Bacterial Citrate Siderophores

H. Budzikiewicz*

Institut für Organische Chemie, Universität zu Köln, Greinstr. 4, 50939 Köln, Germany

Abstract: Soil bacteria as well as those infecting other organisms have developed systems to secure iron necessary for their metabolism which is generally not available in its free ionic form. As one of the possible strategies they learnt to produce secondary metabolites able to bind Fe^{3+} as water soluble complexes, so-called siderophores. A rather small group is based on citric acid. One of the ligand sites is the central C(OH)COOH unit, the two additional ones are carried by substituents bound to one or both of the terminal carboxyl groups. A survey will be presented.

Keywords: Iron metabolism, siderophores, citric acid derivatives.

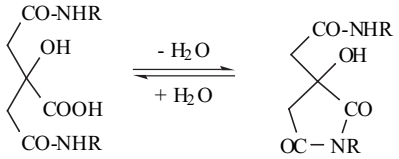
1. INTRODUCTION

In the beginning life on earth developed in a reductive atmosphere where iron was available abundantly in its divalent form. Salts of Fe^{2+} are sufficiently water soluble to provide an adequate supply of this element which is essential for many physiological processes. But as a consequence of the photolytic cleavage of water initiated by cyanobacteria, oxygen was set free and soon only trivalent iron abounded. Due to the low dissociation constants of its various oxide hydrates in the soil the concentration of free Fe^{3+} at *pH*-values around 7 is at best 10^{-17} mol/liter[1] while about 10^{-6} mol/liter would be needed to maintain the necessary supply for living cells. Bacteria infecting animals or man are in a similar situation: here iron is bound strongly to peptidic substances such as transferrins. Bacteria learnt to circumvent this problem either by reduction of Fe^{3+} to Fe^{2+} (cf. [1]) or by the production of Fe^{3+} chelating substances, so-called siderophores, secondary metabolites with a molecular mass below 2000 Da.

Due to its high charge density, small ion radius, and low polarisability Fe^{3+} is a hard *Lewis* acid and can bind strongly hard *Lewis* bases such as oxide ions. Fe^{3+} forms octahedral d^5 high spin complexes providing six coordination sites, which can accommodate three bidentate ligands. The ligand types encountered most frequently among siderophores are catecholate, hydroxamate and α-hydroxy carboxylate units. Mixed systems are not uncommon as in the case of the citrate siderophores. Three bidentate ligands are often connected by aliphatic segments keeping them in place for complexation. This results in an entropic advantage over three non-connected ligands.

Regarding the iron transport into the cell [2-4], small Fe^{3+} complexes can cross the cell membrane through unspecific narrow porins. Larger complexes require a more or less specific transport protein which recognizes the ferri-siderophore at the cell surface. By this interaction the tertiary protein structure is changed to allow an active transport through the cell membrane. Iron is released mostly by reduction to the less strongly bound Fe^{2+}, and the free siderophore is re-exported. This transport process into the cell is referred to as "shuttle mechanism".

Several citrate siderophores were found to be accompanied by derivatives formed by the loss of H_2O. They have a cyclic imide structure and are possibly artifacts formed during the isolation process (Scheme **1**). They are usually designated by the name of the siderophore followed by an A (e.g., schizokinen A).



Scheme 1. Cyclization to amidic citrate structures.

2. CITRIC ACID

Free citric acid has been encountered occasionally as a true siderophore, as in the case of *Bradyrhizobium japonicum* [6,7]. Other bacterial strains as e.g. *Escherichia coli* [8,9], *Mycobacterium smegmatis* [10], *Neisseria meningitidis* [11,12], and *Pseudomonas aeruginosa* [13,14] can use citric acid as so-called heterologous siderophore when it is is supplied from outside. For *Mycobacterium* and *Neisseria* it was shown [10,12] that ^{55}Fe but not labelled citrate was transported into the cell.

3. SIDEROPHORES WITH TWO HYDROXAMATE UNITS

Siderophores of this group have 1,3-diaminopropane, 1,5-diaminopentane or lysine molecules bound amidically to the two outer carboxyl groups of citric acid. To their free amino groups acid residues are connected. They also carry a hydroxyl group thus forming hydroxamic acids. With one exception the two amino ligands are identical and the whole molecules therefore not chiral.

a. Schizokinen (1)

Schizokinen (named after its bacterial cell division promoting ability [15]) is produced as a siderophore by the

*Address correspondence to this author at the Institut für Organische Chemie, Universität zu Köln, Greinstr. 4, 50939 Köln, Germany; E-mail: aco88@uni-koeln.de

[1]In a recent theoretical study it is claimed that the concentration of Fe^{3+} in an aqueous solution at *pH* 7 is $1.4 \cdot 10^{-9}$ mol/liter. Experimental evidence is not given [5].

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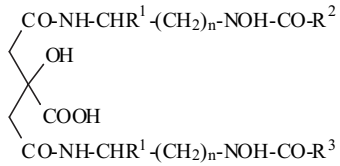


1: R^1 = H, R^2 = R^3 = CH_3, n = 2
2: R^1 = H, R^2 = R^3 = CH_3, n = 3
3: R^1 = H, R^2 = R^3 = CH_3, n = 4
4: R^1 = H, R^2 = R^3 = *E*-CH=CH-$(CH_2)_4$-CH_3, n = 2
5: R^1 = H, R^2 = CH_3 R^3 = *E*-CH=CH-$(CH_2)_6$-CH_3, n = 2
6: R^1 = COOH, R^2 = R^3 = CH_3, n = 4
7: R^1 = COOH, R^2 = R^3 = *E*-CH=CH-C_6H_5, n = 4

Fig. (1). Structures of siderophores with two hydroxamate units.

bacteria *Bacillus megaterium* [16] and *Ralstonia solanacearum* [17] as well as by several species of the cyanobacterium *Anabaena* [18-21]. Schizokinen was also found in the soil of flooded rice fields [22]. It can be used as a siderophor by some non-producers such as strains of *Salmonella typhimurium* not able to produce their own siderophore enterobactin [23], by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Aureobacterium flavescens* [24]. Both natural and synthetic schizokinen is accompanied by the imidic schizokinen A (cf. Scheme **1**) [17, 25-27].

Chemical degradation allowed to identify citric acid, acetic acid and 1-amino-3-hydroxyaminopropane [25]. Detailled ^{1}H-and ^{13}C-NMR studies of schizokinen and schizokinen A were published [17, 26, 28]. Several ways of synthesis for schizokinen and schizokinen A are reported in literature [17, 26, 27].

To confirm the binding sites for Fe^{3+} schizokinen with an acetylated central OH group was prepared. While schizokinen forms a 1:1 metal-to ligand complex the spectral data for the O-acetyl derivative are consistent with a 2:3 ratio. The central α-acetoxy carboxyl unit does not act any more as a ligand. Nevertheless, both complexes are accepted by *Anabaena* with preference however of the original schizokinen complex [29].

The schizokinen mediated Fe^{3+} transport into the cells of *Bacillus megaterium* was studied with doubly labelled material ($^{59}Fe^{3+}$ and ^{3}H). The following sequence is proposed. Ferri schizokinen is bound to the cell surface and subsequently transported into the cell, where iron is released reductively (cf. [30]). Free schizokinen is pooled and eventually re-exported. Re-export does not occur at low temperatures [31]. In the case of *Anabaena* sp. evidence was brought forth that ATP is the energy source for the transport of ferric schizokinen into the cells [21]. [^{59}Fe] uptake studies were also performed with *Ralstonia solanacearum* [17].

From the culture of *Bacillus megaterium* in addition to schizokinen and schizokinen A a further compound, viz. a mono-*N*-deoxyschizokinen was isolated. Despite the fact that it contains only one hydroxamic unit it can bind Fe^{3+}, but it is not clear whether it can act as a siderophor. It may be a precursor or a degradation product of schizokinen, the latter being more likely as it was found during the stationary growth phase of the bacterium. Small amounts of *N*-deoxyschizokinen A were encountered, but it could not be decided which of the ligands formed the imide cycle or whether a mixture of both was present [28].

b. Homoschizokinen (2) and Arthrobactin (3)

Arthrobactin (**3**) was described originally as a growth factor for *Arthrobacter terregens* ("terregens factor") [32,33]. Later it was isolated from the culture filtrate of *A. pascens*. The structure was deduced from the ^{1}H-NMR spectrum and the fact that citric acid and 1,5-diaminopentane was obtained by reductive hydrolysis [34].

Homoschizokinen (**2**) and homoschizokinen A have only been obtained by synthesis[1] [26]. Although the goal of the synthesis was to compare the complex stability with respect to the increasing distance of the hydroxamic acid units from the citrate part no such data are reported for **2**. Also the synthesis of arthrobactin is described [27, 35].

Synthetic analogs of **1** and **3** with an "inversed" hydroxamic acid unit (-NH-$(CH_2)_n$-CO-NOH-C_2H_5, n = 2,3,5) showed none to low siderophore activity for *Escherichia coli* and *Arthrobacter flavescens* [36].

Derivatives of **3** were synthesized where the β-lactam antibiotics carbacephem or LY 163892 are attached to one of the hydroxamic acid units (-NOH-CO-CH_2-CH_2-CO-antibiotic replacing -NOH-CO-CH_2-CH_2-CO-CH_3). They showed antibiotic activity against *E. coli* [35]. Thus the ferric siderophore acted as a Trojan hors [37] still being recognized at the cell surface.

c. Acinetoferrin (4)

Acinetoferrin (**4**) is produced by *Acinetobacter haemolyticus* [38]. Chemical degradation revealed the constituents citric acid, 1,3-diaminopropane and *E*-2-octenoic acid. The structure was finally established by NMR and mass spectroscopy. As other representatives of this group, the Fe^{3+} complex of acinetoferrin shows a broad charge transfer band with a maximum at 486 nm in methanol.

Total syntheses of **4** and of acinetoferrin A [39], of the *E*-cinnamoyl analog (cf. nannochelins below) of **4** and of its higher homolog with a 1,4-diaminobutane chain (cf. homoschizokinen above) are reported [40]. These compounds (and nannochelin A, **7**) were used for growth stimulation tests with *Mycobacterium paratuberculosis* in view of their use as carriers for antibiotics ("Trojan horses"). Most of them gave about the same results as mycobactin J (a hydroxamate/salicylate siderophor [41]), the native Fe^{3+} chelator, with the exception of nannochelin A (**7**) and especially acinetoferrin A which gave higher values [40].

d. Rhizobactin (5)

Rhizobactin (**5**) is the siderophore of the *Medicago sativa* (alfalfa) symbiont *Rhizobium meliloti* [42]. The color of a

[1] In Ref [35] homoschizokinen is referred to as awaitin B as "a proposed member of the citrate-based siderophores". Awaitin B (as its A and C congeners, see below) were just "awaited" to be found in nature. Nature seems to think otherwise. Steric reasons in Fe^{3+} complexation are probably the reason that only siderophores with 3 or 5 C-atoms between the two N-atoms are found.

neutral aqueous solution of the Fe^{3+} complex is golden-brown. The charge transfer absorption maximum shifts from 399 nm at pH 7.0 to 426 nm at pH 3.6 in agreement with the other siderophores of this group [29, 43]. Structure elucidation was effected by chemical degradation, NMR and mass spectrometry. A CD spectrum suggests a (prevailing) Λ configuration of the Fe^{3+} complex, and there is evidence that in aqueous solution there exists an equilibrium between a monomeric and a dimeric form. The absolute configuration of the central carbon of the citrate part has not been determined (citric acid derivatives become chiral when the two terminal carboxyl groups carry different substituents, cf. rhizoferrin below).

e. Aerobactin (6)

Aerobactin (**6**) had been isolated first from *Aerobacter aerogenes* [43,44]. It was found subsequently to be produced also by various genera of the Enterobacteriaceae (*Enterobacter* [45], *Escherichia* [46], *Salmonella* [47], *Shigella* [48], *Yersinia* [49]) as well as by *Erwinia carotovora* [50], *Vibrio* spp. [51] and by a halophilic *Pseudomonas* strain living together with the cyanobacterium *Spirulina platensis* [52]. It can also be used by *Anabaena* sp. in addition to its native siderophore schizokinen (**1**) [20]. Aeroactin is an important virulence factor for enterobacteria [53]. *I. a.* it can remove Fe^{3+} from transferrin, though not as efficiently as enterobactin [54], a catecholate siderophore produced by various enterobacteria [62]. The aerobactin mediated iron transport was studied by double labelling ($^{59}Fe^{3+}$, ^{3}H) and is probably analogous to that reported for schizokinen (see above) [31].

By reductive hydrolysis with HI lysine was obtained while hydrolysis with HCl gave N^6-hydroxylysine and citric acid. The structure elucidation was completed using mass and ^{1}H-NMR spectroscopy [44]. The stereochemistry of lysine was not determined. The total synthesis of (-)-aerobactin containing L-lysine and of its constituent amino acid, N^6-acetyl-N^6-hydroxy-L-lysine is described [55].

The bright orange Fe^{3+} (and also the Cr^{3+} complex) were investigated in detail [43]. The absorption maximum due to a charge transfer transition shifts from 399 nm at pH 7.0 to 450 nm at pH 2.7 (cf. rhizobactin above). The CD spectrum suggests an octahedral structure with preferentially Λ configuration. At pH 7.0 the redox potential is -336 mV vs. the normal hydrogen electrode. Also the ligand protonation and the metal-ligand equilibria were investigated.

f. Nannochelins

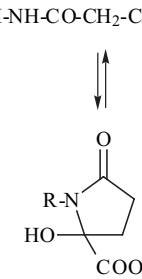
For the structure elucidation of the siderophore of the myxobacterium *Nannocystis exedens* there exists only a preliminary report [56] giving the UV/Vis and the IR spectrum and the optical rotation (due to the incorporation of L-lysine). Actually, three compounds are reported, the free carboxylic acid (**7**) (nannochelin C), its mono- (B) and dimethyl ester (A), though the esters may be artifacts from the work-up with methanol. A full account was planned to appear in *Liebigs Ann. Chem.*, but has not been published so far[2].

[2] Private communication by Prof. G. Höfle, Braunschweig.

A total synthesis of nannochelin A was effected [57]. The ^{1}H-NMR spectrum of the synthetic compound is reported to be consistent with the structure proposed for the natural product. A synthesis of the ornithine analog of nannochelin C ("awaitin A", cf. footnote[2]) is mentioned but no details are given [35].

4. SIDEROPHORES WITH 2-OXOGLUTARIC ACID UNITS

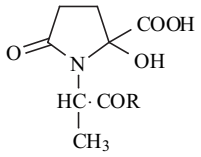
N−alkylated 2-oxoglutaric acid exists usually as an equilibrium of several hydrated and cyclic structures. At neutral pH values the 2-hydroxy-5-oxoproline form seems to prevail (Scheme **2**). In this way an α-hydroxy carboxylic acid grouping is formed which can act as a ligand in the complexation of Fe^{3+}.



Scheme 2. Cylization of 2-oxoglutaric acid side chains.

a. Vibrioferrin (8)

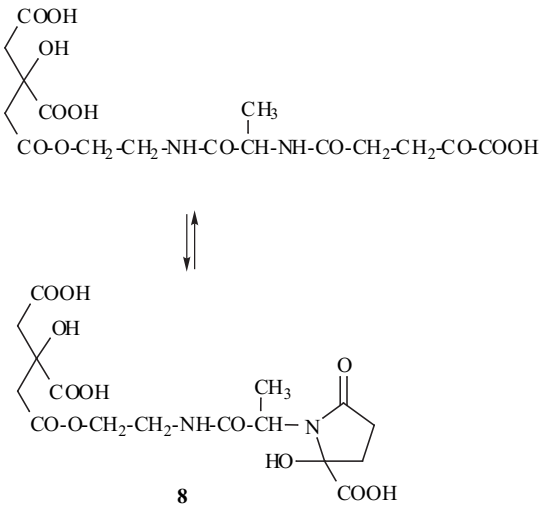
Vibrioferrin was obtained from the culture medium of *Vibrio parahaemolyticus* [58]. Partial acid hydrolysis yielded citric acid, 1-amino-2-hydroxyethane, and the compounds **9** and **10** as methyl esters. Complete hydrolysis gave *i. a.* L-alanine. The molecular mass of vibrioferrin as determined by FAB mass spectrometry revealed that only one carboxyl group of citric acid is substituted by **10**. NMR spectroscopy confirmed the cyclic structure **8**, consisting of an equilibrium of the two epimers at C-2 of the pyrrolidone ring (Scheme **3**). The chirality of the central C-atom of citric acid was not determined [58].



9: R = OH
10: R = NH-CH_2-CH_2OH

Fig. (2). Degradation products of vibrioferrin.

The vibrioferrin molecule offers only five ligand sites for the complexation of Fe^{3+}, viz. the two α-hydroxy-carboxylate groups and the free terminal carboxyl group of citric acid. The eighth position of an octahedral complex must then be satisfied by a solvent molecule unless one considers metal-to-ligand ratios other than an 1:1. Studies with the ^{55}Fe labelled ferric siderophore proved that vibrioferrin acts as a siderophore [58].



Scheme 3. Cyclization equilibrium of vibrioferrin.

b. Staphyloferrin B (11)

From *Staphylococcus hyicus* and from several other *Staphylococcus* spp. the siderophor staphyloferrin B (**11**) in addition to staphyloferrin A (see below) was obtained [59]; it is also produced by *Ralstonia eutropha* [60]. Hydrolysis yields citric acid, 1,2-diaminoethane and L-2,3-diaminopropionic acid. NMR and mass spectral studies completed the structure elucidation [60,61]. Comparison of the CD curve with model compounds showed that staphyloferrin B has *S*-configuration at the central C-atom of citric acid [60].

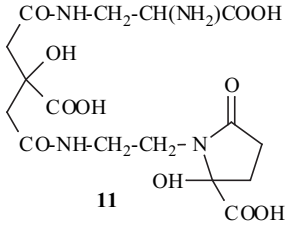


Fig. (3). Staphyloferrin B.

Mass spectrometric [60] and CD studies [61] indicate an 1:1 Fe^{3+}-to-ligand ratio. As discussed for vibrioferrin also staphyloferrin possesses only two bidentate ligands sites and a free carboxylate group. To get more information about the complexing sites of staphyloferrin B its Ga^{3+} complex was investigated by NMR (Fe^{3+} complexes are not amenable to NMR, but Ga^{3+} forms also octahedral complexes and the two metals have almost identical ion radii) [60]. Comparison of the chemical shifts of the Ga^{3+} complex and the free ligand in their ^{13}C NMR spectra allows to recognize the binding sites. This shows that the three carboxyl groups are involved. The chemical shifts of the carbon atoms carrying the carboxylate groups as well as the two hydroxyl and the amino function are negligibly influenced by the metal ion in agreement with the retention of the OH/NH_2 hydrogens (cf. rhizoferrin below). However in the ^1H-NMR spectrum of the Ga^{3+} complex two sharp H-signals of the amino group can be seen indicative of their fixed position as a consequence of the coordination of the nitrogen with the metal ion.

Uptake studies with $^{55}Fe^{3+}$ labelled staphyloferrin B showed that it accepted as a siderophore, but it is less efficient than staphyloferrin A (below) [61].

c. Achromobactin (12)

Erwinia chrysanthemi produces two siderophores, viz. the catecholate chrysobactin [62] and the citrate derivative achromobactin [63]. Its name was chosen since no color reaction was observed after addition of Fe^{3+}. HCl hydrolysis yielded citric acid, L-2,4-diaminobutanoic acid, succinic acid (the typical decomposition product of 2-oxoglutaric acid) and 1-amino-2-hydroxyethane. The cyclic structure **12** was finally established by detailled ^1H- and ^{13}C-NMR studies. Achromobactin thus has two different branches with 2-oxoketoglutaric bound to and 1-amino-2-hydroxyethane and to L-2,4-diaminobutanoic acid. Citric acid thus becomes assymmetric but the chirality of its central C-atom has not been determined.

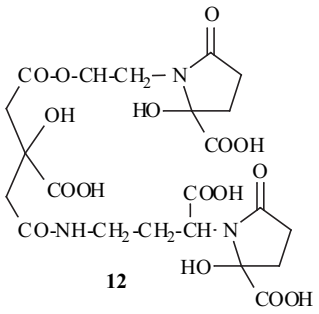


Fig. (4). Achromobactin.

5. SIDEROPHORES WITH TWO CATECHOLATE UNITS

a. Petrobactin (13) and Petrobactin Sulfonate

In the siderophore of the marine bacterium *Marinobacter hydrocarbonoclasticus* two catecholate units bound to spermidine are linked by the 1,3-carboxyl groups of a citrate unit. Originally it had been assumed [64] that 2,3-dihydroxybenzoic acid (DHB) is the constituent, but synthetic studies [65] revealed that in petrobactin (**13**) 3,4-dihydroxybenzoic acid is incorporated. Recently also a perobactin sulfonate was isolated [66].

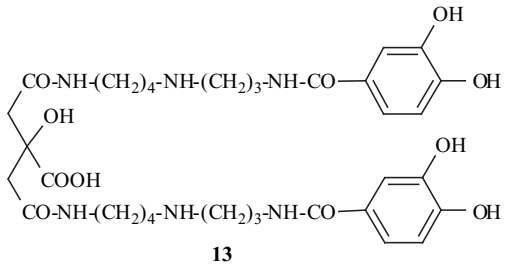
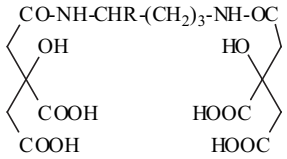


Fig. (5). Petrobactin.

6. SIDEROPHORES WITH TWO CITRIC ACID UNITS

Siderophores of this group have only two typical complexing ligands, *viz*. the two central α-hydroxy carboxylic acid parts of the citrate molecules. The free outer

carboxyl groups must then satisfy the two remaining ligand sites in an octohedral Fe^{3+} complex.



14: R = H
15: R = COOH

Fig. (6). Rhizoferrin and staphyloferrin A.

a. Rhizoferrin (14)

R,R-Rhizoferrin was isolated from cultures of the fungus *Rhizopus microsporus* var. *rhizopodiformis* [67] and was found subsequently to be the common siderophore of the zygomycetes [68]. Interestingly, ferri-rhizoferrin (as well as various ferrioxamines) can also be used by the bacterial spcies *Pasteurella multocia* and *P. haemolytica* from which so far no genuine siderophores have been isolated [69]. Its structure was established by chemical degradation yielding citric acid and 1,4-diaminobutane, mass and NMR spectroscopy as **14**. It is accompanied by two dehydration products (formation of one or two imide rings, cf. Scheme **1**). CD spectroscopy revealed *R,R*-configuration at the central carbon atoms of the citric acid units and Λ configuration around the metal center. Above pH 3 the iron-to-ligand ratio is 1:1 [70]. The complexing constant of **14** was determined as log k_{LFe} = 25.3. ^{13}C NMR spectroscopic examination of the Ga^{3+} complex of **14** and comparison of the chemical shifts with those of the free ligand showed two things. First, the twofold symmetry of the free ligand is not lost upon complexation, since there is not duplication of the signals. Second, major changes of the chemical shifts are observed only for the carboxylate carbon signals. Those of the carbon atoms carrying the carboxylate and the hydroxyl group are not influenced. It is concluded that the hydroxyl groups - though participating as ligands in the octahedral complex - are not deprotonated between pH 5.5 and 9.0 [71].

The uptake mechanism was studied with the Fe^{3+} and other metal complexes including the kinetically inert Cr^{3+} and the reductively inert Ga^{3+} complexes. The results suggest a so-called shuttle mechanism. *R,R*-Rhizoferrin can also be used by the bacterium *Morganella morganii* [72,73].

From the bacterium *Ralstonia pickettii* the optical antipode *S,S*-rhizoferrin (*enantio*-rhizoferrin) was obtained [74]. Uptake studies showed that *Ralstonia* does not differentiate between the two enantiomeric forms in contrast to the fungus *Rhizopus* which has a pronounced preference for the *R,R*-enantiomer.

b. Staphyloferrin A (15)

Staphyloferrin A (**15**), the second siderophore of the bacterial genus *Staphylococcus*, was originally obtained from *S. hyicus* and subsequently also from other *Staphylococcus* spp. [75,76]. Chemical degradation yielded citric acid and D-ornithine. Mass and NMR spectroscopy completed the structure elucidation. The metal:ligand ratio of the Fe^{3+} complex is 1:1, it conformation is preferentially Λ as shown by CD. As a consequence of the asymmetric structure of the molecule three dehydration products were found, two mono-imidic and one di-imidic. Experiments with the $^{55}Fe^{3+}$ labelled ferric complex proved that staphyloferrin A is a true siderophore. The configuration of ornithine is D (*R*), that of the two citrate centers was not determined.

7. CONCLUSION

Citrate is readily available to all microorganisms. It can complex Fe^{3+} and it can be used for iron transport into bacterial cells, but under conditions of severe iron limitation more potent siderophores are synthesized by attaching additional ligand sites to the citric acid molecule. It could be observed that several citrate siderophores are produced by unrelated microorganisms. Parallel biogenesis of structurally related representatives or inheritance from common evolutionary ancestors is one possibility, horizontal gene transfer especially between soil bacteria is another one.

REFERENCES

[1] Budzikiewicz, H. *Biodegrad.* **2003**, *14*, 65.
[2] Braun, V., Braun, M. *Curr. Opin. Microbiol*. **2002**, *5*,194.
[3] Buchanan, S.K. *Curr. Opin. Struct. Biol.* **1999**, *9*, 455.
[4] Hancock, R.E.W.; Brinkman, F.S.L. *Annu. Rev. Microbiol*. **2002**, *56*, 17.
[5] Chipperfield, J.R.; Ratledge, C. *BioMetals* **2000,** *13*, 165.
[6] Guerinot, M. L., Meidl, E. J., Plessner, O. *J. Bacteriol.* **1990**, *172*, 3298.
[7] Lesueur, D., Diem, H. G., Meyer, J. M. *J. Appl. Bacteriol.* **1993**, *74*, 675.
[8] Frost, G.E., Rosenberg, H. *Biochim. Biophys. Acta* **1973**, *330*, 90.
[9] Wagegg, W., Braun, V. *J. Bacteriol.* **1981**, *145*, 156.
[10] Messenger, A. J., Ratledge, C. *J. Bacteriol.* **1982**, *149*, 131.
[11] Archibald, F.S., DeVoe, I. W. *Inf. Immun.* **1980**, *27*, 322.
[12] Simonson, C., Trivett, T., DeVoe, I. W. *Inf. Immun.* **1981**, *31*, 547.
[13] Cox, C. D. *J. Bacteriol.* **1980**, *142*, 199.
[14] Harding, R. A., Royt, P. W. *J. Gen. Microbiol.* **1990**, *136*, 1859.
[15] Lankfort, C. E., Walker, J. R., Reeves, J. B., Nabbut, N. H., Byers, B. R., Jones, R. J. *J. Bacteriol.* **1966**, 91, 1070.
[16] Byers, B. R., Powell, M. V., Lankford, C. E. *J. Bacteriol.* **1967**, *93*, 286.
[17] Budzikiewicz, H., Münzinger, M., Taraz, K., Meyer, J.-M. *Z. Naturforsch.* **1997***, 52c,* 496.
[18] Simpson, F.B., Neilands, J. B. *J. Phycol.* **1976**, *12*, 44.
[19] Clarke, S. E., Stuart, J, Sanders-Loehr, J. *Appl. Environ. Microbiol.* **1987**, *53*, 917.
[20] Goldman, S. J., Lammers, P. J., Berman, M. S., Sanders-Loehr, J. *J. Bacteriol.* **1983**, *156*, 1144.
[21] Lammers, P. J., Sanders-Loehr, J. *J. Bacteriol.* **1982**, *151*, 288.
[22] Akers, H. A. *Appl. Environm. Microbiol.* **1983**, *45*, 1704.
[23] Luckey, M., Pollack, J. R., Wayne, R., Ames, B. N., Neilands, J. B. *J. Bacteriol.* **1972**, *111*, 731.
[24] Rabsch, W., Winkelmann, G. *Biol Metals* **1991**, *4*, 244.
[25] Mullis, K. B., Pollak, J. R., Neilands, J. B. *Biochem.* **1971**, *10*, 4894.
[26] Milewska, M. J., Chimiak, A., Glowacki, Z. *J. Prakt. Chem.* **1987**, *329*, 447.
[27] Lee, B. H., Miller, M. J. *J. Org. Chem.* **1983**, *48*, 24.
[28] Hu, X., Boyer, G.L. *BioMetals* **1995**, *8*, 357.
[29] Plowman, J.E., Loehr, T.M., Goldman, S. J., Sanders-Loehr, J. *J. Inorg. Biochem.* **1984**, *20*, 183.
[30] Smarrelli, Jr. J., Castignetti, D. *Biochim. Biophys. Acta* **1986**, *882*, 337.
[31] Arceneaux, J. E. L., Davis, W. B., Downer, D. N., Haydon, A. H., Byers, B. R. *J. Bacteriol.* **1973**, *115*, 919
[32] Lochhead, A. G., Burton, M. O. *Canad. J. Bot.* **1953**, *31*, 7 (quoted in [34]).
[33] Burton, M. O., Sowden, F. J., Lochhead, A. G. *Canad. J. Biochem.* **1954**, *32*, 400 (quoted in [34]).

[34] Linke, W.-D., Crueger, A., Diekmann, H. *Arch. Microbiol.* **1972**, *85*, 44.
[35] Ghosh, A., Miller, M. J. *J. Org. Chem.* **1993,** *58*, 7652.
[36] Milewska, M. J., Chimiak, A., Neilands, J. B. *Z. Naturforsch.* **1991,** *46b,* 117.
[37] Budzikiewicz, H. *Curr. Top. Med. Chem.* **2001**, *1*, 73.
[38] Okujo, N., Sakakibara, Y., Yoshida, T., Yamamoto, S. *BioMetals* **1994**, *7*, 170.
[39] Wang, Q. X., Phanstiel, IV, O. *J. Org. Chem.* **1988,** *63*, 1491.
[40] Guo, H., Naser, S. A., Ghobrial, G., Phanstiel, IV, O. *J. Med. Chem.* **2002**, *45*, 2056.
[41] Barclay, R., Ewing, D. F., Ratledge, C. *J. Bacteriol.* **1985**, *164*, 896.
[42] Persmark, M., Pittman, P., Buyer, J. S., Schwyn, B., Gill, P. R., Neilands, J. B. *J. Am. Chem. Soc.* **1993**, *115*, 3950.
[43] Harris, W. R., Carrano, C. J., Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2722.
[44] Gibson, F., Magrath, D. I. *Biochim. Biophys. Acta* **1969**, *192*, 175.
[45] Van, Tiel-Menkveld, G. J., Mentjox-Vervuurt, J. M., Oudega, B., de, Graaf, F. K. *J. Bacteriol.* **1982**, *150*, 490.
[46] Warner, P. J., Williams, P. H., Bindereif, A., Neilands, J. B. *J. Infect. Immun.* **1981**, *33*, 540.
[47] McDougall, S., Nielands, J. B. . *J. Bacteriol.* **1984**, *159*, 300.
[48] Payne, S. M., Niesel, D. W., Peixotto, S. S., Lawlor, K. M. *J. Bacteriol.* **1983**, *155*, 949.
[49] Stuart, S. J., Prpic, J. K., Robims-Browne, R. M. *J. Bacteriol.* **1986**, *166*, 1131.
[50] Ishimaru, C. A., Loper, J. E. *J. Bacteriol.* **1992**, *174*, 2993.
[51] Okujo, N, Yamamoto, S. *FEMS Microbiol. Lett.* **1994**, *118*, 187.
[52] Buyer, J. S., de, Lorenzo, V., Neilands, J. B. *Appl. Environ Microbiol.* **1991**, *57*, 2246.
[53] de, Lorenzo, V., Martinez, J. L. *Eur. J. Clin. Microbiol. Infect. Dis.* **1988,** *7*, 621.
[54] Konopka, K., Bindereif, A., Neilands, J. B. *Biochemistry* **1982,** *21*, 6503.
[55] Maurer, P. J., Miller, M. J. *J. Am. Chem. Soc.* **1982**, *104*, 3096.
[56] Kunze, B., Trowitzsch-Kienast, W., Höfle, G., Reichenbach, H. *J. Antibiot.* **1992**, *45*, 147.
[57] Bergeron, R. J., Phanstiel, IV, O. *J. Org. Chem.* **1992***, 57*, 7140.
[58] Yamamoto, S., Okujo, N., Yoshida, T., Matsuura, S., Shinoda, S. *J. Biochem.* **1994**, *115,* 868.
[59] Haag, H., Fiedler, H.-P., Meiwes, J., Drechsel, H., Jung, G., Zähner, H. *FEMS Microbiol. Lett.* **1994**, *115*, 125.
[60] Münzinger, M., Taraz, K., Budzikiewicz, H. *Z. Naturforsch.* **1999**, *54c*, 867.
[61] Drechsel, H., Freund, S., Nicholson, G., Haag, H., Jung, O., Zähner, H., Jung, G. *BioMetals* **1993**, *6*, 185.
[62] Budzikiewicz, H. *Mini-Rev. Org. Chem.* **2004**, *1*, 163.
[63] Münzinger, M., Budzikiewicz, H., Expert, D., Enard, C., Meyer, J.-M. *Z. Naturforsch.* **2000**, *55c*, 328.
[64] Barbeau, K.; Zhang, G.; Live, D.H.; Butler, A. *J. Am. Chem. Soc.* **2002**, *124*, 378.
[65] Bergeron, R.J.; Huang, G.; Smith, R.E.; Bharti, N.; McManis, J.S.; Butler, A. *Tetrahedron* **2003**, *59*, 2007.
[66] Hickford, S. J., Kupper, F. C., Zhang, G., Carrano, C. J., Blunt, J. W., Butler, A. *J. Nat. Prod.* **2004**, *67*, 1897.
[67] Drechsel, H., Metzger, J., Freund, S., Jung, G., Boelaert, J. R., Winkelmann, G. *Biol. Metals* **1991,** *4*, 238.
[68] Thieken, A., Winkelmann, G. *FEMS Microbiol. Lett.* **1992**, *94*, 37.
[69] Reissbrodt, R., Erler, W., Winkelmann, G. *J. Basic Microbiol.* **1994**, *1*, K61.
[70] Drechsel, H., Jung, G., Winkelmann, G. *BioMetals* **1992,** *5*, 141.
[71] Carrano, C. J., Drechsel, H., Kaiser, D., Jung, G., Matzanke, B., Wimkelmann, G., Rochel, N., Albrecht-Gray, A. M. *Inorg. Chem*. **1996**, *35*, 6429.
[72] Carrano, C. J., Thieken, A., Winkelmann, G. *BioMetals* **1996,** *9*, 185.
[73] Kühn, S., Braun, V., KösterW. *J. Bacteriol.* **1996**, *178*, 496.
[74] Münzinger, M., Taraz, K., Budzikiewicz, H., Drechsel, H., Heymann, P., Winkelmann, G., Meyer, J.-M. *BioMetals* **1999,** *12*, 189.
[75] Meiwes, J., Fiedler, H.-P., Haag, A., Zähner, H., Konetschny-Rapp, S., Jung, G. *FEMS Microbiol. Lett.* **1990**, *67*, 201.
[76] Konetschny-Rapp, S., Jung, G., Meiwes, J., Zähner, H. *Eur. J. Biochem.* **1990,** *191*, 65.

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