 10-fold (to 300 mM) or $MgSO_4$ (to 32 mM) in the growth medium had no effect on resting cell activity. Thus, the poor pigment production of growing cells at high concentrations of phosphate and $MgSO_4$ is due to inhibition of pigment synthases but not to repression or inactivation of these enzymes.

(b) Trace metals. In previous growing cell studies, three trace metals (Zn^{++}, Mn^{++} and Fe^{++}) were stimulatory for both growth and pigment production [12]. The optimum concentrations were 70 μM, 35 μM and 35 μM respectively. Data given above show that 2 μM $ZnSO_4$, 20 μM $MnSO_4$ and 20 μM $FeSO_4$ stimulate pigment production by resting cells. To determine whether the trace metals also affect formation or stability of pigment-forming enzymes, we eliminated the three trace metals from the growth medium and examined the effect on resting cell pigment production; no effect was observed (data not shown). Thus the higher pigment production by growing cells in the presence of a combination of the three trace metals is not due to an induction or stabilization of pigment-forming enzymes but to a stimulation of action of the pigment synthase(s). The overall conclusion is that trace metals act by stimulating both growth and enzyme action.

(c) Fructose. In the studies of carbon source effects on red pigment production by growing cells, it was found that glucose, maltose and fructose were the three best sugars supporting growth and pigment production, being better than other carbon sources such as galactose, lactose, and sucrose [12]. Since both growth and volumetric pigment production were lower with fructose than with glucose and maltose, specific pigment production supported by fructose was only slightly lower than with the other two sugars. This indicated that fructose was taken up and/or metabolized less efficiently than glucose and maltose. If this is true, fructose should not inhibit pigment production by resting cells but merely support lower rates than glucose or maltose. As shown in Table 3, this is what was found, no matter what was the source of carbon for growth.

DISCUSSION

Inhibition of resting cell formation of red pigments by cerulenin, iodoacetamide and ethionine shows that *Monascus* pigments are produced by resting cells via polyketide biosynthesis and methyl transfer from *S*-adenosylmethionine, rather than merely by excretion of preformed pigment. Inhibition of extracellular soluble red pigment formation by carbonyl reagents (hydroxylamine, phenylhydrazine) and stimulation by vitamin B_6 indicates that the conversion of the endogenous water-insoluble orange pigments to soluble red pigments involves an enzymatic Schiff base reaction.

The resting cell system allowed us to define the mode of action of a number of nutritional effectors of pigment biosynthesis. Thus, the negative effect of high concentrations of phosphate and Mg^{++} and the positive effect of trace metals (Zn^{++}, Mn^{++}, Fe^{++}) are due to inhibition and stimulation of pigment synthase action respectively and not

TABLE 3

Effect of fructose on pigment production

Carbon source for growth	Resting cell system	Specific extracellular pigment production (ΔOD_{500} g DCW^{-1} h^{-1})
Glucose	no sugar	155
	glucose	230
	fructose	187
Maltose	no sugar	157
	glucose	227
	fructose	188
Fructose	no sugar	133
	glucose	171
	fructose	161

to effects on synthase formation or stability. The poorer performance of fructose, as compared to glucose or maltose, as carbon source for fermentation is due to poorer or less efficient uptake or metabolism but not to inhibition of pigment synthase action.

Trace metals have important effects on secondary metabolism [26]. It had been shown earlier [1] that pigment production by *Monascus purpureus* is promoted by zinc sulfate. With respect to other polyketides, a higher concentration of zinc is needed for production of aflatoxin than is required for growth [7,10]. Excess manganese is necessary for patulin production [18].

With regard to the vitamin B_6 stimulation, the superiority of pyridoxamine may be due to two possible reasons. One is that the transport of pyridoxamine into cells might be better than that of pyridoxal or pyridoxine. It has been noted that the yeast *Saccharomyces carlsbergensis* has two distinct uptake systems for vitamin B_6 compounds. One with an optimum pH of 3.5 transports pyridoxal effectively but not pyridoxamine; the other with an optimum pH of 6.0 transports pyridoxamine effectively but not pyridoxal. Both systems transport pyridoxine, while neither transports pyridoxal-5′-phosphate [19]. The other possible reason is that pyridoxal (i.e. intracellular pyridoxal phosphate) might not be the coenzyme involved; pyridoxamine (i.e. intracellular pyridoxamine phosphate) may be the coenzyme of the Schiff base reaction.

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REFERENCES

1 Bau, Y.S. and H.C. Wong. 1979. Zinc effect on growth, pigmentation and antibacterial activity of *Monascus purpureus*. Physiol. Plant. 46: 63–67.

2 Birch, A.J., A. Cassera, P. Fitton, J.S. Holker, H. Smith, G.A. Thompson and W.B. Whalley. 1962. Studies in relation to biosynthesis. Part 30. Rotiorin, monascin and rubropunctatin. J. Chem. Soc. 3583–3586.

3 Carels, M. and D. Shepherd. 1977. The effect of different nitrogen sources on pigment production and sporulation. Can. J. Microbiol. 23: 1360–1372.

4 Chen, F.C., P.S. Manchand and W.B. Whalley. 1971. The chemistry of fungi. Part 54. The structure of monascin: the relative stereochemistry of the azaphilones. J. Chem. Soc. (C) Org. Chem. 3577–3579.

5 Cordes, E.H. and W.P. Jencks. 1962. On the mechanism of Schiff base formation and hydrolysis. J. Amer. Chem. Soc. 84: 832–837.

6 Demain, A.L. and Y.M. Kennel. 1978. Resting-cell studies on carbon source regulation of β-lactam antibiotic biosynthesis. J. Ferment. Technol. 56: 323–328.

7 Gupta, S.K., K.K. Maggon and T.A. Venkitasubramanian. 1976. Zinc-dependence of glycolytic enzymes in an aflatoxigenic strain of *Aspergillus parasiticus*. Microbios. Lett. 3: 89–92.

8 Hadfield, J.R., J.S.E. Holker and D.N. Stanway. 1967. The biosynthesis of fungal metabolites. Part II. The β-oxo-lactone equivalent in rubropunctatin and monascorubrin. J. Chem. Soc. (C) Org. Chem. 751–755.

9 Jencks, W.P. 1969. Carbonyl- and acyl-group reactions. In: Catalysis in Chemistry and Enzymology, pp. 463–554. McGraw-Hill, New York.

10 Lee, E.G.H., P.M. Townsley and C.C. Walden. 1966. Effect of bivalent metals on the production of aflatoxin in submerged cultures. J. Food Sci. 31: 432–436.

11 Lin, T.F. 1991. Studies on the formation of *Monascus* red pigments. Ph. D. Thesis, Massachusetts Institute of Technology, Cambridge, MA.

12 Lin, T.F. and A.L. Demain. 1991. Effect of nutrition on formation of *Monascus* red pigments. Appl. Microbiol. Biotech. 36: 70–75.

13 Lin, T.F., K. Yakusijin, G. Büchi and A.L. Demain. 1992. Formation of water-soluble *Monascus* red pigments by biological and semi-synthetic processes. J. Ind. Microbiol. 9: 173–179.

14 Lowry, T.H. and K.S. Richardson. 1987. Reactions of carbonyl compounds. In: Mechanism and Theory in Organic Chemistry, 3rd edn, pp. 661–735, Harper & Row, New York.

15 Manchand, P.S. and W.B. Whalley. 1973. Isolation and structure of ankaflavin; a new pigment from *Monascus anka*. Phytochemistry 12: 2531–2532.

16 McGuire, J.C., G. Glotfety and R.J. White. 1980. Use of cerulenin in selecting improved mutants of a daunorubicin-producing streptomycete. In: Advances in Biotechnology. Vol 1, (Moo-Young, M., C.W. Robison and C. Vezina, eds), pp. 57–61, Pergamon Press, Toronto.

17 Omura, S. 1976. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. Bacteriol. Rev. 40: 681–697.

18 Scott, R.E., A. Jones and G.M. Gaucher. 1986. Manganese and antibiotic synthesis. III. The site of manganese control of patulin production in *Penicillium urticae*. Can. J. Microbiol. 32: 273–279.

19 Shane, B. and E.E. Snell. 1976. The transport of vitamin B_6 in the yeast *Saccharomyces carlsbergensis* 4228. J. Biol. Chem. 25: 1042–1051.

20 Streitwieser, A. Jr and C.H. Heathcock. 1985. Aldehydes and ketones. In: Introduction to Organic Chemistry, 3rd edn, pp. 383–386, Macmillan, New York.

21 Tomoda, H., A. Kawaguchi, S. Omura and S. Okuda. 1984. Cerulenin resistance in a cerulenin-producing fungus. II. Characterization of fatty acid synthase from *Cephalosporium cerulens*. J. Biochem. 95: 1705–1712.

22 Tryfiates, G.P. 1986. Pyridoxal phosphate and metabolism. In: Vitamin B_6. Pyridoxal Phosphate. Chemical, Biological, and Medical Aspects. Part B (Dolphin, D., R. Poulson and O. Avramovic, eds), pp. 421–447, John Wiley & Sons, New York.
23 Turner, W.B. and D.C. Aldridge. 1983. Fungal Metabolites II, pp. 55–223, Academic Press, New York.
24 Walsh, C. 1979. Enzymatic reactions requiring pyridoxal phosphate. In: Enzymatic Reaction Mechanisms, pp. 777–827, W.H. Freeman and Co., San Francisco.
25 Wang, H.L. and C.W. Hesseltine. 1979. Mold-modified foods. In: Microbial Technology. Vol. 7, 2nd edn (Peppler, H.J. and D. Perlman, eds), pp. 95–129, Academic Press, New York.
26 Weinberg, E.D. 1989. Roles of micronutrients in secondary metabolism of actinomycetes. In: Regulation of Secondary Metabolism in Actinomycetes (Shapiro, S., ed.), pp. 239–261, CRC Press, Boca Raton.
27 Whalley, W.B. 1963. The sclerotiorin group of fungal metabolites: their structure and biosynthesis. Pure Appl. Chem. 7: 565–587.
28 Zollner, H. 1989. Handbook of Enzyme Inhibitors, pp. 322–323, VCH Weinheim, Germany.