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Formation of water-soluble *Monascus* red pigments by biological and semi-synthetic processes

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SUMMARY

New water-soluble red pigments were produced by *Monascus* sp. in a chemically defined fermentation medium containing glutamate as nitrogen source. They were isolated and characterized as glutamate derivatives of the well-known orange *Monascus* pigments (monascorubrin and rubropunctatin). The new pigments have several advantages over the known red *Monascus* pigments (rubropunctamine and monascorubramine) including very high water-solubility, higher absorption coefficient, and greater resistance to decoloration by light. Adding glutamate, glycine or leucine to a resting-cell system led to the formation of specific water-soluble red pigments corresponding to the exogenous amino acid. The water-soluble red pigments produced by resting-cells have retention times identical to those of the corresponding red derivatives made chemically from the orange pigments in methanol–phosphate buffer at pH 7. The hydrophobicities of the amino acid sources correspond to the HPLC retention times of the red pigments derived from them.

INTRODUCTION

Monascus species have been used for a long time as a meat colorant, disinfectant and a Chinese folk medicine. Presently, they are mainly used as a natural food colorant. More than 50 patents have been issued in Japan, United States, France and F.R.G. concerning the use of *Monascus* pigments for food [25]. *Monascus* pigments are stable in the pH range of 2 to 10, heat stable and can be autoclaved [7]. The six well-known *Monascus* pigments are produced mainly in the cell-bound state and are insoluble in water. Thus, many patents have focused on the extraction and solubilization of *Monascus* pigments. Although toxicity studies reveal that *Monascus* pigments are safe for human consumption [9,18], the water-insolubility and sensitivity to decoloration by sunlight restrict the wide use of the *Monascus* pigments in the beverage and confectionary industries [4,19]. In previous studies on the development of a chemically-defined medium supporting rapid and intensive red pigment production, use of glutamate as nitrogen source stimulated red pigment production and shifted the site of pigment from predominantly cell-bound to mainly extracellular [12]. The major pigments produced in the glutamate medium represent a new type of water-soluble *Monascus* red pigments. The discovery and properties of the new water-soluble pigments as well as their formation by both biosynthesis and semi-synthesis are described.

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MATERIALS AND METHODS

Microorganism

Monascus sp. strain TTWMB 6093, which is a high pigment producer obtained by screening [11], was used.

Media

The chemically-defined medium developed in our previous report [12] was used as the fermentation medium. It contains: maltose, 50 g; monosodium glutamate (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 of *Monascus* sp. were used for preparing seed cultures. The inoculated seed cultures were grown in 250-ml flasks containing 40 ml of YM broth (which contains the same nutrients as YM agar but without agar) at 30 °C, 250 rpm

for 4 days. Then 4 ml seed culture was inoculated into 250-ml flasks containing 40 ml of fermentation medium at 30 °C, 250 rpm for 6 days.

Preparation of resting-cell suspension

After 36 h 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 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 one min to make a more homogenous 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 1.0% glucose, 8 μM $ZnSO_4$, 80 μM $MnSO_4$ and 160 mM amino acid at pH 7.0. The 20 ml inoculated mixture was incubated at 30 °C, 250 rpm for 1.5 h.

HPLC analysis of pigments

The apparatus used was a Waters liquid chromatograph equipped with a model 481 LC spectrophotometer monitoring absorbance at 500 nm for red and orange pigments and at 400 nm for yellow pigments. A μBondapak C_{18} column (39 mm × 30 cm) 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.

Isolation and purification of new red pigments from fermentation broth

The dark red filtrate of the 6-day fermentation broth was concentrated under vacuum and extracted with ethyl ether to remove any water-insoluble impurities. Some of the red pigments precipitated in the aqueous layer. After filtration, the red aqueous solution was mixed with five vol. of 100% ethanol and most of the water-soluble red pigments precipitated. After a second filtration, both precipitates were combined and dissolved in methanol. The resulting dark red methanol solution was injected into a μBondapak C_{18} column installed on a Waters liquid chromatograph equipped with a model 481 LC spectrophotometer monitoring the absorbance at 500 nm under ambient conditions. Elution was carried out with an initial 35% aqueous solution of acetonitrile gradually increasing to 70% within 15 min at a flow rate of 1.0 ml/min. Two well-separated fractions of red acetonitrile solutions were evaporated to very deep red amorphous powders and subjected to structural identification by ultraviolet spectra (Perkin-Elmer Hitachi No. 200 instrument), IR spectra (Perkin-Elmer No. 397 infrared instrument) and ^{1}H-NMR (Varian NMR instrument model 300).

Semi-synthesis of glutamate derivative of monascorubrin

A crude orange pigment mixture containing about 70% of monascorubrin (C_7 side chain) and 30% of rubropunctatin (C_5 side chain) was streaked on preparative silica gel thin layer plates. The plates were developed with dicholoromethane five times to separate the mixture into two pure compounds. To a mixture of purified monascorubrin (50 mg) and L-glutamic acid (200 mg) in 10 ml of dichloromethane was added triethylamine (0.3 ml) drop-wise at room temperature. The reaction mixture was stirred under an argon atmosphere at the same temperature for 20 h. The solvent and the triethylamine were removed by evaporation under reduced pressure. The solid red residue was purified by flash column chromatography on silica gel and eluted with 7 : 3 CH_2Cl_2/MeOH to give 60 mg (90% yield) of a water-soluble pigment as a very deep red amorphous residue.

Semi-synthesis of red pigments derived from amino acids

A crude orange pigment mixture containing about 70% of monascorubrin (C_7 side chain) and 30% of rubropunctatin (C_5 side chain) was added to 10 ml aqueous solution of 50% methanol containing three different buffers (36 mM K_2CO_3, pH 11.7; 100 mM MOPS, pH 7.0; 100 mM phosphate, pH 7.0) in an amount of 1.2 mg (about 0.32 mM). To each buffer system, glutamate (glut), glycine (gly) or leucine (leu) was included at a final concentration of 8 mM. The reaction mixtures were incubated at 30 °C and 250 rpm for varied periods of time.

Solubility of red pigments

Different amounts of a mixture of the known C_5 and C_7 *Monascus* red pigments (rubropunctamine and monascorubramine) and a C_5 and C_7 mixture of the new red pigments were separately dissolved in water at 20 °C. The undissolved residual pigments were recovered by filtration and weighed after drying at 80 °C for 24 h [15].

Absorption spectra of red pigments

Ten-mg samples of the known red pigment mixture and the new red pigment mixture were separately dissolved in 10 ml of pure methanol and placed in standard 1-cm depth glass cuvettes for absorbance measurements within the region of visible light in a Gilford model 2600 spectrophotometer.

Resistance to UV light

Aqueous pigment mixtures of the known C_5 and C_7 *Monascus* red pigments (rubropunctamine and monascorubramine), and the C_5 and C_7 mixture of the new water-

soluble red pigments were separately dissolved in 20% aqueous methanol solutions containing 0.01 M disodium phosphate-citrate buffer at both pH 3.0 and pH 7.0, at a concentration giving an $O.D._{500}$ value of approximately 0.8. These pigment solutions were placed in Pyrex tubes and exposed to long wavelength ultraviolet light at a wavelength of 366 nm with a Black-Ray lamp (Ultra-Violet Productions Inc.) located 15 cm from the top of the sample tubes, i.e., the UV rays entered through the opening of the tubes. The $O.D._{500}$ values of these solutions were determined before exposure and every 12 h after exposure.

RESULTS AND DISCUSSION

Isolation and characterization of red glutamate derivatives

Comparison of the HPLC profiles of the pigments produced in an early chemically-defined medium and in our newly developed medium showed a large difference. The whole broth of the early medium contained the six well-known *Monascus* pigments, most of which was cell-bound [12] and all of which had retention times of longer than 21 min (Table 1). However, the major part of the pigments produced in the new fermentation broth was extracellular [12] and formed two peaks which eluted very early, having retention times of 4 to 8 min under the HPLC conditions used.

We successfully isolated the two major pigments produced in the new medium. The spectral data for these two water-soluble pigments were identical in the following respects; IR(Njuol) 3400, 1720, 1630, 1545, 1470, 1380, 1210, and 1160 cm^{-1}; UV (MeOH) (log ε) 501 (4.54), 422 (4.36) nm; ^1H NMR (CD_3OD) ∂ 0.89 (3H, t, J-7 Hz), 1.33 (8H, m), 1.60 (2H, m), 1.67 (3H, s), 1.98 (3H, d, J = 4 Hz), 2.27 (1H, m), 2.60 (1H, m), 2.82 (2H, t, J = 7 Hz), 4.97 (1H, t, J = 7 Hz), 6.56 (2H, bs), 6.73 (1H, s), 7.06 (1H, s), 8.28 (1H, s). The spectral data of the natural pigments isolated from fermentation broths were almost identical to those of a mixture of the semi-synthetic C_5 and C_7 soluble red colorants as well as the semi-synthetic C_7 compound. The latter two samples were prepared chemically from insoluble orange pigments (see below and *Materials and Methods*). The only difference was the intensity of the 'methylene envelope' centered at ∂1.33 in the ^1H NMR spectrum due to different ratios of the C_5 and C_7 side-chain pigments among the three different samples. We conclude that the new red pigments are the glutamate derivatives of the two well-known orange pigments (Fig. 1). The conventional red pigments have NH in the ring instead of glutamate nitrogen.

TABLE 1

Retention time of *Monascus* pigments in HPLC analysis

Kinds of pigment	Retention time (min)
Rubropunctatin	21.2
Monascorubrin	23.2
Rubropunctamine	24.6
Monascorubramine	26.2
Monascin	30.8
Ankaflavin	37.4
New pigments	4–8

Properties of the red glutamate derivatives

Since water-insolubility restricts the use of the conventional *Monascus* pigments, several chemical processes have been patented to semi-synthesize water-soluble red pigments in the presence of ethanol by exchanging the 'replaceable oxygen' in the orange pigments with the nitrogen of the primary amino group of various compounds. The postulated reaction is shown in Fig. 2 [13]. These compounds include amino acids, peptides, water-soluble

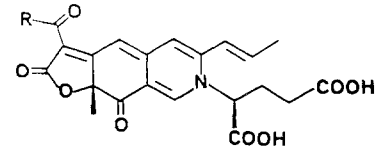

Fig. 1. Red water-soluble glutamate derivatives of orange pigments isolated from fermentation broth. The R group for the orange pigment monascorubin is a C_7 side chain; that for the orange pigment rubropunctatin is a C_5 side chain.

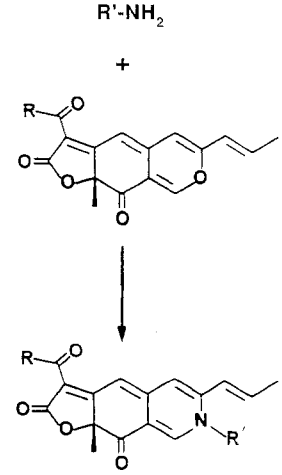

Fig. 2. Postulated reaction between orange pigments and compounds containing a primary amino group.

proteins [26], amino sugars, amino alcohols, chitosan [13], nucleotides [14] and nucleic acids [23]. Other patents involve adding protease [21a,22] or RNAase [21b] to the pigment-protein or pigment-RNA complex. Lipase has also been added to the ethanol extract of *Monascus* pigments [6] so that the enzyme would bind to the pigments to form a pigment-protein complex or shorten the aliphatic side-chain. Water-soluble pigments produced by chemical modification are claimed to be more stable to heat, light or pH changes [20,25]. No scientific literature exists on the isolation and characterization of the new natural water-soluble red pigments. Direct production of water-soluble pigments by fermentation offers a more acceptable alternative to the semi-synthetic processes, since it avoids the use of 'chemical additives' in foods [17].

A comparison of important properties of the new red pigments and the known red pigments, rubropunctamine and monascorubramine, is summarized in Table 2. The absorption spectra of these two types of red pigments are quite similar, but the new red pigments have over double the absorption coefficient of the known red pigments at their maximum absorption wavelength, 501 nm, which is 11 nm shorter than the maximum absorption wavelength of the known red compounds. There are also other advantages of the new red compounds. They have 500 times greater water-solubility, and are more resistant to decoloration by UV light. Under irradiation with 366 nm UV light for 36 h, more than 92% of the known red pigments was destroyed at pH 7.0 or 3.0 but the new red compounds lost only 25% of their original absorbance (Fig. 3). Both the new and the known red pigments are resistant to heat and can be autoclaved. They are also

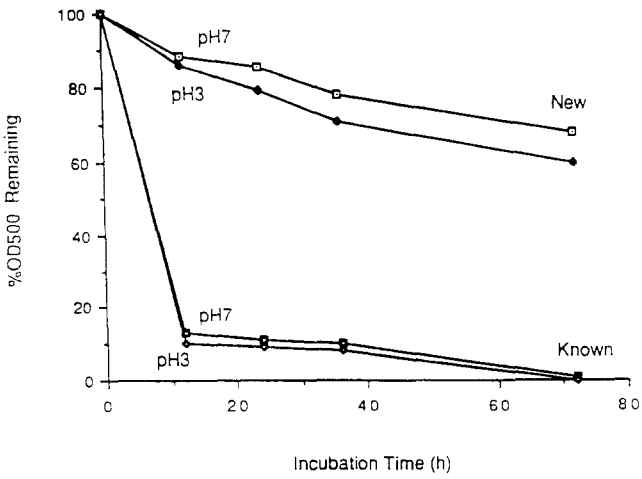

Fig. 3. Ultraviolet light decoloration of new and known red pigments.

TABLE 2

Comparison of properties of *Monascus* red pigments

Property	New pigments	Known pigments
Water solubility (g/l)	>50	<0.1
Absorption coefficient (cm^2/mol)	35,064 @ λ_{501}	15,709 @ λ_{512}
Fading at 366 nm, at pH 3 ($t_{1/2}$)	90 h	6 h
Stability to autoclaving	stable	stable
Stability at pH 3 to 12	stable	stable

resistant to pH changes, no appreciable color change being observed in the range of pH 3.0 to 12.0. These excellent properties make the new red pigments promising for wide use in the food, beverage and cosmetic industries.

Semi-synthesis of red amino acid derivatives of orange pigments

The orange *Monascus* pigments are members of a group of mold metabolites known as azaphilones because of their facile reaction with ammonia and methylamine [2,8,24]. Several chemical processes are used to convert orange pigments into red pigments in alcoholic solutions [10,13,26]. Three methanol-buffer systems were chosen to study the chemical conversion of 0.32 mM orange pigments into their red amino acid derivatives. Increase in $O.D._{500}$ and HPLC analyses of the red pigments were used to monitor the process of the chemical reaction.

Methanol-buffer systems were found to be very good for the conversion of orange pigments to their red amino acid derivatives. Among the three buffers tested, neutral buffers supported better conversion than did the pH 11.7 buffer (Table 3). This observation suggests that a nucleophilic addition to form Schiff base intermediates followed by dehydration is involved in the formation of red derivatives (Fig. 4), since the nucleophilic addition is favored by alkaline conditions but the dehydration is normally catalyzed by an acidic environment [1,3,5,16]. The reaction was over by 2 h; during an additional 10 h in methanol buffers, there was almost no further change in $O.D._{500}$ reading in the three systems tested. Methanol-phosphate buffer at pH 7.0 was chosen to synthesize glut, gly and leu derivatives of the orange pigments. The leu reaction showed an $O.D._{500}$ similar to those of the glut and gly derivatives, i.e., 8.82 (see Table 3).

HPLC analysis (Fig. 5) showed that there were no detectable orange pigments left in the glut and leu reaction mixtures after 2 h of incubation in methanol-phosphate buffer and only a trace left in the gly reaction mixture. The red pigments produced in the methanol-phosphate system

TABLE 3

Semi-synthesis of red glutamate and glycine derivatives in different 50% methanol reaction systems

Amino acid added	Buffer[a]	Δ OD_{500} reading after 2 h[b]
None	K_2CO_3	0.04[c]
	MOPS	0.00[c]
	Phosphate	0.00[c]
Glut	K_2CO_3	0.88
	MOPS	2.20
	Phosphate	2.62
Gly	K_2CO_3	0.48
	MOPS	1.84
	Phosphate	2.20

[a] K_2CO_3 was at pH 11.7; MOPS and phosphate were at pH 7.0.
[b] The zero time reading was 6.44 due to the reading of the orange pigments added. No further reaction occurred after 2 h.
[c] These were orange in color, but the other two systems were scarlet red at 2 h and 12 h.

using the three amino acids as reactants appeared to be specific derivatives of the orange pigments (Fig. 5d,e,f). The most hydrophilic amino acid, glut, formed two peaks with the shortest retention times (Rt's) (3.0 and

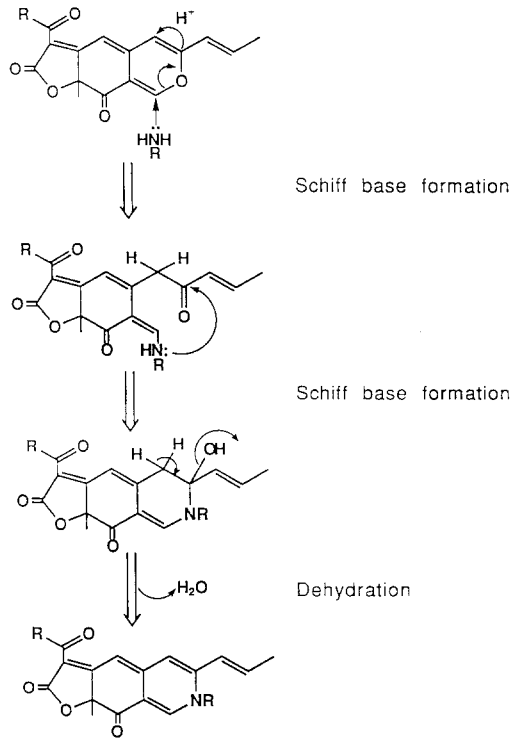

Fig. 4. Proposed reaction steps for the formation of red amino acid derivatives of orange pigments.

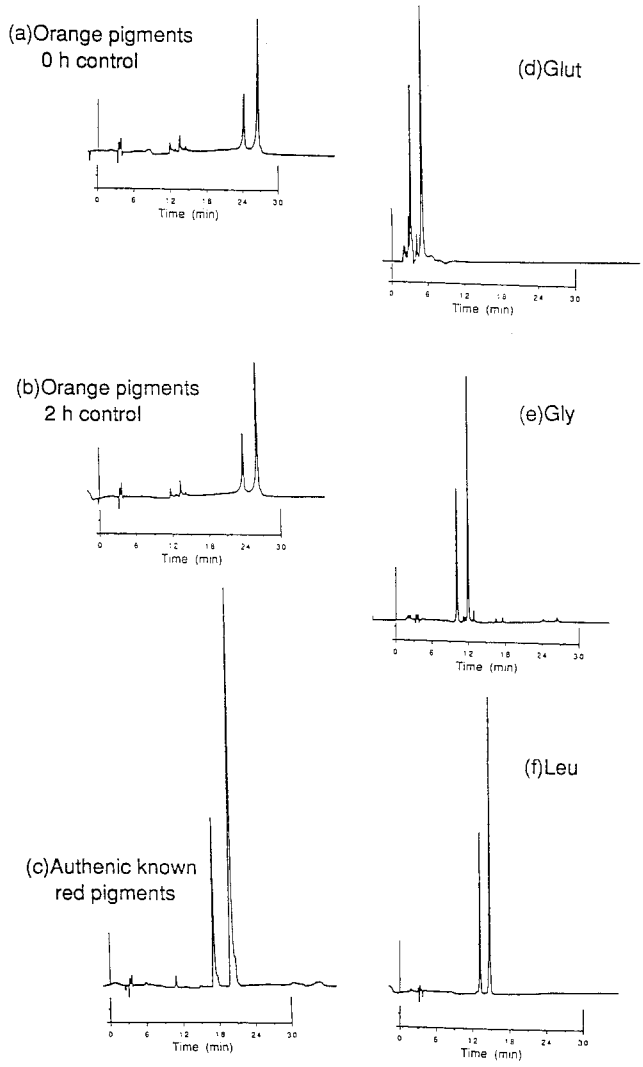

Fig. 5. HPLC profile of chemical reaction mixtures after 2 h incubation of orange pigments with an amino acid. The profile of the well-known red pigments (c) is shown for comparison.

4.5 min). The most hydrophobic amino acid, leu, supported formation of red pigments with the longest Rt's (13 and 15 min). The red pigments formed from gly were intermediate, with Rt's of 10 and 12 min. The known orange pigments have Rt's of 24 and 26 min and those of known red pigments are 18 and 21 min.

Since the reactions with amino acids were virtually complete in the methanol-phosphate buffer system and no other red side products were observed by HPLC analysis, the similarity in $O.D._{500}$ readings among the three amino acids (Table 4) indicates that the soluble pigments made from the three amino acids have similar molar absorption coefficients.

Effects of different amino acids on resting-cell production and identity of extracellular pigments with semi-synthetic pigments

A resting-cell system containing 40 mM amino acid was developed to study the effect of different amino acids (glut, gly, leu) on the production of extracellular pigments in the absence of protein synthesis. HPLC analysis of the resting-cell systems in the presence of the three amino acids showed different pigment profiles (Fig. 6). When there was no amino acid added to the system, four major red pigment peaks were found. The Rt's of the first two peaks were 3.0 min and 4.2 min, which are similar to the retention times of the two glut-derived pigments. The later two minor peaks with Rt's of 19 min and 22 min represent the two known red pigments, rubropunctamine and monascorubramine. When glut was added to the system (no matter whether glucose was present or not), there were only two major peaks with Rt's of 3.0 and 4.2 min. When there was gly in the system, two new major peaks were seen at 11 and 12 min accompanied by two minor peaks corresponding to the glut derivatives. When leu was used as amino acid source, two major peaks were seen at 13 and 15 min; two minor peaks corresponding to glut derivatives were also present. This indicates that resting-cells produce specific types of red pigment corresponding to the exogenous amino acids. Hydrophobic amino acids lead to the formation of pigments with longer retention times, i.e., more hydrophobic pigments. The Rt's observed are similar to the Rt's of the soluble red pigments synthesized by the chemical method in the presence of the corresponding amino acid (Fig. 5). All these red amino acid derivatives have shorter Rt's than the well-known red pigments, rubropunctamine and monascorubramine.

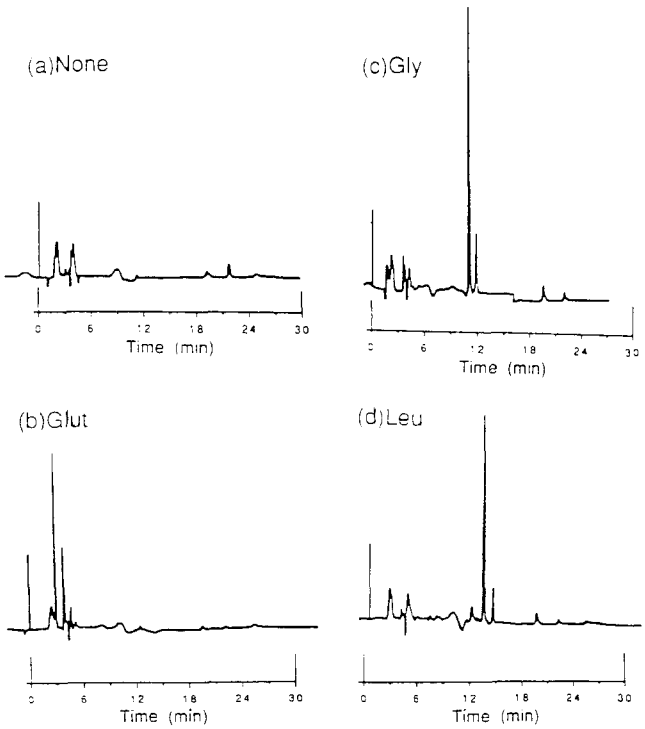

Fig. 6. Extracellular red pigments produced by resting-cells in the presence of different amino acid sources.

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REFERENCES

1 Bender, M.L., R.J. Bergeron and M. Komiyama. 1984. General acid-base catalysis: organic reactions. In: The Bioorganic Chemistry of Enzymatic Reactions. pp 71–115. John Wiley & Sons, New York.

2 Büchi, H., J.D. White and G.N. Wogan. 1965. The structures of mitorubrin and mitorubrinol. J. Am. Chem. Soc. 87: 3484–3489.

3 Carey, F.A. and R.J. Sundberg. 1984. Advanced Organic Chemistry. Part A: Structure and Mechanism. pp. 403–454. Plenum Press, New York.

4 Chang, R.Q. 1977. Studies on the stability of *Monascus* pigment. Masters Thesis. National Taiwan University, Taipei, Taiwan (in Chinese).

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

6 Endo, N. and S. Koyama. 1977. Water soluble pigment of *Monascus*. Japan Patent Kokai 77,034,986.

7 Francis, F.J. 1987. Lesser-known food colorants, Food Technol. 41: 62–68.

8 Haws, E.J., J.S.E. Holker, A. Kelly, A.D.G. Powell and A. Robertson. 1959. The chemistry of fungi. Part 37. The structure of rubropunctatin. J. Chem. Soc. 3598–3610.

9 Huang, T.L. 1981. Fermentative production and toxic test of natural pigment-*Monascus* pigments. Masters Thesis, National Taiwan University, Taipei, Taiwan (in Chinese).
10 Kumasaki, S., K. Nakanishi, E. Nishikawa and M. Ohashi. 1962. Structure of monascorubrin. Tetrahedron 18: 1171–1184.
11 Lin, T.F. and C.T. Huang. 1983. The zymotic properties of *Monascus* mold. The production of extracellular amylases. Ann. Rpt. Res. Inst. Wines 157–167 (in Chinese).
12 Lin, T.F. and A.L. Demain. 1991. Effect of nutrition of *Monascus* sp. on formation of red pigments. Appl. Microbiol. Biotechnol. 36: 70–75.
13 Moll, H.R. and D.R. Farr. 1976. Red pigment and process. U.S. Patent 3,993,789.
14 Nakagawa, N., S. Watanabe and J. Kobayashi. 1980. Nucleotide treatment of *Monascus* pigments to produce meat-coloring agents. Japan Patent Kokai 80,09,682.
15 Pellian, K.A. and R.E. Tellis. 1971. Dyes, methods of analysis. In: Encyclopedia of Industrial Chemical Analysis. Vol 12, (F.R. Snell and L.S. Ettre, eds.) 11–13. Interscience, New York.
16 Ritchie, C.D. 1990. Physical Organic Chemistry. 2nd edn., 227–252. Marcel Dekker, New York.
17 Spears, K. 1988. Developments in food colorings: the natural alternatives. Tibtech 6: 283–288.
18 Su, Y.C. and W.H. Wang. 1983. Chinese red rice: Anka. In: Handbook of Indigenous Fermented Foods. (K.H. Steinkraus, R.E. Cullen, C.S. Pederson, L.F. Nellis and B.K. Gavitt, eds.), 547–553. Marcel Dekker, New York.
19 Sweeny, J.G., M.C. Estrada-Valdes, G.A. Iacobucci, H. Sato and S. Sakamura. 1981. Photoprotection of the red pigments of *Monascus anka* in aqueous media by 1,4,6-trihydroxynaphthalene. J. Agric. Food Chem. 29: 1189–1193.
20 Tezuka, T. and M. Kashino. 1979. Heat-stable food-coloring agent. Japan Patent Kokai 79,086,669.
21 Toyo Jozo Co., Ltd. 1976a,b. Water-soluble *Monascus* pigment. Japan Patent Kokai 76,091,937 and 76,091,938.
22 Toyo Jozo Co., Ltd. 1978. Food coloring. Japan Patent 5,306,003.
23 Toyo Jozo Co., Ltd. 1980. *Monascus* pigment production. Japan Patent Kokai 80,102,660.
24 Whalley, W.B. 1963. The sclerotiorin group of fungal metabolites: their structure and biosynthesis. Pure & Appl. Chem. 7: 565–587.
25 Wong, H.C. 1982. Antibiotic and pigment production by *Monascus purpureus*. Ph. D. Thesis, University of Georgia, Athens.
26 Yamaguchi, Y., H. Ito, S. Watanabe, T. Yoshida and A. Komatsu. 1973. Water-soluble *Monascus* pigment. U.S. Patent 3,765,906.