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The catabolism and heterotrophic nitrification of the siderophore deferrioxamine B

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Summary. Three bacteria, two of which were previously noted as active heterotrophic nitrifiers, were examined for their ability to grow and nitrify with the siderophore deferrioxamine B as the carbon source. *Pseudomonas aureofaciens* displayed limited growth and nitrification while a heterotrophic nitrifying *Alcaligenes* sp. was without action concerning its metabolism of deferrioxamine B. The third bacterium, a unique Gram-negative soil isolate, was unable to nitrify deferrioxamine B but grew well when the siderophore was employed as the sole C source. The Gram-negative bacterium removed deferrioxamine B from the medium and left only residual amounts of the compound in solution at the termination of its growth. The organism was without action when the ferrated analogue of deferrioxamine B, ferrioxamine B, sereved as either the C source for growth, for metabolism by resting cells, or as the substrate for cell-free extracts. Deferrioxamine B, by contrast, was rapidly metabolized by resting cells. Cell-free extracts of the bacterium synthesized a monohydroxamate(s) when deferrioxamine B was the substrate. The catabolism of deferrioxamine B, which is synthesized by soil microbes, suggests that soil microflora have the ability to return deferrioxamine B, and perhaps other, siderophores to the C cycle.

Key words: Deferrioxamine B - Catabolism - Heterotrophic nitrification

Introduction

Iron is an essential nutrient for most organisms. The element is of importance because of its role in intermediary metabolism (Emery 1982; Neilands 1988), nucleic acid (Fontecave et al. 1987) and chlorophyll biosynthesis (Emery 1982). The majority of aerobic and facultatively anaerobic bacteria and fungi examined acquire the element when it is present at low concentrations by synthesizing and excreting the avid ferric-ion-chelating compounds knwon as siderophores. The acquisition of ferric ion from aerobic environments is greatly enhanced by siderophores due to their extremely efficient binding, as exemplified by their formation constants which range over approximately 10^{28}-10^{52} (Neilands 1981; Raymond and Carrano 1979). Once the ferric ion is bound by the siderophore, microbes utilize specific energy-requiring transport systems to assimilate the ferrisiderophores (Emery 1982; Lodge et al. 1982; Neilands 1981, 1988; Straka and Emery 1979).

Siderophores are crucial to iron acquisition by a number of microbes in aerobic environments. The compounds are thus thought to be virulence factors for certain microbial pathogens (Enard et al. 1988 Neilands 1988; Sokol 1986) and to play a role in ferric ion assimilation in a variety of soils. All of 67 different soils from across the United States contained measurable concentrations of siderophores (Powell et al. 1980). In addition, the siderophore schizokinen was recovered and identified from a rice paddy soil (Akers 1983) and wood-decaying basidiomycetous fungi were recently shown to produce iron-chelating molecules which are probably siderophores (Fekete et al. 1989).

The ferric ion of ferrisiderophores is available not only to microbes: plants have the ability to incorporate the ferric ion of siderophores into their biomass (Becker et al. 1985; Reid et al. 1984) and at least one plant enzyme, NADH:nitrate reductase, can function as a ferrisiderophore reductase (Castignetti and Smarrelli 1986; Smarrelli and Castignetti 1986). While perhaps not the only mechanism, siderophores of a plant symbiont aid in the reduction of plant pathogenesis by sequestering the available ferric ion and thus denying plant pathogens sufficient iron to establish themselves in the rhizosphere and cause disease (Kloepper et al. 1980; Simeoni et al. 1987).

Although a number of studies have addressed siderophore synthesis, transport and Fe^{3+} acquisition

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Abbreviations. DFB, deferrioxamine B; FB, ferrioxamine B; $PhMeSO_2F$, phenylmethylsulfonyl fluoride

(Emery 1982; Neilands 1981), little is known with respect to the recycling of siderophore carbon and nitrogen. Warren and Neilands (1964, 1965) demonstrated that a soil pseudomonad was capable of growth when the siderophores ferrichrome and ferrichrome A served as sole sources of C and N and limited growth was observed when the siderophore coprogen was the C and N source. These authors suggested that siderophore catabolism was initiated by a peptidase and that the ferrisiderophore functioned as the preferred substrate. Confirmation of this hypothesis came when Villavicencio and Neilands (1965) isolated an alkaline peptidase from the bacterium which cleaved the ferrichrome A ring at an acyl-serine bond and yielded a linear hexapeptide.

The current study examines the capacity of another pseudomonad, *Pseudomonas aureofaciens,* to use the siderophore DFB as a sole C source. *P. aureofaciens* was noted also to nitrify some of the DFB. As neither *P. aureofaciens* nor another active heterotrophic nitrifier (an *Alcaligenes* sp.) demonstrated substantial metabolism of DFB, enrichments based on the ability of the microflora to use DFB as a sole C source, were initiated. The isolation of a Gram-negative soil bacterium active in the dissimilation of DFB as a sole C source (trivially given the designation DFBC 5, i.e. deferrioxamine B catabolizer no. 5) was accomplished and the metabolism of the compound by the microbe was characterized.

Materials and methods

Bacteria and growth conditions

Pseudomonas aureofaciens (American Type Culture Collection 13985) and an *Alcaligenes* sp. originally isolated from soil (Castignetti and Gunner 1980) are active heterotrophic nitrifiers/denitrifiers and have been characterized previously (Castignetti and Gunner 1980; Castignetti and Hollocher 1981, 1984). DFBC 5 was obtained from a local garden soil (Glenview, Illinois). The enrichment, isolation and growth procedures used to obtain the bacterium were essentially those of Castignetti and Gunner (1980) except as follows. Growth of the organism was in the mineral salts medium described by Castignetti and Gunner (1980) which was supplemented with 0.1% NH_4Cl, 5.0 ml vitamin solution (Wolin et al. 1963), 0.05% $Mg(SO_4) \cdot 7\ H_2O$, and 0.1–0.3% (1.5–4.5 mM) DFB (the sole C source) for 1 l mineral salts medium. These nutrients were filter (0.2 μm) sterilized and aseptically added to the sterile mineral salts medium. After the initial characterization of DFBC 5, it was determined that the microbe grew satisfactorily in this medium when the vitamins were omitted. As our primary interest was the examination of DFB as the sole C source for the bacterium, growth experiments were thus conducted in some cases without added vitamins. For purposes of obtaining isolated colonies, the above DFB mineral salts medium (DFB medium) was solidified by the addition of 1.8% agar. The growth of DFBC 5 on agar plates was best when the mineral salts medium components were dissolved at twice normal concentration, sterilized and then added to double-strength sterile agar and the filter-sterilized NH_4Cl, $MgSO_4 \cdot 7\ H_2O$ and DFB.

The bacterium was grown, as were *P. aureofaciens* and the *Alcaligenes* sp., with vigorous aeration, i.e. 280 min of 100 ml culture medium in a 500-ml conical flask, at 30° C. Growth was measured by correlating absorbance at 660 nm to either cell protein concentration or to dry cell mass concentration. All growth experiments and cultures prepared for either resting cell or cell-free extract studies were grown in the above medium. A primary inoculum was grown in the medium from cultures maintained on agar-solidified plates, i.e. DFB NH_4^+ mineral salts plates in the case of DFBC 5 and DFB 0.05% yeast extract mineral salts plates in the case of the *P. aureofaciens* and the *Alcaligenes* sp. This inoculum was then used to initiate the growth of cells used in either the growth, resting cell or cells prepared for cell-free extracts experiments. Studies where the DFB mineral salts medium was supplemented with yeast extract (0.05%) were performed with the *P. aureofaciens* and the *Alcaligenes* sp. and are noted as such. Growth experiments were conducted on at least two separate occasions and contained three replicate cultures of each bacterium or uninoculated control. The data presented represent the means of the triplicate values obtained for each parameter monitored.

Resting cell and cell-free extract studies were performed on the pooled cells of DFBC 5 harvested during mid-logarithmic growth in the DFB NH_4^+/mineral salts medium and were repeated on at least two additional occasions. Representative results of these experiments are presented.

Morphological or biochemical tests were performed as described by either Stanier et al. (1966), Palleroni (1984), the Manual of Methods for General Bacteriology (Gerhardt 1981), Lelliot et al. (1966), the Difco Manual (9th and 10th editions, 1972 and 1984), API oxidase test kit (API Corp., New York), Microbiological Applications (Benson 1979), Laboratory Text in Microbiology for the Health Sciences Student (Norrell 1985), Laboratory Exercises in Microbiology (Pelczar and Chan 1972), or with the various buffers (pH 3.6–8.5) supplemented with 0.3% each of yeast extract (Difco) and glucose (Gomori 1955).

DFBC 5 displayed the following characteristics. The bacterium is a motile, singularly flagellated rod (1.0×2–3 μm, uranyl acetate transmission electron microscopy as described by Gerhardt 1981), an obligate aerobe (Hugh-Leifson method as described in the Manual of Methods for General Bacteriology), catalase, oxidase, β-lactamase and lipase positive, grew in yeast extract glucose media at pH 6.0–8.5, demonstrated a very limited dull green fluorescence when grown on either King B medium or the medium of Luisetti et al. (as described by Palleroni 1984), arginine dihydrolase, phenazine pigments, gelatinase, starch hydrolysis, acid-fast and capsule-negative, unable to produce levan from sucrose, to grow at 4° or 41° C or on *Pseudomonas* isolation agar (Difco), or to reduce nitrate to nitrate or to atmospheric nitrogen (denitrification negative), did not use either 2-ketogluconate or trehalose as C sources but good growth of the bacterium was noted when glucose was the carbon source. Fatty acid analysis performed by two different firms (Microbial ID, Inc., Newark, DE and Microcheck, Inc., Northfield, VT) indicated that DFBC 5 is novel and unlike anything previously catalogued by the firms, which included classification of a number of soil bacteria such as *Alcaligenes, Pseudomonas* and *Xanthomonas.* The bacterium has been deposited with the American Type Culture Collection and assigned the number ATCC 49538.

Chemicals

All chemicals used were reagent grade or better. DFB (Desferal) was generously supplied by the Ciba-Geigy Corporation (Summit, NJ), Chelex 100 resin was from Bio-Rad (Richmond, CA), DL-alanine-β-napthylamide·HCl was from Aldrich (Milwaukee, WI), hide powder azure was from Sigma (St. Louis, MO) and azocoll was purchased from and used as described by the manufacturer (Calbiochem-Behring, La Jolla, CA). Proteolytic enzymes were from Sigma and were microbial in origin. They were type XIV (pronase E, from *Streptomyces griseus,* EC, 3.4.21.4), type XXVII (Nagarse, a non-specific protease from *Bacillus* species, EC, 3.4.21.14), and type XXIII (from *Aspergillus oryzae,* EC, 3.4.24.4). The peptidase inhibitors used were leupeptin, $PhMeSO_2F$, pepstatin A (Sigma) and Na_2EDTA (Fisher). They were prepared and used as described by the protease inhibitors technical bulletin of Boehringer Mannheim Biochemicals (Indianapolis).

Extracellular enzymes, resting cells and cell-free extracts

Resting cells were prepared and experiments conducted as described (Castignetti et al. 1985) with the exception that either DFB or ferrioxamine B (FB), made by ferrating DFB with 95 mol% of $FeCl_3 \cdot 6H_2O$, 10 mM HCL, served as the substrate. Extracellular enzymes were examined by using the culture medium of the organism from which mid-logarithmic cells had been removed by centrifugation. Cell-free extracts were prepared either by grinding with alumina (type 305 from Sigma; Castignetti et al. 1983) or by using sonication (Sonic 300 Dismembrator, Artex Systems Corp., Farmingdale, NY). The latter was done by placing an appropriate suspension of cells in 0.1 M phosphate pH 7.0 in an ice bath and by sonicating for 1-min bursts followed by 0.5-min rest until 75–95% breakage, as noted by a decrease in the absorbance at 660 nm, was accomplished (usually 3–5 min). The unbroken cells and cell debris were removed by centrifugation at 4° C for 25 min and $3000 \times g$.

Assays and procedures

Hydroxylamine was determined by the method of Magee and Burris (1954) as described (Castignetti et al. 1983). Nitrite was measured via diazotization (Van't Riet et al. 1968) and nitrate was monitored by use of the chromotropic acid assay of Rand et al. (1975). DFB and FB were determined via the use of the $Fe(ClO_4) \cdot 6H_2O$ 0.1 M $HClO_4$ assay of Leong and Neilands (1982).

To determine the amount of DFB, the sample was added to the $FeClO_4$ reagent; in the case of FB determinations, the FB sample was prepared as described above and, at the appropriate time, was added to the $FeClO_4$ reagent which stops any further metabolism as the acidity is sufficient to lower the pH to 1.5–2.0. To determine the synthesis of monohydroxamate(s) from the trihydroxamate siderophore, i.e. either DFB or FB, a modification (Castignetti et al. 1988) of the spectral assays of Warren and Neilands (1964) and Leong and Neilands (1982) was developed. This assay allows the differentiation of the trihydroxamate FB, and thus DFB, from monohydroxamates based on the absorption characteristics of these compounds at a pH 1.5–2.0. In addition, the cyanide-dependent assay of Emery (1984) was also used to distinguish between mono-, di- and trihydroxamates. All assays were repeated at least twice on separate days and the data given is the mean of the results collected with the exceptions of Table 2 and Fig. 3, which are representative of the results collected.

Protein was determined by the method of Herbert at al. (1971). Proteolytic assays were conducted as described by Kersters and DeLay (1971; DL-alanine-β-naphthylamide·HCl method), McKellar and Cholette (1984; hide powder azure method), or the manufacturer of azocoll (Calbiochem-Behring). To determine if resting cells and cell-free extract of DFBC 5 or commercial proteolytic enzymes hydrolyzed either DFB or FB, a solution of 4.0 mM DFB in 100 mM phosphate (pH 7.0) was mixed with an equal volume of 3.75 mM $FeCl_3 \cdot 6\ H_2O$, 5.0 mM HCl to yield a 1.87 mM FB, 0.12 mM DFB solution. This solution was then mixed with an equal volume of the resting cells, cell-free extract or the particular protease (1.0 unit ml^{-1}) dissolved in 100 mM phosphate pH 7.0 and assayed by the method described above (Castignetti et al. 1988). Duplicate solutions were prepared and aliquots were removed at 0, 30 and 60 min. The degradation of DFB was monitored in the same manner except that ferration with $FeCl_3$ was not performed and 2.0 mM DFB in 100 mM phosphate buffer was added to an equal volume of either the cell-free extract (in 100 mM phosphate buffer) or the protease phosphate buffer (100 mM) solution. With both the FB and DFB phosphate buffer solutions, controls consisted of the same solutions except that buffer alone was added instead of the protease buffer solution. Boiled (5 min) resting cells or cell-free extracts served as controls in experiments where resting cells or cell-free extract were employed. As DFBC 5 was unable to grow in FB medium, resting cells and cell-free extracts were prepared as described above from mid-logarithmic cells grown in the DFB NH_4^+ mineral salts medium.

The use of Chelex 100 to remove ferric ion from various solutions and buffers was as described by Davey et al. (1970). Glassware was soaked in EDTA, to remove iron, as detailed by Lodge and Emery (1984). Osmotic shock was performed according to the method of Neu and Heppel (1965).

Results

Ability of Alcaligenes sp. and P. aureofaciens to use DFB

The examination of the *Alcaligenes* sp. and *P. aureofaciens* as heterotrophic nitrifiers of the siderophore DFB stems from their active nitrification of other *N*-oxygenated compounds (Castignetti and Gunner 1980; Castignetti et al. 1983; Castignetti and Hollocher 1984). The *Alcaligenes* sp. was unable to use DFB as a sole C source or to nitrify the DFB even if the medium contained 0.05% yeast extract as a supplement to the 0.2% DFB and NH_4^+. *P. aureofaciens*, however, was capable of using DFB as a sole C-N source although growth was limited and approximately 95% of the DFB remained in the growth medium after 21 days of culturing. The best growth and the most active consumption of DFB by *P. aureofaciens* was noted when the bacterium was grown in 2.4 mM DFB 0.05% yeast extract mineral salts medium (Table 1). *P. aureofaciens* grown in DFB mineral salts medium without supplements yielded approximately 10% of the biomass and nitrite synthesized as when the medium was supplemented with yeast extract. The nitrification of DFB performed by *P. aureofaciens* was not due to the oxidation of yeast extract nitrogenous compounds as the microbe failed to produce any nitrite or nitrate when grown in a yeast extract (0.05%) mineral salts medium.

Table 1. Growth and nitrification of *P. aureofaciens* in 2.4 mM DFB/0.05% yeast extract/mineral salts medium

Time (days)	Growth (mg cell protein ml^{-1})	DFB (mM)	Hydroxylamine (mM)	Nitrite (mM)	Nitrate (mM)
0.0	0.00	2.4	0.04	0.04	0.0
0.25	0.06	2.4	0.03	0.011	0.0
1.0	0.11	2.3	0.06	0.014	0.0
3.0	0.12	2.3	0.19	0.113	0.0
7.0	0.13	2.2	0.35	0.550	0.0
14.0	0.12	2.2	0.02	1.440	0.0
21.0	0.12	2.1	0.03	1.820	0.0

Uninoculated controls demonstrated no growth, no consumption of DFB, no production of nitrate, and maxima of 0.34 mM hydroxylamine (on the 14th day of the experiment) and 0.024 mM nitrite (on the 21st day). The *Alcaligenes* sp. and DFBC 5 also failed either to consume DFB, or to produce nitrate, nitrite or hdroxylamine when cultured in DFB-containing media

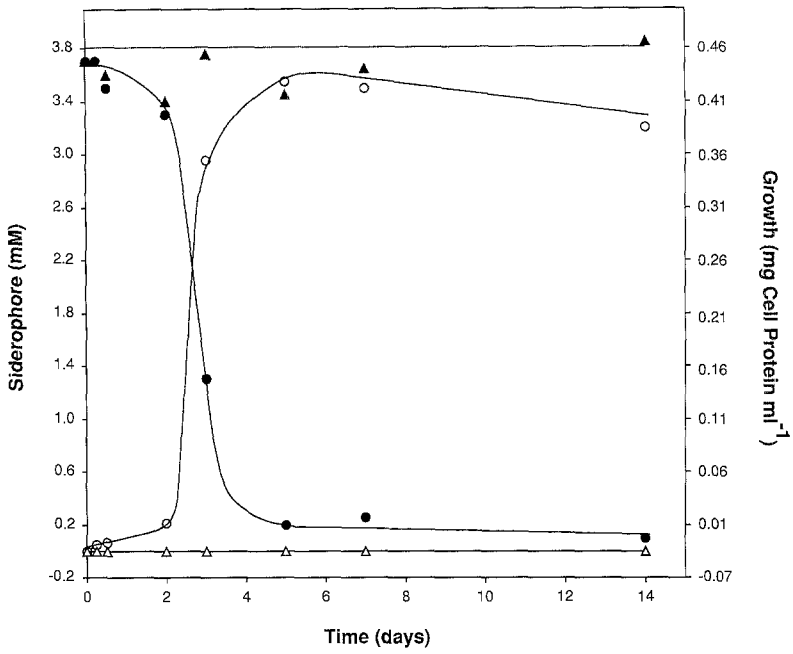

Fig. 1. Growth and DFB consumption of DFBC 5 in DFB/NH_4^+ vitamins mineral salts medium. (○) Growth of DFBC 5; (△) growth of uninoculated controls; (●) DFB concentration of the DFBC 5 cultures; (▲) DFB concentration of uninoculated controls

Metabolism of DFB by a soil microbe

The sparse growth and nitrification of *P. aureofaciens* with DFB as a C and oxygenated N source prompted the isolation of a DFB-catabolizing microbe from the soil. While a number of microbes were present in the DFB enrichment cultures, only two displayed reasonable growth on DFB mineral salts agar plates. Of these two isolates, the one designated DFBC 5 was the superior DFB catabolizer as judged by its growth and consumption of DFB.

DFBC 5 demonstrated no growth in an FB mineral salts medium but did grow well on either DFB mineral salts medium or DFB NH_4^+ vitamins mineral salts medium (Fig. 1). No nitrification of the siderophore in either medium was observed. Indeed, residual concentrations of nitrite and hydroxylamine were lower in the inoculated medium as compared to uninoculated controls, presumably due to the removal of these compounds from the medium by the bacterium. Only minor amounts of siderophore, approximately 0.1 mM, remained after the growth of the bacterium reached stationary phase.

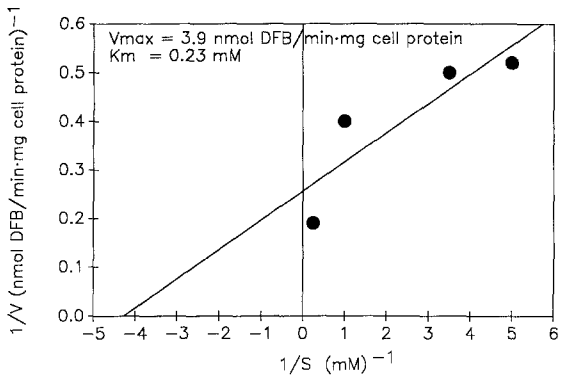

Fig. 2. DFB metabolism by resting cells of DFBC 5. Cells were grown in DFB medium and harvested during mid-logarithmic growth. Cell densities ranged over 1.3–7.0 mg cell protein ml^{-1}. The data is presented in the form of a Lineweaver-Burk plot to establish the overall ability of the bacterium to metabolize DFB

Examination of culture medium during logarithmic growth for the presence of mono- and dihydroxamates, possible trihydroxamate (DFB) degradation products, was negative. Extracellular proteins present in the culture supernatant were unable to degrade either DFB or FB and did not display any proteolytic activity. DFBC 5, however, did contain peptidase activity, as determined by the method of Kersters and DeLay (1971), and proteins released by osmotic shock resulted in the hydrolysis of hide powder azure.

Resting cells from DFB-grown cultures demonstrated no synthesis of either mono- or dihydroxamates when either FB or DFB was the substrate. Resting cells did, however, actively consume DFB (Fig. 2), but not FB. The apparent V_{max} and K_m for DFB metabolism by resting cells of DFBC 5 were 3.9 nmol DFB min^{-1} mg cell protein^{-1} and 230 μM, respectively.

Cell-free extract, prepared from mid-logarithmic cells grown in DFB medium by either grinding with alumina or by sonication, synthesized a monohydroxamate(s) when DFB (Fig. 3), but not FB, was the substrate. Such extracts were positive for the formation of a monohydroxamate by either the spectral assay (Castignetti et al. 1988) or with the cyanide assay of Emery (1984). With the latter assay, however, the blue color of the monohydroxamate–cyanide complex was observed within the first few minutes of mixing the cell-free extract and DFB and then disappeared, perhaps due to competing reactions in the extract system.

Cell-free extracts from DFBC 5 which actively converted DFB into a monohydroxamate(s) were incapable of general protease activity as measured by the degradation of azocoll. These cell-free extracts, however, were at least 85% inhibited with respect to DFB degradation

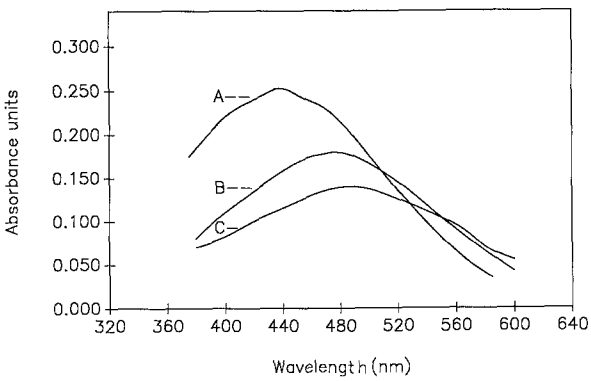

Fig. 3. Production of a monohydroxamate by cell-free extracts of DFBC 5 grown in 0.2% DFB/NH_4^+ mineral salts medium. Extracts were prepared by sonication as described in Materials and methods and were present at 2.5 mg protein ml^{-1}. The concentration of DFB was 1 mM. The assay was performed as described by Castignetti et al. (1988), i.e. longer wavelength absorption maxima represent increasing concentrations of monohydroxamates and decreasing concentrations of trihydroxamates, in this case, DFB. A, B, and C are the spectra obtained at 0, 30 and 60 min, respectively. Boiled cell-free extract served as the control and yielded spectra indistinguishable from that of the 0-min spectrum regardless of the time of measurement

Table 2. Inhibition by the protease inhibitors leupeptin, pepstatin A, $PhMeSO_2F$ and EDTA with respect to the degradation of DFB to monohydroxamates by cell-free extract of DFBC 5

Time (min)	Ratio of monohydroxamate synthesized to trihydroxamate (DFB) remaining		Inhibition (%)
	control	experimental	
0	0.25	0.25	0
30	15.0	1.5	90
60	>100.0	15.0	>85

Assays were performed as described in Materials and methods. Zero inhibition is defined as having the same monohydroxamate/trihydroxamate ratio in the experimental samples, at a given time interval, as in the control samples. The final concentration of cell-free extract was 4.0 mg protein ml^{-1}. Controls were prepared by incubating the cell-free extract with DFB devoid of inhibitors while the experimental treatments contained the four inhibitors. The protease inhibitors were confirmed as being active as they inhibited the degradation of azocoll by >85% when present with the substrate and the protease pronase E

when the peptidase inhibitors leupeptin, pepstatin A, $PhMeSO_2F$ and EDTA were present (Table 2).

The broadly catalytic proteases nagarse and pronase E, as well as a protease from *Aspergillus oryzae*, were given DFB and FB as substrates and assayed (Castignetti et al. 1988) to determine if the siderophore was either consumed or converted to monohydroxamates. The proteolytic enzymes were incapable of degrading either DFB or FB, as judged by chromophore disappearance, nor were they able to synthesize any monohydroxamates from either DFB or FB, even though the enzymes were quite active in the degradation of the proteolytic substrate azocoll.

Discussion

The siderophore deferrioxamine B is a poor nitrification substrate for the bacteria examined in this study, i.e. two avid heterotrophic nitrifiers and DFBC 5. Two of these bacteria failed to nitrify DFB while *P. aureofaciens* demonstrated a limited ability to produce nitrite from the compound. Less than a third of the hydroxamate nitrogen (DFB is a trihydroxamate with 3 mol hydroxamate N/mol DFB) was converted to nitrite and growth of *P. aureofaciens* was limited in the DFB yeast extract mineral salts medium.

Neither the *Alcaligenes* sp. nor *P. aureofaciens* demonstrated vigorous growth with DFB as the C source. Conversely, DFBC 5 grew well under these conditions. DFB was utilized readily and the removal of the siderophore from the medium paralleled the growth of the bacterium. DFB utilization by resting cells demonstrated that the deferrisiderophore is readily catabolized by DFBC 5. It is noteworthy that the bacterium had no action, with respect to growth, resting cell and cell-free extract catabolism, when FB was the substrate.

Warren and Neilands (1964, 1965) and Villavicencio and Neilands (1965) observed that ferrichrome A was more readily catabolized by a soil *Pseudomonas* sp. than was deferriferrichrome A. Ferrichrome dissimilation occurred more slowly than did that of ferrichrome A while deferriferrichrome was unable to support the growth of the pseudomonad. The bacterium studied by these authors excreted monohydroxamates, but apparently not extracellular siderophore-dissimilating enzymes, into the growth medium. The binding of Fe^{3+} by deferriferrichrome A was thought to expose a peptide bond of the molecule which facilitated its catabolism.

DFBC 5 produced monohydroxamates only when cell-free extracts were employed. No evidence of extracellular DFB-catabolizing enzymes was observed. Furthermore, no FB dissimilation was noted when it was either the substrate for growth, resting cell catabolism or degradation by cell-free extracts. Analysis of these results suggests that the two bacteria both use either periplasmic or cellular enzymes to metabolize their respective siderophores. They employ different strategies, however, with respect to the dissimilation of their respective siderophore substrates, i.e. the *Pseudomonas* sp. preferentially catabolized ferrisiderophores while DFBC 5 only dissimilated the deferrisiderophore.

As a general characteristic, Warren and Neilands (1964) observed that their pseudomonad did not degrade hydroxamates nor did it possess a broadly catalytic amidase activity. Furthermore, of four additional natural hydroxamates tested, only the siderophore coprogen supported any growth of the bacterium. The enzyme(s) responsible for the degradation of DFB to hydroxamates in cell-free extract of DFBC 5 also was somewhat specific for its substrate. While this extract produced monohydroxamate(s) from DFB, it was unable to degrade the general peptidase substrate azocoll. DFB itself appears not to be easily catabolized as three

different broadly catalytic proteases, all active with the commercial peptidase substrate azocoll, were unable to degrade DFB or to produce monohydroxamates from it. While the current study clearly demonstrates that DFB is catabolized by DFBC 5, further study is warranted to ascertain whether siderophores, as a group, are recalcitrant molecules and to enumerate the microbial flora capable of siderophore dissimilation.

Siderophore-producing microbes have been suggested as biological control agents as they apparently limit the amounts of iron available to potential plant pathogens (Bakker et al. 1986; Loper 1988; Simeoni et al. 1987). The ability of bacteria, such as that of this study, to degrade siderophores raises the question of whether such microbes could be of agricultural benefit. That is, if the siderophores of plant pathogens can be eliminated via an active catabolic microflora, then control of agriculturally deleterious microbial agents may be possible and deserves investigation.

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