JOURNAL OF BACTERIOLOGY, Sept. 1970, p. 722-733
Vol. 103, No. 3

Rhodotorulic Acid from Species of *Leucosporidium*, *Rhodosporidium*, *Rhodotorula*, *Sporidiobolus*, and *Sporobolomyces*, and a New Alanine-Containing Ferrichrome from *Cryptococcus melibiosum*

C. L. ATKIN, J. B. NEILANDS, AND H. J. PHAFF

Department of Biochemistry, University of California, Berkeley, California 94720, and Department of Food Science and Technology and Department of Bacteriology, University of California, Davis, California 95616

Received for publication 16 June 1970

An examination of 142 strains within 19 genera of yeasts and yeastlike organisms for formation of hydroxamic acids in low-iron culture showed production of hydroxamates by two unclassified strains and by 52 strains among the genera *Aessosporon* (3 of 3 strains), *Cryptococcus* (1 of 43), *Leucosporidium* (3 of 11), *Rhodosporidium* (4 of 4), *Rhodotorula* (27 of 39), *Sporidiobolus* (2 of 2), and *Sporobolomyces* (12 of 13). Crystalline rhodotorulic acid was isolated in amounts sufficient to account for most or all of the measured hydroxamate in culture supernatants of 16 strains representative of the five last-mentioned hydroxamate-producing genera. A new alanine-containing ferrichrome was isolated from one strain of *Cryptococcus melibiosum*. Rhodotorulic acid was a major metabolic product of many of the positive strains when grown in low-iron media, and iron was shown to repress its synthesis and excretion into the culture medium. The taxonomic significance of production of hydroxamic acids is described in connection with the position of these yeast species in the subclass *Heterobasidiomycetidae*.

Numerous nonporphyrin iron-chelating natural products have been isolated from low-iron cultures of bacteria, actinomycetes, and fungi [16, 26-28; J. B. Neilands Microbial iron transport compounds. *In* G. Eichhorn (ed.), Inorganic Biochemistry, Elsevier Publishing Co., Amsterdam, the Netherlands, *in press*]. Most of these compounds contain three secondary hydroxamic acid groups; collectively, they are termed siderochromes. Those members which are antibiotics (i.e., albomycin) and those with growth-factor or antibiotic-antagonist activities (i.e., ferrichrome), or both, are called sideromycins and sideramines, respectively (3). Sideramines block the permeation of sideromycins [the antibiotic effects of which may involve several different mechanisms or sites of action (28)] into cells (39); however, more importantly, sideramines appear to be of widespread importance in microbial iron transport and metabolism.

We recently reported (1) the structure and biological activities of a new dihydroxamate sideramine, rhodotorulic acid (RA), obtained from low-iron cultures of a *Rhodotorula pilimanae* strain. RA is a diketopiperazine of δ-*N*-acetyl-L-δ-*N*-hydroxyornithine. In ferrichrome-like sideramines, L-δ-*N*-hydroxyornithine occurs (acylated on the δ-nitrogen with either acetyl or with various mevalonyl-related groups) as a tripeptide within a cyclic hexapeptide. In the present paper are reported several experiments on the physiology of induction of RA synthesis in *Rhodotorula*, an investigation of other representative yeast genera for production of hydroxamic acids and the discovery of a new alanine-containing sideramine of the ferrichrome type.

MATERIALS AND METHODS

Cultures were maintained on 4% malt extract agar (Difco) or on 5 to 10% Dry Diamalt 20 (Fleischmann) with 2% agar. Growth was generally much more vigorous on the latter medium. Most of the strains were obtained from the yeast collection of the Department of Food Science and Technology, University of California, Davis (UCD); some of these cultures originated from the Central Bureau voor Schimmelcultures (CBS), Delft, the Netherlands; from the Northern Regional Research Laboratory (NRRL), Peoria, Ill.; or from the Institute for Fermentation at Osaka (IFO), Japan. Other strains were obtained from the American Type Culture Collection (ATCC),

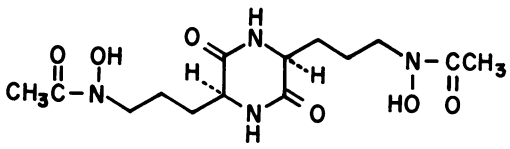

FIG. 1. *Structure of rhodotorulic acid.*

Rockville, Md., and from the Prairie Regional Laboratory (PRL), Saskatoon, Saskatchewan. *Sphacelotheca holci* and *Ustilago cynodontis* were obtained from C. I. Kado, Department of Plant Pathology, University of California, Davis. *U. sphaerogena* was from stocks maintained at Berkeley for production of ferrichromes; this strain is from the same source (P. J. Allen) as *U. sphaerogena* ATCC 12421. *R. pilimanae* UCD 67-64, the original source of RA, arose as a contaminant found to produce notable amounts of ferric-complexing material (1).

Low-iron media and cultural conditions. The low-iron sucrose medium of Garibaldi and Neilands (13) was used for preliminary experiments; however, to insure adequate growth of a broader range of species, several vitamins were supplied in addition to thiamine, and sucrose was replaced by other carbon sources. The ammonium acetate and sugar levels were increased to improve hydroxamate production (*see below*).

The following stock medium was prepared without carbohydrate and was stored at 3 C with $CHCl_3$ or toluene as preservative: 1 g of K_2SO_4, 3 g of $K_2HPO_4 \cdot 3H_2O$, 6 g of ammonium acetate, 1 g of citric acid, 0.8 g of $MgSO_4 \cdot 7H_2O$, 9 mg of $ZnSO_4 \cdot 7H_2O$, 1 ml of trace elements solution ["carbon-free trace elements" of Barnett and Ingram (2), minus iron, made up as a 1,000-fold concentrated solution], a 10-ml portion of a stock vitamin solution (containing per 10 ml: 0.1 mg of biotin, 2 mg of *myo*-inositol, 2 mg of thiamine chloride hydrochloride, and 0.5 to 1.0 mg each of *p*-aminobenzoic acid, calcium pantothenate, nicotinamide, and pyridoxine hydrochloride), plus distilled water and concentrated NH_4OH to 1 liter at *p*H 6.8 to 7.0. Three different media were made from this stock by addition of 30 to 40 g of either glucose, glycerol, or maltose per liter before autoclaving for 15 min at 121 C. These will be named, respectively, as low-iron glucose, low-iron glycerol, or low-iron maltose medium. Glucose medium in which the sugar was separately autoclaved generally gave both poorer growth and hydroxamate production. A fourth medium contained 10 g each of glucose, glycerol, and sodium succinate hexahydrate per liter, and 20 mg each of DL-histidine, DL-methionine, and DL-tryptophan per liter. It was necessary to use distilled water, reagent-grade carbohydrates, and other chemicals to minimize iron content. Acid-washing of the glassware was not necessary for good hydroxamate production.

For the various growth and physiological experiments with *R. pilimanae* and for production of hydroxamate from other strains in quantities sufficient for isolation, 10 to 200 ml of medium in Erlenmeyer flasks was inoculated with a 1% volume of a previous 2-day culture. The organisms were grown at 24 to 27 C or at 30 C on a gyrotory shaker at 500 cycle/min for 2 weeks or more. For the survey of yeasts, 5-ml cultures in tubes were grown 2 weeks or longer at various temperatures (*see below*) on inclined rotating racks (New Brunswick Scientific "Rollordrums"). Serial cultures were initially started from malt agar slants, whereas subsequent tubes were inoculated with two drops of the previous cultures.

For experiments on the effect of iron on hydroxamate production, portions of 316 μg of Fe/ml of solution (5.67 mM ferric ammonium sulfate-60 mM sodium citrate, stored in the dark) were added to low-iron medium before autoclaving.

Assay for hydroxamic acids. The standard assay consisted of mixing 0.5 ml of culture supernatant, or a suitable dilution thereof, with 2.5 ml 5 mM $Fe(ClO_4)_3$-0.1 M $HClO_4$ in a cuvette, followed by measurement of the maximum optical density (OD_{max}) versus a blank similarly prepared from sterile medium. The reported OD_{480} values are the experimental readings multiplied by the dilution factor. The ferric complex of RA shows the following spectral properties: λ_{max} = 480 nm, absorbancy linear to RA concentration up to $OD_{480} \geqq 1.5$, and an apparent molar extinction coefficient ϵ_{480} = 1,800 [calculated on the basis of iron-free RA with a molecular weight of 344 (1)]. Assays of spent culture supernatants lacking any hydroxamate still showed OD_{480} values of 0.01 to 0.05, and a stronger yellowish color than the blank;

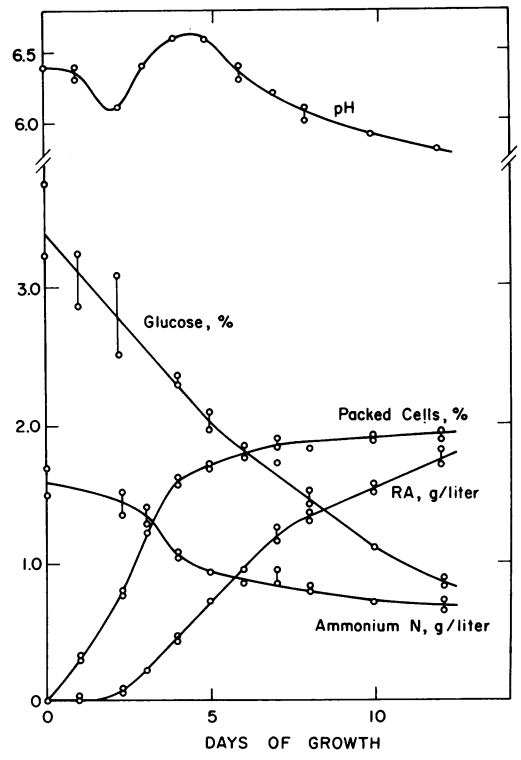

FIG. 2. *Time course of duplicate low-iron glucose cultures of R. pilimanae UCD 67-64 grown at 24 to 27 C.*

presumably this was due to some concentration by evaporation during growth and to the presence of various nonhydroxamate fermentation products. Because of this background absorption, the characteristic red color of ferric rhodotorulate was detected reliably only when the assay gave $OD_{480} \geqq$ ca. 0.07, although in some cases smaller amounts of hydroxamate were detectable either by eye or by difference spectra versus blanks.

Ferrichrome and ferrichrome A, for comparison in the assay, were extracted and crystallized by published procedures (13, 26) from supernatants of low-iron sucrose *U. sphaerogena* cultures.

Other assays. Packed cell volumes of culture portions were determined by centrifugation in Kimax 46815 sedimentation tubes for 10 min in a tabletop centrifuge. The supernatants from the time course experiment (Fig. 2) were quickly frozen and saved for glucose and ammonium assays. Samples of 100-fold dilutions of the collected supernatants were assayed for glucose with "Glucostat" (Worthington Biochemical Corp, Freehold, N.J.), and for ammonium ion by the quantitative ninhydrin assay of Spies (34). Controls showed that neither assay was affected by RA equivalent to several grams per liter of culture supernatant.

Isolation and identification of RA. Supernatants of 2-week low-iron cultures, plus several drops of *n*-hexanol to prevent frothing, were vacuum-evaporated at 40 C to one-tenth of the original volume. RA crystallized directly from many of these concentrates after they were cooled and allowed to stand for several days. Concentrates from the less productive strains (indicated by footnote *i* in Table 3) failed to precipitate RA, and were therefore extracted with $CHCl_3$-phenol (1:1, v/v). Aqueous solutions of the hydroxamates were again obtained by dilution of the organic phase with excess ether and extraction with water; after washing with ether and concentration, these aqueous solutions often precipitated a small amount of amorphous, grayish nonhydroxamate material which was removed by centrifugation before RA crystallized. One extract required special treatment. The final aqueous extract of the hydroxamate of *Sporidiobolus ruinenii* UCD 67-67 formed a gel; extraction of the gel with $CHCl_3$ resulted in removal of an ether-insoluble lipid and yielded an aqueous phase from which RA was crystallized as above. Crude RA was recrystallized as previously described (1) and characterized by infrared spectra in KBr discs, and, in the case of the product from *R. rubra* UCD 67-121, by melting-point determination and electrometric titration. The isolated products were assumed to contain the L-configuration of δ-*N*-hydroxyornithine.

RESULTS

Spectra of siderochromes in the hydroxamate assay. The present assay with acid and iron was used rather than former assays (13, 26) or other chemical or biological assays (*see below*) because of its simplicity, lower background color, and greater spectral differentiation between types of hydroxamates. Various siderochromes—including mono-, di-, and trihydroxamic acids—are known to show characteristic values for λ_{max} and ϵ_{max}, depending on which chelate types (ferric mono-, bis-, or tris-hydroxamate complexes) or combination of complexes form under different conditions (26).

The spectral properties of the complex formed

TABLE 1. *Effect of medium carbon and nitrogen levels on hydroxamate production by 2-week low-iron cultures*[a]

Strain	Medium[a]		Iron-free siderochromes[b]	Percentage of medium constituents incorporated into siderochromes	
	Sucrose	Ammonium acetate		C[c]	N
	g/liter	*g/liter*	*g/liter*	%	%
Rhodotorula pilimanae UCD 67-64	20	3	1.8	9.5	54
	20	6	2.8	13	42
	60	9	3.1	5.4	31
R. rubra UCD 67-121	20	3	1.8	9.5	54
	20	6	3.4	16	51
	60	9	3.8	6.6	38
Ustilago sphaerogena	20	3	ca. 1.4	ca. 7	ca. 30
	20	6	ca. 1.0		
	60	9	ca. 0.65		

[a] Low-iron sucrose medium of Garibaldi and Neilands (13), except with varying sugar and ammonium levels, and *p*H adjusted with NaOH rather than NH_4OH. Growth was at 30 C.

[b] Calculated from OD_{480} in the hydroxamate assay using $\epsilon = 1800$ for the two RA-producing yeasts; ϵ_{480} was assumed to be ca. 3,000 for the ferrichrome-producing smut (26).

[c] Calculation includes carbon from acetate and citrate.

by RA in the assay were previously given. In the Cary 14 recording spectrophotometer, ferrichrome-type siderochromes show flat-topped absorption curves under conditions of this assay, probably because of the presence of mixtures of mononuclear ferrichromes, binuclear complexes, and perhaps other chelated species (35); crystalline ferrichrome showed a spectral maximum over the range 475 to 490 nm, crystalline ferrichrome A from 480 to 495 nm, and crude supernatants of low-iron. *U. sphaerogena* cultures (containing ferrichrome and ferrichrome A in ca. 1:10 ratio plus small amounts of uncharacterized hydroxamates) also showed flat-topped curves. Other siderochromes such as aspergillic acids, coprogen, ferrioxamines, fusarinines, and schizokinen also generally exhibit characteristic spectra in this assay.

Low-iron cultures of two smuts, *U. cynodontis* and *S. holci*, showed spectra typical of ferrichromes in the assay with iron. These siderochromes have not been further characterized.

Development of low-iron media and growth of the yeasts. Additions to the low-iron sucrose medium of Garibaldi and Neilands (13) were prompted by the observation that *R. pilimanae* UCD 67-64 used a large fraction of the available nitrogen for synthesis of RA. Table 1 shows that higher sucrose and ammonium acetate levels considerably enhanced RA synthesis by *Rhodotorula*, but decreased synthesis of deferriferrichromes by *U. sphaerogena*. Intermediate concentrations of sugar and ammonium acetate were then chosen for further work with the yeasts.

A number of RA-producing strains excreted no hydroxamate when grown in yeast extract, glucose, or malt extract. These media contain significant concentrations of iron; however, *R. pilimanae* UCD 67-64 produced only barely detectable amounts of RA when grown in 4% malt extract (Difco) which had been extracted with 8-hydroxyquinoline and $CHCl_3$ to remove metals (36) and to which had been added magnesium, zinc, and trace elements (with the exception or iron). This may be partially due to the inability of *R. pilimanae* to assimilate the maltose in this medium. Considerable RA excretion occurred upon addition of ammonium acetate to this low-iron malt extract medium, and it approached the RA levels obtainable in low-iron glucose when ammonium acetate and glucose were added at 6 g/liter and 20 g/liter, respectively. It appears, therefore, that RA production in complex media may require both removal of iron and addition of acetate and extra glucose, and perhaps addition of ammonium ion (*see below*).

Growth and RA production of R. pilimanae UCD 67-64. The rates of growth and of RA production decreased after about 4 and 8 days, respectively; the reason for this decline is probably exhaustion of some essential nutrilite (e.g., iron; see below and Fig. 3), since ample residual levels of glucose and ammonia remained in the medium at this stage. At 21 days (*not shown*), the cultures had utilized nearly all of the glucose, the ammonium nitrogen had decreased to 0.56 g/liter, and RA had leveled off at about 3.9 g/liter. The amount of RA which appeared between 12 and 21 days contains ca. 2.4 times as much nitrogen as was taken up from the medium during the same period, so a considerable amount of RA or nitrogenous precursors, or both, must have been present intracellularly at 12 days. There was no evidence that the RA content of the culture medium declined upon ageing. The rate of RA production of these small shaken cultures was considerably lower than that of the strongly aerated and heavily inoculated cultures used for large-scale preparation of RA (1).

Effects of iron concentration. A series of cultures of *R. pilimanae* UCD 67-64 were made up with various additions of iron to the low-iron glucose medium, which routinely contains roughly 0.01 mg of Fe/liter (ca. 2×10^{-7} M Fe) as contamination from other ingredients. As little as 0.001 mg of Fe added per liter, however, caused earlier and faster RA production (compared to cultures without added iron); however, there was little effect upon total RA or cell mass at 4 weeks (*see* Fig. 3). RA production was sharply repressed when more than 0.01 mg of Fe was added per liter; also, with increasing iron concentration, the color of the cells changed from deep to light salmon and both cell yield and *p*H increased markedly. The cultures with >0.1 mg of additional Fe per liter showed a high proportion of broken, presumably autolyzed, cellular material. Lysis would explain the decreased packed cell volume indicated by the broken portion of the curve, and perhaps it also accounts for the inflection in the *p*H curve.

Production of hydroxamic acids by other yeasts. Although variable, hydroxamic acid production was increased in most strains by serial culture. Some strains would show no detectable hydroxamate until the second or third culture; occasionally a decrease in hydroxamate occurred. Table 2 shows typical results for serial cultures of several productive strains and for one nonproducer. Hydroxamate yields from productive strains were as a rule better from growth at room temperature (24 to 27 C) than at lower temperatures. For this reason, psychrophilic species were cultured close to their maximum growth temperatures (footnotes *c* and *j* in Table 3). All strains were grown

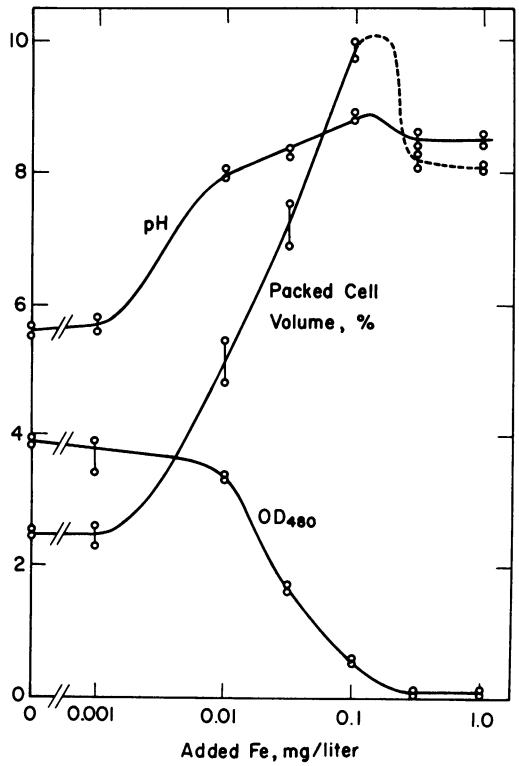

FIG. 3. *Effects of addition of iron to the low-iron glucose medium on pH, cell yield, and hydroxamate content (OD_{480} in hydroxamate assay) of duplicate cultures of R. pilimanae UCD 67-64 grown at 24 to 27 C for 4 weeks. Broken line indicates high proportion of lysed cells.*

in at least two serial cultures, and most were grown in three.

None of the hemiascosporogenous yeasts, or their asporogenous stages, which were grown for 2 weeks on the low-iron glucose medium at 24 to 27 C were found to form hydroxamic acid. The following species were examined: *Candida utilis* ATCC 9950; *Debaryomyces hansenii* UCD 67-120; *Fabospora fragilis* (syn. *Saccharomyces fragilis*) UCD C-351; *Hanseniaspora uvarum* UCD 67-107; *Hansenula ciferri* (haploid mating type) NRRL-Y-1031; *Kloeckera apiculata* (syn. *K. brevis*) UCD 55-45; *Metschnikowia pulcherrima* (perfect stage of *Candida pulcherrima*) strains PRL 2019, UCD 60-317, UCD 64-9 (NRRL-YB 2272, haploid mating type), UCD 67-1005, UCD 67-1012, UCD 67-1024, and UCD 67-1036; *M. zobellii* UCD 67-100; *Saccharomyces carlsbergensis* UCD 57-49; *S. cerevisiae* UCD C-229; *S. chevalieri* UCD 61-22; *S. diastaticus* UCD 57-5; *S. hienepiensis* UCD 68-8; *S. oleaginosus* UCD 66-1051; and *S. sake*, UCD 62-9.

Table 3 lists all of the strains of heterobasidiomycetous yeasts, or their asporogenous stages, which were tested. Hydroxamic acids were produced by all tested strains of the genera *Aessosporon*, *Rhodosporidium*, *Sporidiobolus*, and *Sporobolomyces* [except *S. roseus* (type) CBS 486], and by some strains of the *Leucosporidium* and *Rhodotorula* species (*see below*). Of the 17 RA isolations attempted from species of these genera, one was lost and the remaining 16 gave crystalline RA in amounts sufficient to account for most or all of the measured hydroxamate. Because of these confirmatory results, and since wavelengths scan in hydroxamate assays of a number of other cultures gave visible spectra with λ_{max} values typical of RA, most of the remaining assays consisted of single measurements of OD values at 480 nm, and no attempt was made to analyze for possible mixtures of hydroxamates. Neither *S. gracilis* (type) CBS 71 nor *S. singularis* (type) UCD 60-79 grew well enough in any of the low-iron media to warrant assignment as positive or negative.

Of the 43 tested strains of the genus *Cryptococcus*, only *C. melibiosum* (type) UCD 52-87 produced detectable hydroxamate. An aqueous solution from $CHCl_3$-phenol extraction failed to precipitate RA, and its visible spectrum upon addition of iron showed a flat-topped absorption curve like that of ferrichrome. Addition of iron and extraction with benzyl alcohol, etc., as for

TABLE 2. *Hydroxamate assays in typical 2-week low-iron glucose serial cultures*

Species and strain numbers	Temp	OD_{480}		
		Culture 1	Culture 2	Culture 3
	C			
Cryptococcus lactativorus (type) UCD 68-199	24–27	0.02	0.02	0.01
C. melibiosum (type) UCD 52-87	17–20	0.10	0.14	
	24–27	0.27	0.08	
Rhodotorula pilimanae (type) UCD 54-190	24–27	1.02	1.58	
R. rubra UCD 67-121	17–20	0.25	0.68	0.67
	24–27	0.48	1.07	
Sporobolomyces pararoseus CBS 2637 (in low-iron maltose medium)	24–27	0.06	0.11	0.28

ferrichrome (26) gave a new siderochrome, the infrared spectrum of which was almost identical to that of ferrichrome. However, an analysis of the amino acid composition revealed that one glycine was replaced by alanine. The complete characterization of this compound, which we propose to name ferrichrome C, will be the subject of a separate paper.

Hydroxamate was also produced by two as yet unclassified strains: a ballistosporogenous red yeast (UCD 68-624D) and a psychrophilic white yeast (UCD 69-4). These products have not been further characterized.

DISCUSSION

It was proposed in 1957 by Neilands (25) that ferrichromes are involved in iron transport in microbial metabolism. The presence of two hydroxamate groups in RA, its sideramine activity, and the repression of its biosynthesis by iron all suggest an iron transport role for this substance. Indeed, it appears that most or all aerobic microorganisms require or synthesize low-molecular-weight iron transport agents (J. B. Neilands, Microbial Iron Transport Compounds, *in press*).

The lower limit for detection of RA by the assay with iron corresponds to ca. 0.1 mM RA in the culture supernatant and, hence, various strains of yeast which are reported as negative may have produced small amounts of RA or other sideramines. This assay cannot distinguish between ferric hydroxamates and ferric complexes formed by such fungal products as frequentin and kojic acid. Other types of assays, although more tedious, are specific for hydroxylamine derivatives or are more sensitive. For example, assays of ferrichrome, ferrioxamine B, or RA by (i) oxidation with HIO_4 to form nitrosoalkane dimers (12), (ii) Czäky's procedure involving oxidation to nitrite and formation of a dye via diazonium coupling (8), and (iii) growth response of *Arthrobacter* JG-9 ATCC 25091 (4, 7) are more sensitive by factors of 3, 20, and 300,000 times, respectively. The biological assay is useless for inactive siderochromes (e.g., ferrichrome A), and many sideramines are much less potent than ferrichrome, ferrioxamine B, or RA.

The following discussion is in three parts: (i) the induction/repression and biosynthesis of RA and its similarities with other systems, (ii) the distribution of sideramines in different microorganisms, and (iii) the use of hydroxamate production as a taxonomic criterion.

Excretion of RA into the medium by *R. pilimanae*, like production of the principal groups of siderochromes, is repressed by added iron concentrations over 0.01 mg/liter (Fig. 3). Possibly the increased hydroxamate yields in serial low-iron cultures of the yeasts may have been due to dilution of utilizable iron carried over from the malt extract slants with the inocula. Like coprogen in *Neurospora crassa* (23) and ferrichromes in *U. sphaerogena* (18), small amounts of RA—probably as its ferric complex—accumulate intracellularly in *R. pilimanae* when the organism is grown in iron-containing media, although neither the complex nor the free acid appears extracellularly in readily detectable amounts. Furthermore, this intracellular accumulation, again resembling the above fungal systems, is increased by addition of cobalt salts to the medium (*unpublished data*). Cobalt toxicity in *Neurospora* has been shown to duplicate the effects of iron deficiency on the levels of certain enzymes in the heme biosynthetic pathway (15, 23), and there is evidence that this may also be true in *Ustilago* (18).

There appears to be no direct relationship between carotenoid level and ability to produce RA in low-iron media. Table 3 shows that the nonpigmented *Leucosporidium scottii* strains produced RA and that mutants of *R. glutinis* deficient in certain carotenoid pigments [strains UCD 68-41 and UCD 68-42, "white" and "yellow" mutants, respectively, of Maxwell, Macmillan, and Chichester (21)] produced as much hydroxamate as their wild-type parent strain UCD 48-23T. White and orange carotenoid-less mutants of *R. pilimanae* CBS 4479 behave similarly (H. Akers and J. B. Neilands, *unpublished data*). Furthermore, the hydroxamate-negative cryptococci (Table 3) include several red carotenoid-containing species (*Cryptococcus hungaricus*, *C. infirmo-miniatus*, *C. macerans*), and several species of *Rhodotorula* did not produce hydroxamate.

Recent experiments (H. Akers and J. B. Neilands, *unpublished data*) have shown that the increase in RA production caused by addition of ammonium acetate to low-iron sucrose medium (Table 1) is due mainly to the acetate rather than to the ammonium ion, and Fig. 2 indicates that ammonium ion is not a limiting nutrient in the low-iron glucose medium. In terms of the acetate originally in the medium, the ultimate yield of acetyl groups in RA molecules was $\leq$ 27% for the various entries in Table 1, and 29% for the experiment in Fig. 2. The early stages of biosynthesis of RA in *R. pilimanae* (H. Akers and J. B. Neilands, *unpublished data*) are identical to those of ferrichromes in *U. sphaerogena* (11), proceeding from L-ornithine to L-δ-*N*-hydroxyornithine, followed by acylation (acetylation in

TABLE 3. *Hydroxamic acid production by heterobasidiomycetous yeasts, or their asporogenous stages, in low-iron culture*[a]

Strain and synonym or other comment	UCD strain number	Original source or other number	OD_{490}[b]	Hydroxamic acid isolated
Aessosporon salmonicolor (type strain)	69–22	CBS 5937	0.36	
A. salmonicolor (haploid strain)	69–24	CBS 5937-2	0.26	
A. salmonicolor (diploid)	69–25	CBS 5937-3	0.13	
Bullera alba (type)	68–382	CBS 501	Nil	
Cryptococcus[c] *albidus* var. *aerius* (type)	68–172	CBS 155	Nil	
C. albididus var. *albidus* [type; also tested were strains UCD 67-128, 68-160 (CBS 1928), and 68–171 (CBS 4517)]	68–150	CBS 142	Nil	
C. albidus var. *diffluens* [type; UCD 68–175 (CBS 969) also tested]	68–174	CBS 160	Nil	
C. ater (type; UCD 67–76 also tested)	68–182	CBS 4685	Nil	
C. dimennae (type)	68–183		Nil	
C. flavus (type, formerly *Rhodotorula flava*)		CBS 331	Nil	
C. gastricus [type; UCD 68–186 (CBS 2288) also tested]	68–185	CBS 1927	Nil	
C. hungaricus [type, syn. *Dioszegia hungarica*; UCD 68–189 (CBS 4475) also tested]	68–187	CBS 4214	Nil[d,e,h]	
C. infirmo-miniatus [type, formerly *Rhodotorula infirmo-miniata*; also tested were strains UCD 67–104 and 68–190 (CBS 4694)]	68–191	CBS 323	Nil[d,e,h]	
C. kuetzingii [type; UCD 68–198 (CBS 2289) also tested]	68–196	CBS 1926	Nil	
C. lactativorus (type; UCD 68–200 also tested)	68–199	CBS 5771	Nil	
C. laurentii var. *flavescens* (type, syn. *Torula flavescens*)	68–210	CBS 942	Nil	
C. laurentii var. *flavescens* (formerly *Rhodotorula peneaus*)	48–23A	ATCC 13456	Nil[d,h]	
C. laurentii var. *flavescens* (formerly *Rhodotorula aurea*)	68–211	CBS 318	Nil	
C. laurentii var. *flavescens* (formerly *Rhodotorula nitens*)	68–212	CBS 4256	Nil	
C. laurentii var. *laurentii* [type; UCD 68–208 (CBS 4926) also tested]	68–201	CBS 139	Nil	
C. laurentii var. *magnus* [type; also tested was UCD 68–215 (CBS 569), syn. *Torula heveanensis*]	68–214	CBS 140	Nil	
C. luteolus [type; UCD 68–217 (CBS 4937) also tested]	68–216	CBS 943	Nil	
C. macerans (type, formerly *Rhodotorula macerans*; CBS 2425 also tested)	68–219	CBS 2206	Nil[d,e]	
C. melibiosum (type, formerly *Torulopsis melibiosum*)	52–87		0.41	Ferrichrome C
C. melibiosum	52–86		Nil	
C. neoformans [type; UCD 68–231 (CBS 883) also tested—this species causes cryptococcal meningitis]	68–222	CBS 132	Nil	
C. skinneri (type)	60–82		Nil	
C. terreus (type; UCD 62–3 also tested)	57–11	CBS 1895	Nil	
C. uniguttulatus [type, syn. *C. neoformans* var. *uniguttulatus*; UCD 68–244 (CBS 2770) also tested]	68–243	CBS 1730	Nil	
Itersonilia perplexans [type, Sowell strain—this genus is pathogenic for parsnips (33)]		ATCC 15495	Nil[d,e,f]	

TABLE 3—*Continued*

Strain and synonym or other comment	UCD strain number	Original source or other number	OD_{490}[b]	Hydroxamic acid isolated
Leucosporidium mesophiles				
L. capsuligenum (type)	68–248	CBS 4736	Nil	
L. capsuligenum (allotype)	68–249	CBS 4381	Nil	
L. capsuligenum	68–250		Nil	
L. scottii (paratype, self-sporulating)	69–19	CBS 5932	0.65	
L. scottii (allotype, mating type *a*)	69–20	CBS 5931	0.33	RA[i]
L. scottii (mating type α, J. Fell strain 24-269 α)	69–21		0.53	
Leucosporidium psychrophiles[j]				
L. frigidum (Stokes strain P-8)	69–2	CBS 5916	Nil	
L. gelidum (Pederson strain 899–5)	69–6		Nil	
L. nivalis (Stokes strain P-7)	69–1		Nil	
L. stokesii (type)	69–3	CBS 5917	Nil	
L. stokesii (Straka strain)	69–5		Nil	
Rhodosporidium sphaerocarpum (type)	AR-3		0.15	
R. toruloides (mating type *a*)	67–52	IFO 0880	0.08	
R. toruloides (mating type α)	67–53	IFO 0559	0.26	RA[i]
R. toruloides (diploid $a\alpha$)	67–55		0.09	
Rhodotorula aurantiaca[c] (type)	68–251	CBS 317	Nil[d,e,f]	
R. aurantiaca	62–348		0.17	
R. aurantiaca	62–351		0.18	
R. aurantiaca	68–252		0.19	
R. aurantiaca (syn. *Mycotorula colostri*)	68–253	CBS 2201	Nil[d,e]	
R. aurantiaca (syn. *R. crocea*)	68–254	CBS 2029	Nil[d,e]	
R. glutinis var. *glutinis* (type)	68–255	CBS 20	0.20	
R. glutinis var. *glutinis*	48–23T		0.12	
R. glutinis var. *glutinis*	67–102		1.1	RA
R. glutinis var. *glutinis* ["colorless" mutant from UCD 48–23T (21)]	68–41		0.17	
R. glutinis var. *glutinis* ["yellow" mutant of UCD 48–23T (21)]	68–42		0.15	
R. glutinis var. *glutinis* (various strains now considered synonymous and originally designated as follows)				
Torula suganii	68–257		2.2	
T. koishikawensis	68–258		0.06	
R. gracilis	68–259	CBS 14	0.08	
T. minuta var. *americana*	68–261		1.7	
T. saitoi	68–265		0.21	
Myotorula roseo-corrallina	68–268		0.18	
R. glutinis var. *rubescens*	68–270	CBS 2371	0.48	
T. miniata	68–272	CBS 2200	0.11	
R. glutinis var. *dairenensis* (type)	68–274	CBS 4406	>3[d] Nil[f] 2.4[g]	RA
R. graminis (type)	68–275	CBS 2826	1.1	RA
R. graminis	68–276	CBS 5016	0.13	
R. lactosa[c] (type, also has number IFO 1423)	67–62	CBS 5826	Nil	
R. lactosa	67–61	IFO 1424	Nil	
R. marina[c] (type)	48–23P	CBS 2365	Nil[h]	
R. minuta var. *minuta*[c] (type)	68–280	CBS 319	Nil[d,e]	
R. minuta var. *minuta*	68–281	CBS 2373	Nil[d,e]	
R. minuta var. *texensis*[c] (type, syn. *R. texensis*)	48–23U	CBS 2177	Nil[d,e]	
R. minuta var. *texensis*	68–290	CBS 4478	Nil[d,e]	
R. pallida[c] (type)	68–291	CBS 320	Nil[d,e]	

TABLE 3—*Continued*

Strain and synonym or other comment	UCD strain number	Original source or other number	OD_{480}[b]	Hydroxamic acid isolated
R. pallida	68–293	CBS 2623	Nil[h]	
R. pilimanae (type)	54–190	CBS 5804	1.0	
R. pilimanae	67–64		1.0	RA
R. pilimanae		CBS 4479	>3[d]	RA
			2.4[g]	
R. rubra (type)	68–275	CBS 17	1.2	
R. rubra	67–121		1.0	RA
R. rubra	68–299	CBS 330	0.96	
R. rubra [original designation *R. mucilaginosa* (type)]	68–312	CBS 316	1.9	RA
R. rubra (formerly *R. mucilaginosa*)	68–313	CBS 2377	2.4	
Sporidiobolus johnsonii (type)	68–29	CBS 5740	0.48	RA[i]
S. ruinenii (type)	67–67	CBS 5001	0.30	RA[i]
Sporobolomyces albo-rubescens (type)		CBS 482	1.4[d]	RA
			Nil[f]	
			2.4[g]	
S. hispanicus (type)		CBS 2873	0.57	RA
S. holsaticus (type)		CBS 1522	0.13	
S. holsaticus (syn. *S. coralli-formis*)		CBS 4209	0.14	
S. odorus (type)		CBS 2636	0.19	
S. odorus		CBS 483	0.29	
S. pararoseus (type)		CBS 484	0.60[d]	
			0.71[f]	RA
S. pararoseus		CBS 2637	0.11[d]	
			0.27[f]	
S. roseus (type)		CBS 486	Nil[d, f]	
S. roseus	67–112		0.35[d]	RA
			0.13[f]	
S. roseus		CBS 1015	0.47[d]	RA
			Nil[f]	
S. salmonicolor (type)		CBS 490	0.41	
S. salmonicolor		CBS 496	0.16	
Tilletiopsis lilacina Tubaki	67–5		Nil[d, h]	
Trichosporon cutaneum (strain from bay water)	67–114		Nil	

[a] Except as noted, all cultures were grown in low-iron glucose medium at 24 to 27 C for two weeks per serial culture.

[b] Maximum absorbancy observed in hydroxamate assays of serial cultures of each strain. See text for lower limits of assay. One OD_{480} unit corresponds to ca. 1 g of RA/liter.

[c] Because of psychrophilicity, serial cultures of most strains of these species were grown at both 17 to 20 C and 24 to 27 C. All of the *Cryptococcus* species also were grown for 4 weeks per serial culture to exclude possible late hydroxamate production.

[d] Low-iron glucose medium.

[e] Low-iron glycerol medium.

[f] Low-iron maltose medium.

[g] Low-iron sucrose medium of Garibaldi and Neilands (13).

[h] Low-iron glucose-glycerol-succinate-amino acids medium.

[i] $CHCl_3$-phenol extracted.

[j] Grown at 12 to 15 C.

the case of RA) to δ-*N*-acyl-L-δ-*N*-hydroxyornithine.

The ferrichromes or their close relative, the tripeptide coprogen, are produced by numerous members of the *Ascomycetes*, *Basidiomycetes*, and *Fungi Imperfecti*, including species of *Aspergillus*, *Penicillium*, *Neurospora*, the smut *Ustilago*, and probably the smut *Sphacelotheca*. The only

known exceptions to the rule of fungi as the exclusive sources of ferrichrome-type compounds are a few actinomycetes which produce the sideromycin, albomycin. The monomer fusarinine and its ester polymers—also based on δ-*N*-acyl-L-δ-*N*-hydroxyornithine—are made by certain species of *Aspergillus*, *Fusarium*, *Gibberella*, and *Penicillium* (9, 10, 30).

Ferrioxamines, including sideramines and sideromycins [ferrimycins, antibiotic A 22,765, and related compounds (28)], are derivatives of α-amino-ω-hydroxyamino butane or pentane and are produced by virtually all actinomycetes which have been thoroughly investigated (16) and by some bacteria (22). Products of other bacteria complete the list of known ω-hydroxyamino-derived hydroxamates: schizokinen from *Bacillus megaterium* (7) and probably other *Bacillus* species (6); aerobactin from *Aerobacter aerogenes* (14) and a large series of mycobactins from mycobacteria (32), both types of compounds containing ε-*N*-hydroxylysine, and ferribactin, from *Pseudomonas fluorescens* (20), a decapeptide with both D and L forms of δ-*N*-hydroxyornithine.

A smaller group of natural product hydroxamates may be considered as derivatives of α-hydroxyamino acids. At least some of these may be formed at the amide rather than at the amine level (19) and appear to be under different control than the above. Thus, the seven strains of *M. pulcherrima* produced visible amounts of pulcherrimin [a very insoluble ferric hydroxamate, also produced by the imperfect stage *C. pulcherrima* (17), and by *Fabospora ashbyi*, *F. dobzhanskii*, and *F. lactis*, but not by *F. fragilis* (37, 38)] when grown on malt agar, but failed to excrete the iron-free form, pulcherriminic acid, into low-iron medium. The function of these compounds within the producing organisms is unknown. Aspergillic acids, structurally related to pulcherriminic acid, are antibiotics for some organisms (19), but serve as growth factors for sideramine-auxotrophic *Arthrobacter* species (4). Pulcherrimin, on the other hand, does not show this sideramine activity (5).

Although the experiments with sideramine-auxotrophs suggest that many chelators might serve for iron transport, hydroxamic acids are more specific for iron, and it appears that the enzymes for ω-*N*-hydroxylation of lysine and ornithine have been retained over a wide evolutionary range, with preservation of certain acylases and ligases over shorter ranges. (It has yet to be demonstrated whether or not the amino-hydroxyaminoalkanes of the ferrioxamines arise, analogous to the biosyntheses of cadaverine and putrescine, by decarboxylation of ε-*N*-hydroxylysine and δ-*N*-hydroxyornithine.) The widespread occurrence of siderochromes and the increasing number of known structures have made possible their use as taxonomical criteria. Snow and White (32) have taken the first step in this direction by correlating nine groups of *Mycobacterium* species with nine different mycobactins. In the following we have analyzed hydroxamate production as a correlative feature in yeast systematics.

In this publication, the nomenclature of the species of *Rhodotorula* and *Cryptococcus* is based on the proposal by Phaff and Spencer (29) to include in *Cryptococcus* all species which assimilate *myo*-inositol as the sole carbon source and which do not produce pseudo- or true mycelium. The lack of RA production by *R. flava* (now *C. flavus*); *R. infirmo-miniata* (now *C. infirmo-miniatus*); *R. macerans* (now *C. macerans*); *R. aurea*, *R. nitens*, and *Rh. peneaus* (all three now *Cr. laurentii* var. *flavescens*) is in line with the general absence of hydroxamate in *Cryptococcus*. Although some species of *Cryptococcus* are probably derived from the heterobasidiomycetous genus *Tremella* in the order *Tremellales* (31), the lack of known sexual cycles makes it highly probable that the *Cryptococcus* species placed in that genus by H. J. Phaff and J. W. Fell [genus *Cryptococcus* Kützing emend. Phaff et Spencer. *In* J. Lodder (ed.), The yeasts—a taxonomic study, 2nd ed. Elsevier Publishing Co., Amsterdam, the Netherlands, *in press*] constitute a heterogeneous group. A species probably related to *Cryptococcus* is *Trichosporon cutaneum*. Its high percentage of guanosine plus cytosine (59 mole % GC) reported by Nakase and Komagata (24) is taken as evidence that this yeast belongs to the heterobasidiomycetes. It differs from *Cryptococcus* mainly by its mycelium-forming ability. Its lack of hydroxamate production, with one exception, is characteristic of other *Cryptococcus* species. The finding of a single species, *C. melibiosum* (type) UCD 52-87, producing ferrichrome C (instead of RA or no hydroxamate) supports the heterogeneity of *Cryptococcus*. This species, originally named *Torulopsis melibiosum*, was transferred to *Cryptococcus* because it has the above-named characteristics. It is interesting that the other known strain of *C. melibiosum*, UCD 52-86, which—apparently by mutation—had lost the ability to assimilate *myo*-inositol also failed to produce hydroxamate. Species studied of the genus *Rhodotorula* are those placed in that genus by Phaff and Ahearn (H. J. Phaff and D. G. Ahearn, *in press*). Both RA-positive and hydroxamate-negative strains were found. The RA-positive strains varied from those which

produce large amounts of RA to those which produce amounts barely detectable by the optical method with iron perchlorate. The many former species now considered synonymous with *R. glutinis* varied from strong to very weak. Other positive species were *R. graminis* (one strain weak), *R. pilimanae* (all good producers), and *R. rubra* (all good producers), and *R. rubra* (all good producers). Most strains of *R. glutinis* do not require an exogenous source of vitamins for maximum growth, but some are stimulated by thiamine. Many strains were tested for hydroxamate production because of ambiguity in *R. aurantiaca* strains (*see below*), but no correlation was found between level of RA production and thiamine requirement or the ability of *R. glutinis* strains to grow in vitamin-free medium. All strains tested of *R. lactosa*, *R. minuta* var. *minuta*, *R. minuta* var. *texensis*, and *R. pallida* were uniformly negative for hydroxamate production. These strains, unlike the *R. glutinis* strains, have the ability to assimilate 5-ketogluconate as sole carbon source, a property which they share with the species of *Cryptococcus*. *R. aurantiaca*, all strains of which require vitamins, is not a well-defined species and includes both 5-ketogluconate-positive strains (including the type culture, CBS 317) and strains unable to utilize this compound. It is interesting that the former were found to be hydroxamate-negative and the latter positive, but these hydroxamates have not yet been characterized. If these hydroxamates should prove to be RA, the positive strains of *R. aurantiaca* will probably be related to *R. glutinis* strains. Hence, at least for the strains discussed above, there appears to be a correlation between the lack of hydroxamate production and the ability to utilize 5-ketogluconate for growth. *R. marina* is an exception in that hydroxamate was not produced and 5-ketogluconate was not assimilated. However, this species, of which only one strain is available, is known to be very unstable physiologically and subject to mutation (Phaff and Ahearn, *in press*), which could explain the discrepancy.

Since both *Rhodosporidium* species recognized at the present time are sexual stages of various *R. glutinis* strains, RA production by these species is a logical consequence of this relationship. Rhodosporidium is thought to be a genus belonging to the *Ustilaginales*, probably in the family *Ustilaginaceae* (J. W. Fell, H. J. Phaff, and S. Y. Newell, *In* J. Lodder (ed.), *Genus Rhodosporidium banno, in press*).

All species of the genus *Sporobolomyces* (H. J. Phaff. Genus *Sporobolomyces* Kluyver et van Niel. *In* J. Lodder (ed.), *in press*) appeared to produce RA, with the exception of the type culture of *S. roseus* (CBS 486). Since this strain was originally isolated in 1925, it may have mutated in culture because other more recently isolated strains of this well-defined species produced readily detectable amounts of RA. None of the strains of this genus can utilize 5-ketogluconate or *myo*-inositol. The ballistospores discharged by species of *Sporobolomyces* are regarded as asexual conidia. Recently J. P. van der Walt (Genus *Aessosporon* n. gen., Antonie van Leeuwenhoek J. Microbiol. Serol., *in press*) discovered the formation of sexual teliospores in a single strain of *S. salmonicolor*, and established for it the new genus *Aessosporon* in the family *Tilletiaceae* of the order *Ustilaginales*. This strain, and a haploid and a diploid substrain, all produced hydroxamate, presumably RA.

Bullera alba, aside from its ability to produce symmetrical ballistospores, is very similar in its properties to *Cryptococcus laurentii*. Its lack of hydroxamate production agrees with the general absence of this compound in *Cryptococcus* species.

The genus *Sporidiobolus* [H. J. Phaff, Genus *Sporidiobolus* Nyland, *In* J. Lodder (ed.), *in press*], which is probably closely related to *Sporobolomyces*, contains two species, both of which produced RA. Another ballistosporogenous species, *Itersonilia perplexans*, did not produce detectable amounts of hydroxamate. This lack of hydroxamate and the readily detectable concentrations of RA in the two species of *Sporidiobolus* supports Phaff's (*in press*) rejection of the proposal by Sowell and Korf (33) to consider *Sporidiobolus* as a synonym of *Itersonilia*. A similar suggestion (33) to combine the genera *Itersonilia* and *Tilletiopsis* does find indirect support by the lack of hydroxamate formation in the latter genus. Another recently described genus of the *Ustilaginales*, *Leucosporidium* (J. W. Fell, A. Statzell, I. L. Hunter, and H. J. Phaff, Antonie van Leeuwenhoek J. Microbiol. Serol., *in press*) contains one RA-positive species (*L. scottii*, the type formerly *C. scottii*) and several hydroxamate-negative species, most of which are psychrophilic. The species of this genus probably do not constitute a homogeneous group, which is supported by the RA data.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-04156 from the National Institute of Allergy and Infectious Diseases and GM-16370 from the National Institute of General Medical Sciences, and National Science Foundation Grant GB-5276. C. L. Atkin was a National Science Foundation Graduate Fellow.

We thank the following for gifts of compounds or cultures: B. Guirard, C. W. Hesseltine (NRRL), C. I. Kado, M. Llinás, J. C. MacDonald (PRL), and J. R. Pollack. We especially thank

Ellen B. Johnson and H. Akers for much assistance and information.

LITERATURE CITED

1. Atkin, C. L., and J. B. Neilands. 1968. Rhodotorulic acid, a diketopiperazine dihydroxamic acid with growth factor activity. I. Isolation and characterization. Biochemistry 7: 3734–3739.
2. Barnett, J. A., and M. Ingram. 1955. Technique in the study of yeast assimilation reactions. J. Appl. Bacteriol. 18:131–148.
3. Bickel, H., E. Güamann, W. Keller-Schierlein, V. Prelog, E. Vischer, A. Wettstein, and H. Zähner. 1960. Iron-containing growth factors, the sideramines, and their antagonists, the iron-containing antibiotics, sideromycins. Experentia 16: 129–133. Translated from the German.
4. Burnham, B. F., and J. B. Neilands. 1961. Studies on the metabolic function of the ferrichrome compounds. J. Biol. Chem. 236:554–559.
5. Burton, M. O., F. J. Sowden, and A. G. Lochhead. 1954. Studies on the isolation and nature of the "terregens factor." Can. J. Biochem. Physiol. 32:400–406.
6. Byers, B. R., and C. E. Lankford. 1968. Regulation of synthesis of 2,3-dihydroxybenzoic acid in *Bacillus subtilis* by iron and a biological secondary hydroxamate. Biochim. Biophys. Acta 165:563–566.
7. Byers, B. R., M. V. Powell, and C. E. Lankford. 1967. Iron-chelating hydroxamic acid (schizokinen) active in initiation of cell division in *Bacillus megaterium*. J. Bacteriol. 93:286–294.
8. Czäky, T. Z. 1948. On the estimation of bound hydroxylamine in biological materials. Acta Chem. Scand. 2:450–454.
9. Diekmann, H. 1967. Fusigen, a new sideramine from fungi. Arch. Mikrobiol. 58:1–5. Translated from the German.
10. Diekmann, H., and H. Zähner. 1967. Constitution of fusigen and its degradation to Δ^2-anhydromevalonic acid lactone. Eur. J. Biochem. 3:213–218. Translated from the German.
11. Emery, T. 1966. Initial steps in the biosynthesis of ferrichrome. Biochemistry 5:3694–3701.
12. Emery, T., and J. B. Neilands. 1962. Further observations concerning the periodic acid oxidation of hydroxylamine derivatives. J. Org. Chem. 27:1075–1076.
13. Garibaldi, J. A., and J. B. Neilands. 1955. Isolation and properties of ferrichrome A. J. Amer. Chem. Soc. 77:2429–2430.
14. Gibson, F., and D. I. Magrath. 1969. The isolation and characterization of a hydroxamic acid (aerobactin) formed by *Aerobacter aerogenes* 62-1. Biochim. Biophys. Acta 192: 175–184.
15. Healy, W. B., S. Cheng, and W. D. McElroy. 1955. Metal toxicity and iron deficiency effects on enzymes in *Neurospora*. Arch. Biochem. Biophys. 54:206–214.
16. Keller-Schierlein, W., V. Prelog, and H. Zähner. 1964. Siderochrome. Fortsch. Chem. Org. Natur. 22:279–322.
17. Kluyver, A. J., J. P. van der Walt, and A. J. Triet. 1953. Pulcherrimin, the pigment of *Candida pulcherrima*. Proc. Nat. Acad. Sci. U.S.A. 39:583–593.
18. Komai, H., and J. B. Neilands. 1966. Zinc and cobalt: effect on the iron metabolism of *Ustilago sphaerogena*. Science (Washington) 153:751–752.
19. MacDonald, J. C. 1967. Aspergillic acid and related compounds, p. 43–51. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics, vol. 2. Springer Verlag, Berlin.
20. Maurer, B., A. Müller, W. Keller-Schierlein, and H. Zähner. 1968. Ferribactin, a siderochrome from *Pseudomonas fluorescens* Migula. Arch. Mikrobiol. 60:326–339. Translated from the German.
21. Maxwell, W. A., J. D. Macmillan, and C. O. Chichester. 1966. Function of carotenoids in protection of *Rhodotorula glutinis* against irradiation from a gas laser. Photochem. Photobiol. 5:567–577.
22. Müller, A., and H. Zähner. 1968. Ferrioxamine from *Eubacteriales*. Arch. Mikrobiol. 62:257–263. Translated from the German.
23. Mutha Krishan, S., G. Padmanaban, and P. S. Sarma. 1969. Regulation of heme biosynthesis in *Neurospora crassa*. J. Biol. Chem. 244:4241–4246.
24. Nakase, T., and K. Komagata. 1968. Taxonomic significance of base composition of yeast DNA. J. Gen. Appl. Microbiol. 14:345–357.
25. Neilands, J. B. 1957. Some aspects of microbial iron metabolism. Bacteriol. Rev. 21:101–111.
26. Neilands, J. B. 1966. Naturally-occurring non-porphyrin iron compounds. Struct. Bonding (Berlin) 1:59–108.
27. Neilands, J. B. 1957. Hydroxamic acids in nature. Science (Washington) 156:1443–1447.
28. Nüesch, J., and F. Knüsel. 1967. Sideromycins, p. 499–541. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics, vol. 1, Springer Verlag, Berlin.
29. Phaff, H., and J. F. T. Spencer. 1969. Improved parameters in the separation of species in the genera *Rhodotorula* and *Cryptococcus*, p. 59–65. *In* A. Kocková-Kratochvilová (ed.), Proc. 2nd Symp. on Yeasts, Bratislava.
30. Sayer, J. M., and T. F. Emery. 1968. Structures of the naturally-occurring hydroxamic acids, fusarinines A and B. Biochemistry 7:184–189.
31. Slodki, M. E., L. J. Wickerham, and R. J. Bandoni. 1966. Extracellular polysaccharides from *Cryptococcus* and *Tremella:* a possible taxonomic relationship. Can. J. Microbiol. 12:489–494.
32. Snow, G. A., and A. J. White. 1969. Chemical and biological properties of mycobactins isolated from various mycobacteria. Biochem. J. 115:1031–1045.
33. Sowell, G., Jr., and R. P. Korf. 1960. An emendation of the genus *Itersonilia* based on a study of morphology and pathogenicity. Mycologia 52:934–945.
34. Spies, J. R. 1957. Colorimetric procedures for amino acids, 467–477. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press, New York.
35. Turková, J., O. Mikeš, J. Schraml, O. Knessl, and F. Sörm. 1965. Complex of albomycin and ferrichrome with a second iron atom. Fed. Proc. Transl. Suppl. 24:T725–T730.
36. Waring, W. S., and C. H. Werkman. 1942. Growth of bacteria in an iron-free medium. Arch. Biochem. 1:303–310.
37. Wickerham, L. J., and K. A. Burton. 1956. Hybridization studies involving *Saccharomyces lactis* and *Zygosaccharomyces ashbyi*. J. Bacteriol. 71:290–295.
38. Wickerham, L. J., and K. A. Burton. 1956. Hybridization studies involving *Saccharomyces fragilis* and *Zygosaccharomyces dobzhanskii*. J. Bacteriol. 71:296–302.
39. Zimmerman, W., and F. Knüsel. 1969. Permeability of *Staphylococcus aureus* to sideromycin antibiotic 22765. Arch. Mikrobiol. 68:107–112.