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Extracellular Siderophores from *Aspergillus ochraceous*

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A large number of iron-chelating compounds (siderophores) were isolated from supernatants of iron-deficient cultures of a mold isolate, subsequently identified as *Aspergillus ochraceous*. Siderophores in their iron chelate form were purified to homogeneity by using Bio-Gel P2, silica gel, and C-18 bonded silica gel (reverse-phase) columns. Most of these compounds, as identified by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy and X-ray crystallography, belong to the ferrichrome family. The organism produces ferrirubin and ferrichrysin as the predominant and the second major compound (62 and 15% of the total siderophores), respectively. Ferrichrysin appears as the first siderophore in the medium on day 2 of growth. Several of the other siderophores are novel and ranged in quantities from 0.2 to 5% of the total. The trivial names asperchrome A, B1, B2, C, D1, D2, and D3 are proposed for these novel compounds, which are all members of the ferrichrome family, and all but the first one contain a common Orn_1-Orn_2-Orn_3-Ser_1-Ser_2-Gly cyclic hexapeptide ring with three dissimilar ornithyl δ-*N*-acyl groups. Another compound which appeared late in the growth period was similar to fusarinine C (fusigen). All of these compounds showed growth factor activity to various extents in bioassays with *Arthrobacter flavescens* Jg-9. None of these compounds showed antibacterial activity against *Escherichia coli* or *Bacillus megaterium*.

Iron as a nutritionally essential trace element functions catalytically in many metabolic processes in aerobic organisms. Although abundant in nature, it occurs in the extremely insoluble form of ferric hydroxide polymer under most biological conditions at neutral pH. Therefore, under such iron-limiting conditions ($\sim 10^{-7}$ M Fe) microorganisms excrete a variety of high-affinity, low-molecular-weight (500 to 1,500), iron-sequestering agents (formation constant, $\sim 10^{30}$) called siderophores which specifically solubilize ferric iron in an extracellular aqueous environment and make the metal available for active transport through the hydrophobic membrane (23). Siderophores with enormous structural diversity have been isolated from culture broths of various bacteria and fungi (ferrichromes, ferrioxamines, fusarinines, rhodotorulic acid, aerobactin, enterobactin, mycobactins, pseudobactins, etc.) and have also been shown to act as growth factors, antibiotics, antibiotic antagonists, tumor inhibitors, cell division growth factors, etc. (24). However, the majority of these naturally occurring siderophores, whether linear or cyclic, use either hydroxamate or catecholate functional groups to form very stable octahedral complexes with iron where the iron atom is d^5, high spin, and readily exchangeable (23).

The production pattern of iron chelates in fungi, as a typical and highly strain-specific phenomenon, has been reported to be a useful trait in fungal taxonomy (3, 29). Also, elaboration of ferrichrome-type siderophores by many strains of the genus *Aspergillus* has been observed by several workers (3, 8, 18, 29, 31). Ferrichrome-type compounds have a cyclohexapeptide ring containing three residues of δ-*N*-acyl-δ-*N*-hydroxyornithine and a tripeptide of neutral amino acids consisting of Tris-glycine (ferrichrome), produced by *Aspergillus niger* and *A. quadricinctus*; glycyl-seryl-glycine (ferricrocin), produced by *A. fumigatus*, *A. nidulans*, *A. humicola*, *A. versicolor*, and *A. viridi-nutans*; or seryl-seryl-glycine (ferrichrysin), produced by *A. melleus*, *A. terreus*, and *A. oryzae*, as a major component in their culture filtrates (18, 29, 31). However, several other minor compounds, such as ferrirubin, ferrirhodin, and fusarinine, and many unknown minor components, depending on the composition of the cultivation medium, have also been observed in culture broths of different aspergilli (5, 7, 17, 29). Characterization of many of the unknown minor components has often not been pursued due to the problems associated with poor yields (7, 9, 17). During a screening program of aerobic cultures conducted in this laboratory, it was observed that a laboratory mold contaminant, a strain of *Aspergillus ochraceous*, produces a large number of extracellular siderophores in an iron-deficient medium. We report here for the first time the isolation, purification, structures, and biological activities of these siderophores. Many of the minor components are novel and hitherto undescribed compounds.

MATERIALS AND METHODS

Cultures and maintenance. A laboratory mold isolate, identified as a strain of *A. ochraceous* on the basis of colony characteristics and morphological details of the spore-bearing structures on different media (26), was used in the present studies. The fungal strain designated gold was maintained on slants of chemically defined Grimm-Allen iron-limited medium (15) with the following composition: K_2SO_4, 1.0 g; K_2HPO_4, 3.0 g; NH_4CH_3COO, 3.0 g; citric acid, 1.0 g; thiamine, 2.0 mg; sucrose, 20.0 g; $CuSO_4 \cdot 5H_2O$, 0.005 mg; $ZnSO_4 \cdot 7H_2O$, 2.0 mg; $MnSO_4 \cdot H_2O$, 0.035 mg; $MgSO_4 \cdot 7H_2O$, 80 mg; and double glass-distilled water to 1 liter (pH 6.8).

The siderophore-requiring organism *Arthrobacter flavescens* Jg-9, obtained from the American Type Culture Collection, Rockville, Md. (ATCC 25091), was maintained on yeast extract-Casamino Acids-sucrose slants (14) supplemented with 100 μg of Desferal (CIBA Pharmaceutical Co., Summit,

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N.J.) per liter of medium. Wild-type cultures of *Bacillus megaterium* and *Escherichia coli* obtained from the culture collection of the Department of Botany and Microbiology, University of Oklahoma, Norman, were maintained on nutrient agar slants.

Production of siderophores. The organism was grown in 700-ml batches of Grimm-Allen minimal iron medium in 2.0-liter Erlenmeyer flasks at 24 ± 1°C for a specified period on a gyrotary shaker at 100 rpm. The medium was rendered iron deficient ($\sim 10^{-7}$ M) (4) by passing it through a column of Chelex-100 (Na^+; 100-200 mesh; Bio-Rad Laboratories) before the addition of divalent salts, although this step was later found to be unnecessary when high-purity chemicals and double glass-distilled water are used. A 48-h culture of the organism grown on the same medium was used as the inoculum. For studies on the production pattern and the relative percentage yields of the siderophores up to a period of 16 days, each flask (700 ml of medium) was inoculated with a constant inoculum of 10^9 conidiospores per 100 ml of medium. Siderophore production in culture supernatants was routinely followed by the addition of 0.5 ml of the supernatants to 4.5 ml of 2% (wt/vol) $FeCl_3 \cdot 6H_2O$ in 5 ml of HCl and measurement of the absorbance at 440 nm. Measurement of growth was performed by harvesting the mycelia by filtration through Whatman no. 4 paper and drying to constant weight at 80°C.

Extraction and purification. At the end of the incubation the mycelia were removed by filtration through Whatman no. 4 paper, and the culture filtrates were collected. Excess $FeCl_3 \cdot 6H_2O$ was added to the cooled filtrate to convert siderophores to iron chelates, and the filtrate was centrifuged at 1,500 × *g* for 20 min. The clear supernatant was then evaporated under vacuum at 40 ± 1°C to about 1/10 of the original volume. After evaporation, the concentrated solution was recentrifuged, and the chelates were extracted from the supernatant with phenol-chloroform (1:1 [wt/vol]) and back into water after the addition of 4 to 5 volumes of diethyl ether to the organic mixture. The aqueous extract was concentrated and initially purified by gel filtration through a column of Bio-Gel P2. Individual components of the extract were then separated and purified by the method shown in Fig. 1.

Analytical methods. Total siderophore was measured at regular intervals during a growth period of 16 days. Samples were harvested, extracted, and partially purified through Bio-Gel P2 as described above. The amount of total siderophore was then measured in this aqueous extract spectrophotometrically. The absorbance of a siderophore solution (4 ml) was measured at 440 nm against a blank, and the concentration was calculated from a standard curve prepared with aqueous ferrirubin solutions. The aqueous extract was then evaporated to dryness, dissolved in a small quantity of methanol, filtered, and chromatographed on precoated layers of silica gel 60 H using chloroform-methanol-water (35:12:2) and butanol-acetic acid-water (4:1:5), upper phase, as solvent systems to reveal the individual siderophores. Paper electrophoresis of the extract and the individual compounds was carried out at pH 2.0, 4.5, and 6.9 using a field strength of 1,000 V for 1 h. Molecular structures of ferric siderophores were determined by low-temperature (138 K) single-crystal X-ray diffractometry on an Enraf-Nonius CAD-4 diffractometer using MoKα radiation as described earlier (16).

Proton and ^{13}C nuclear magnetic resonance (NMR) spectroscopy of deferriated siderophores was performed on a Varian XL-300 instrument. Deferriation of iron complexes was accomplished by incubating an aqueous solution with excess 8-hydroxyquinoline at 45°C for 24 h and removing the unreacted reagent and its ferric complex by chloroform extraction or by the KCN sodium dithionite method (13) using 0.1 M potassium phosphate buffer (pH 7.0).

Biological activities. Siderophores (5 μg/ml) were examined for growth-promoting activity for the siderophore auxotroph *A. flavescens* Jg-9 by the method described by Estep et al. (14) using 7-mm (diameter) filter paper disks. Blank and ferrichrome-treated disks were used as negative and positive controls, respectively.

Susceptibility tests were performed against *E. coli* and *B.*

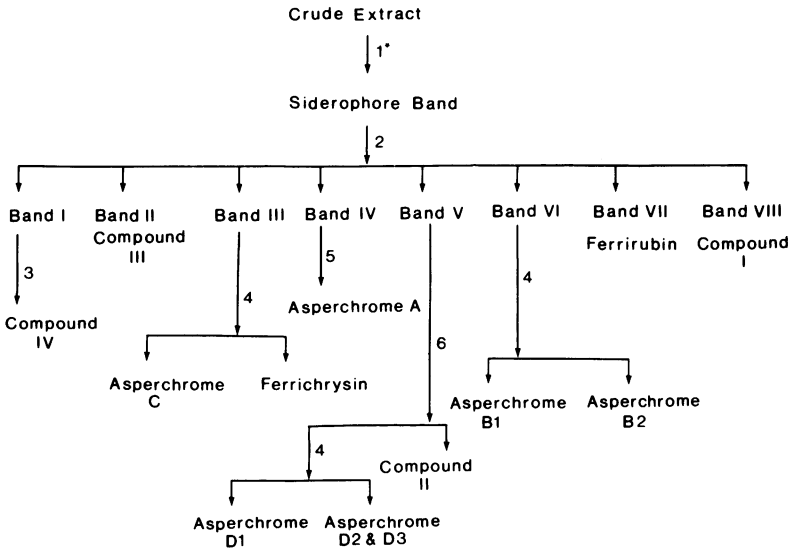

FIG. 1. Purification steps for various siderophores from *A. ochraceous*. Liquid chromatography systems: 1, Bio-Gel P2 (100-200 mesh), 2.5 × 36.2 cm, water; 2, silica gel 60 H (thin-layer chromatography grade), 3.5 × 65 cm and 2 × 90 cm, chloroform-methanol-water (35:12:2); 3, silica gel 60 H (thin-layer chromatography grade), 2 × 40 cm, hexane-chloroform-methanol (1:1:1); 4, octadecyl silica gel (reversed phase, 37 to 53 μm), 2.2 × 30 cm, methanol-water gradient; 5, silica gel 60 H (thin-layer chromatography grade), 2 × 40 cm, chloroform-methanol-water (28:20:2); 6, silica gel 60 H (thin-layer chromatography grade) 2 × 90 cm, chloroform-methanol (1:3).

megaterium (2×10^7 cells per ml in each case) by the agar diffusion technique using 1 mg/ml solutions of ferric siderophores. Plates were incubated at 37°C for 24 to 48 h before the results were recorded.

RESULTS

Isolation and identification. Siderophores were extracted from several batches of cultures at their maximal production stage, and the individual components were purified to homogeneity by the purification procedure outlined in Fig. 1. Ferrirubin and ferrichrysin (Fig. 2 and Table 1) were initially identified by cochromatography with authentic samples in silica gel layers developed with chloroform-methanol-water (35:12:2) and butanol-acetic acid-water (4:1:5; upper phase) (Table 2). Other solvent systems were also used (Fig. 1). Ferrirubin was then crystallized in two different crystal forms: (i) from water and (ii) by vapor diffusion of acetonitrile into an ethanolic solution. The X-ray structures of both of these forms were determined (C. L. Barnes, M. A. F. Jalal, R. Mocharla, M. B. Hossain, D. R. Powell, and D. van der Helm, Abstr. Winter Meet. Am. Cryst. Assoc., 1983, N6, p. 40). Ferrichrysin crystallized from a methanolic solution equilibrated with benzene and its unit cell parameters [a = 8.884(3), b = 15.224(9), c = 29.774(8) A°, volume 4,026.76 $A^{\circ 3}$, orthorhombic, space group $P2_12_12_1$] were close to the published results (21), which were, however, determined on crystals obtained from slow evaporation of an alcoholic solution. Compound I was cationic (all others were neutral), moved 5.4 cm/h toward the cathode when subjected to paper electrophoresis at pH 2.0, and was found to contain the same constituents as fusarinine C (fusigen), but not to be identical to this compound on the basis of proton and ^{13}C spectroscopy. It crystallized from ethanol equilibrated with ethyl acetate in thin needles, but the crystals were not sufficiently large for X-ray diffraction studies. Further work is in progress to confirm its identity.

A number of the minor siderophores were purified to homogeneity. The structures of most of these were determined using single-crystal X-ray diffraction studies and high-resolution NMR spectroscopy (X-ray and NMR data will be published). All of these were novel compounds (Fig. 2). Asperchrome A is similar to ferrirubin, but with the serine residue at position 5 of the hexapeptide ring replaced by an alanine. In asperchrome C, one of the ferrirubin-type acyl groups is esterified. Asperchromes B1 and B2 are isomers,

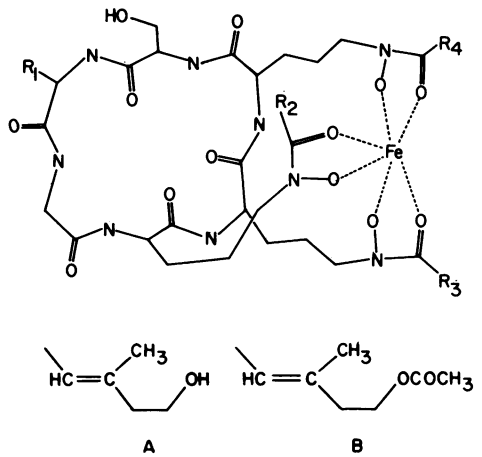

FIG. 2. Structures of siderophores isolated from *A. ochraceous* gold. Structural components are given in Table 1.

TABLE 1. Structural components of siderophores isolated from *A. ochraceous* gold

Siderophore	Group[a]			
	R_1	R_2	R_3	R_4
Ferrichrysin	CH_2OH	CH_3	CH_3	CH_3
Ferrirubin	CH_2OH	A	A	A
Asperchrome A	CH_3	A	A	A
Asperchrome B1	CH_2OH	CH_3	A	A
Asperchrome B2	CH_2OH	A	1A and	$1CH_3$
Asperchrome C	CH_2OH		2 A and 1 B	
Asperchrome D1	CH_2OH	A	CH_3	CH_3
Asperchrome D2	CH_2OH	CH_3	A	CH_3
Asperchrome D3	CH_2OH	CH_3	CH_3	A

[a] See Fig. 2.

with two ferrirubin-type and one ferrichrysin-type acyl group. Asperchromes D1, D2, and D3 are also isomers, but with two ferrichrysin-type and one ferrirubin-type acyl group.

Asperchromes A and B1 crystallized from water, and their X-ray structures have been determined (D. van der Helm, M. A. F. Jalal, R. Mocharla, M. B. Hossain, C. L. Barnes, and D. R. Powell, Abstr. Winter Meet. Am. Cryst. Assoc., 1983, PB12, p. 29).

Structures of asperchromes B2, C, D1, D2, and D3 were determined based on information obtained with ^1H and ^{13}C NMR spectroscopy and X-ray diffraction studies (M. A. F. Jalal, R. Mocharla, C. L. Barnes, M. B. Hossain, D. R. Powell, B. A. Benson, and D. van der Helm, Proc. 8th Am. Peptide Symp., 1983, in press, and abstracts 7 and 6, p. 182). It was extremely difficult to separate asperchromes D2 and D3 from each other by conventional as well as reverse-phase chromatography. These compounds were found to be isomers of asperchrome D1 on the basis that their ^1H and ^{13}C NMR was identical to that of asperchrome D1.

Compound IV crystallized from water, and its crystal system was monoclinic (space group $P2_1$) with the following unit cell parameters: a = 18.202(5), b = 13.729(11), c = 11.493(12) A°, β = 106.58(7), volume, 2,752.55 $A^{\circ 3}$. A complete molecular structure of compound IV could not be determined due to the small size of these crystals. The minor band III was found to be a mixture of at least three compounds, and their identification was not pursued further.

Production pattern. Growth, pH changes, and production of siderophores as a function of incubation period are shown in Fig. 3. Maximum total siderophore production (1.05 g/liter of medium) was observed after 9 to 10 days of incubation, when the organism reached the stationary phase. The concentration of total siderophore in the medium decreased considerably as the culture grew older.

Table 2 shows the percentage yields of the individual siderophores isolated from the cultures at the maximal production stage. Ferrirubin and ferrichrysin appeared to be the major (≈62% of total) and the second major (≈15%) components, respectively, compared with the minor compounds, which ranged from 0.2 to 5.0% of the total. The first siderophore to appear in the medium (on day 2 of incubation) was ferrichrysin, which remained as the major component until day 4 (Table 3). Ferrirubin, along with most of the asperchromes, appeared on day 4 and remained predominant throughout the incubation period starting from days 5 to 6. The last compounds to appear in the medium were asperchrome C (day 8) and compound I (day 10). Another unknown polar compound appeared on day 8 and disappeared on day 11. Some of the minor components complete-

TABLE 2. R_f values and relative yields of various siderophores produced by *A. ochraceous*

Siderophore	R_f value on silica gel 60 thin layers in solvent system[a]:		Yield	
	1	2	Mg/liter of medium at 10 days	% of total siderophores
Ferrirubin	0.25	0.22	557	61.8
Asperchrome B1	0.27	0.20	33	3.7
Asperchrome B2	0.27	0.17	45	5.0
Asperchrome D1	0.30	0.13	23	2.5
Asperchrome D2 and D3	0.30	0.16 and 0.15	36	4.0
Asperchrome A	0.32	0.23	2	0.2
Asperchrome C	0.35	0.25	29	3.2
Ferrichrysin	0.35	0.11	135	15.0
Unidentified compounds				
I	0.09	0.17	11	1.2
II	0.30	0.21	20	2.2
III	0.39	0.22	6	0.7
IV	0.46	0.28	5	0.5

[a] Solvent system 1, chloroform-methanol-water (35:12:2); solvent system 2, 1-butanol-acetic acid-water (4:1:5) (upper phase).

ly disappeared and others declined in concentration as the culture grew older.

Biological activity. None of the ferric siderophores showed any antimicrobial activity against *E. coli* or *B. megaterium*, even when the concentration of the test compound was increased to 100 μg per disk. However, all of them exhibited growth-promoting activity toward *A. flavescens* Jg-9 to various extents. At a concentration of 5 μg/ml their activity ranged from 42 to 96% when compared with ferrichrome as a control (100%) (Table 4).

DISCUSSION

A large number (more than 12) of extracellular siderophores were isolated from the iron-starved culture medium of *A. ochraceous* gold. All of the compounds identified thus far belong to the family of ferrichromes and show closely related structural features. The major and the second major compounds are ferrirubin and ferrichrysin, which have been reported earlier from other fungal sources (17, 18); the rest of the identified siderophores are novel compounds. All of these siderophores except asperchrome A have a common hexapeptide ring composed of three δ-*N*-acyl-δ-*N*-hydroxyornithines, two serines, and one glycine arranged in the same order, but they differ in the constitution of their ornithyl ε-*N*-acyl groups. Ferrichrysin and ferrirubin have three similar *N*-acyl groups derived from either acetic acid (former) or 3-methyl-5-hydroxy-pent-2-enoic acid (latter). A novel feature of asperchromes B1, B2, C, D1, D2, and D3 is the presence of dissimilar ornithyl acyl groups. In asperchromes B1 and B2, two of these groups are derived from 3-methyl-5-hydroxy-pent-2-enoic acid (as in ferrirubin), and the remaining one is derived from acetic acid (like ferrichrysin). The reverse situation is observed in asperchromes D1, D2, and D3, which contain two ferrichrysin-like and one ferrirubin-like acyl group. The isomers differ from one another on the basis of the position of their odd acyl group. X-ray diffraction studies revealed that the dissimilar acyl group is attached to ornithine-1 in asperchromes B1 and D1. The presence of all these compounds in the medium suggests that the fungus is capable of producing most, and probably all, of the possible hybrids of ferrirubin and ferrichrysin.

Dissimilarity between the acyl groups of a ferrichrome-type siderophore has not previously been observed. Every member of the ferrichrome family thus far reported in the literature contains three identical δ-*N*-acyl groups in its ornithine side chains, although the structure of the acylating acid may differ in different ferrichromes. The ferrichromes also vary in their peptide ring constitution. The presence of different acyl groups in the same siderophore raises some questions regarding the mechanism of biosynthesis of these compounds, the specificity of the enzyme system or systems involved, and the role of these variously modified siderophores in iron transport.

According to Emery (10), ornithines are first hydroxylated and subsequently acylated before their incorporation into the peptide ring of ferrichromes. The last step in the biosynthesis has been assumed to be carefully controlled so that only those ornithines which are similarly acylated are selected to take part in the formation of a specific type of

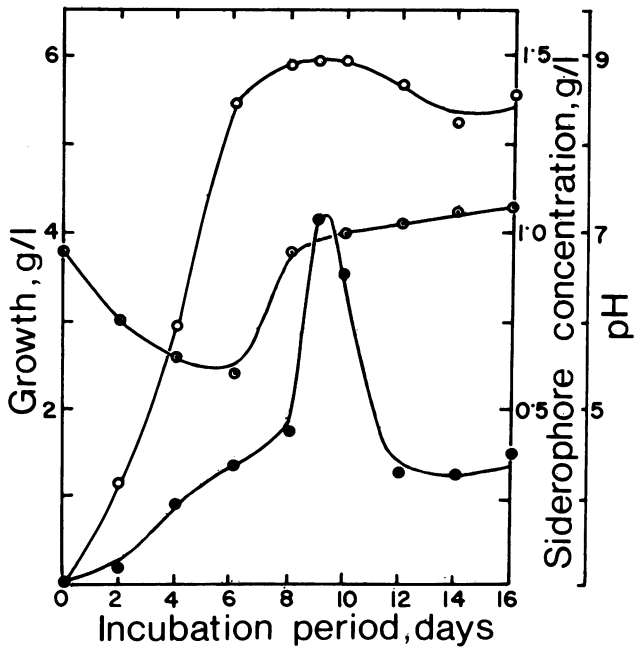

FIG. 3. Mycelial growth (open circles), pH change (dotted circles), and total siderophore production (closed circles) during an incubation period of 16 days in *A. ochraceous* gold.

TABLE 3. Production sequence of extracellular siderophores in *A. ochraceous*

Incubation period (days)	Intensity of the spots on thin-layer chromatography[a]											
	Ferrirubin	Asp B1	Asp B2	Asp D1	Asp D2 and D3	Asp A	Asp C	Ferrichrysin	Compound I	Compound II	Compound III	Compound IV
2	−	−	−	−	−	−	−	t	−	−	−	−
4	++	+	+	+	+	t	−	+++	−	+	−	−
6	++++	+	+	+	+	t	−	++	−	+	t	t
8	++++	+	+	+	+	t	−	++	−	+	t	t
10	++++	+	+	+	+	t	+	++	+	+	t	t
12	++++	+	+	+	+	+	+	++	+	+	t	t
14	++++	+	+	t	+	+	t	++	+	t	t	t
16	++++	+	+	−	+	+	t	++	+	t	−	−

[a] Spot intensity on the basis of visual comparison of chromatograms: ++++, intense; +++, moderately intense; ++, clearly visible; +, visible; t, faintly visible; −, absent. Asp, Asperchrome.

ferrichrome. However, the enzyme complex operating in this step in *A. ochraceous* must have nonspecific selectivity so as to allow for the random insertion of ornithines, acylated by different acids, into the three ornithyl positions in the same peptide ring. It is also possible, although unlikely, that each of these closely related compounds is produced by a specific enzyme complex. The production of asperchrome C, which has an acetylated *N*-acyl ornithine, may occur as in the other asperchromes, but it is not unlikely that this compound is produced by selective acetylation of ferrirubin, as it appears late in the growth period, when the ferrirubin concentration in the medium reaches its maximum value. Acetyl derivatives of other siderophores, such as those of fusarinine C (triacetyl fusarinine C) (20), coprogen B (coprogen) (6), and ferrioxamine B (ferrioxamine D1) (25) are known to occur naturally in microorganisms. However, asperchrome C is unique in being the only acetylated ferrichrome isolated so far and in having an *O*-acetyl group instead of the more common *N*-acetyl function.

Another compound isolated from this fungus, asperchrome A, is similar to ferrirubin in the constitution of the ornithyl *N*-acyl groups, but differs from the latter in having an alanine residue in place of the second seryl residue in the peptide ring. Three other alanine-containing ferrichromes, ferrichrome C, Saké colorant A, and malonichrome, have been isolated previously from *Cryptococcus melibiosum* (1), *A. oryzae* (28), and *Fusarium roseum* (11), respectively. The peptide backbone of asperchrome A is identical to that of Saké colorant A (in which all *N*-acyl groups are derived from acetic acid) in having a Ser-Ala-Gly sequence in the neutral tripeptide portion (19). In addition to this similarity in biosynthesis, the close biosystematic relationship of *A. ochraceous* to *A. oryzeae* is also exhibited by the production of (i) the same tripeptide backbone (Ser-Ser-Gly) in their major siderophores (ferrirubin in the former and ferrichrysin in the latter) and (ii) a large number of extracellular siderophores in both the species (more than 12 in the former and at least 5 in the latter) (27).

TABLE 4. Growth-promoting activity of *A. ochraceous* siderophores toward *A. flavescens* Jg-9[a]

Ferric siderophore or compound	Relative growth-promoting activity (%)[b]
Ferrichrome (standard)	100
Ferrichrysin	96 (3)
Asperchrome D2 and D3	89 (3)
Asperchrome D1	87 (5)
Asperchrome A	80 (4)
Asperchrome C	64 (3)
Asperchrome B1	64 (4)
Asperchrome B2	42 (3)
Ferriubin	42 (3)
Unidentified compound	
I	63 (2)
II	56 (3)
III	85 (3)
IV	53 (4)
Blank	0

[a] Based on the diameter of the growth zone.

[b] The numbers in parentheses are estimates of standard deviation for the last digit based on three determinations.

It is interesting that *A. ochraceous* produces so many neutral siderophores based on a common cyclic hexapeptide platform with many variations in the *N*-acyl side chains that surround the iron atom in the ferric complex, apparently to perform the single but vital function of capturing and transporting ferric iron in an extremely iron-deficient environment. Such versatility in biosynthetic capabilities is rare among microorganisms, and obviously it is tempting to speculate about the advantage to the organism in producing a large number of siderophores where just one would be sufficient to perform the function. When tested on a limited array of microorganisms (*B. megaterium* and *E. coli*), none of these compounds showed any antibiotic activity. In fact, some ferrichromes are known to work as antibiotic antagonists by reverting the inhibition caused by albomycin-type antibiotics (22). However, ferrirubin has been shown to inhibit coprogen transport by *Neurospora crassa* (30), and it is possible that others of these compounds actively inhibit the growth of competitors in a natural ecosystem. It is also likely that the iron complexes of some of these compounds are more efficiently transported by *A. ochraceous* than by other microorganisms, many of which (including bacteria, which are not producers of ferrichromes) have ferrichrome transport systems in their outer membranes, and that this constitutes an advantage to *A. ochraceous*. Kinetic studies on the specificity of chelate iron uptake in some species of *Aspergillus* have shown that ferrichrysin-producing strains favored iron uptake from ferrichrysin compared with other ferrichromes (29). Growth promotion studies with the siderophore-requiring bacteria *A. flavescens* Jg-9, which has been generally used as a model system due to its flexible iron transport system (2, 12, 14), revealed that these siderophores are not equally efficient in transporting iron to this organism. Although caution should be taken to correlate these results to the fungal systems (the transport assembly may be totally different), it reveals some interesting phenomena of ferri-

chrome transport in this microorganism. First, the efficiency of the siderophore seems to increase when the 3-methyl-5-hydroxy-2-pentenyl residues in the ornithines are gradually replaced by the acetyl residues (ferrichrysin > asperchromes D1, D2, and D3 > asperchrome B1 > ferrirubin). Efficiency seems to depend also on (i) the position of the odd acyl group when the compounds are isomeric (asperchrome B1 > asperchrome B2) and (ii) the hydrophobicity of the acyl groups (asperchromes > ferrirubin) and of the peptide ring (asperchrome A > ferrirubin). These results suggest that the efficiency of the tested ferrichromes to function as iron transport agents in this organism depends on minor structural variations and that the acyl part, as well as the peptide ring of the siderophore, is involved in hydrophobic interactions with the siderophore-binding protein and possibly with the outer lipid membrane in which the transport protein nests.

ACKNOWLEDGMENT

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