Current Trends in Microbiology
Vol. 16, 2022

Review

The history of siderophores

Günther Winkelmann*
Institute of Microbiology and Biotechnology, University of Tuebingen, Morgenstelle 28, 72076 Tuebingen, Germany.

ABSTRACT

From the beginning of 1952 until today many laboratories and scientists have reported on the function and occurrence of iron-binding molecules in fungi and bacteria which we call siderophores (iron carrier). Although these compounds were initially described as growth-promoting agents in microbes, it turned out that they are excreted for collecting iron from the environment under iron-limiting conditions. Iron uptake systems are essential to survive for all kinds of organisms. This review on the history of siderophores lists some of the most characteristic siderophore structures and the authors involved in their detection and description. The finding of siderophores and their membrane receptors are reported and cited consecutively, giving an overview of the state of knowledge. In addition it has finally a focus on the contribution of siderophore research at the laboratory in Tuebingen, Germany, where some of the novel siderophore structures have been elucidated.

KEYWORDS: siderophore detection, siderophore structures.

INTRODUCTION

All begun with the detection of fungal growth factors like coprogen [1], ferrichrome [2] and ferrichrome A [3], which turned out to be iron complex compounds containing hydroxamate ligands which were later named sideramines or siderochromes but eventually named siderophores (iron-carrier). A growth factor activity was found in *Bacillus megaterium* [4, 5] named schizokinen, the structure of which turned out to be a hydroxamate with iron transport properties. These first observations paved the way for further intensive search of new siderophore structures. Aerobic life is not possible without iron, a statement which is generally accepted with only few exceptions in the lactobacterial groups, where manganese seems to fullfil some enzymatic roles. Today most of the aerobic bacteria excrete siderophores to capture iron from the environment. This is not a continuous activity of iron uptake, but as soon as iron is taken up in sufficient amounts uptake is halted. The mechanism behind this regulated siderophore uptake in bacteria is the ferric uptake regulation (Fur) which was coined by Joachim Ernst in 1978 during his postdoctoral stay in the lab of Bennet and Rothfield [6]. Cloning of the Fur gene in *E. coli* was subsequently obtained by Hantke [7]. A recent review of the group of Michaud-Soret described some molecular details of *Fur* in the pathogen *Francisella tularensis* [8].

Siderophore production in various microbial genera

The history of new siderophore structures continued at several places and the transport of hydroxamate and catecholate siderophores were studied within many microbial genera [9]. Siderophores of *Bacillus antracis*, *Bacillus cereus* and *Bacillus thuringiensis* revealed new structures like bacillibactin and petrobactin [10]. Among the fungal siderophores fusigen [11] and fusarinines were elaborated by the group of Emery [12] and van der Helm [13]. Siderophore transport genes in *Sacharomyces cerevisiae* were reported as members of the major facilitator superfamily [14, 15, 16, 17], although

*Email id: winkelmann@uni-tuebingen.de

Saccharomyces does not synthesize own siderophores. Mycobactins, carboxymycobactins and exochelins, were described by Snow [18] and Ratledge [19], while vibriobactin was described by Payne [20]. Enterobactin was first described by Pollack and Neilands [21] and aerobactin in *E.coli* was described by deLorenzo *et al.* [22]. Marinobactins were studied by Butler [23] and protochelin by Duhme [24] with the group of Hider at King's college in London. Peptide sequences of a variety of Pyoverdines from different *Pseudomonas* strains were studied by the group of Budzikiewicz and Meyer [25] which are now continued by Isabelle Schalk [26] at Illkirch, France. Novel ornibactins (Fig. 1) with different side-chain length, were identified as a new family of siderophores [27] from Pseudomonas strains which are now classified as *Burkholderia* species. It is interesting to note that the marinobactins have a similar basic structure like the ornibactins. In San Francisco at the department of chemistry in Berkeley, many students with Ken Raymond worked with siderophores and its analogs. One of his students was Carl Carrano who solved the structure of rhodotorulic acid and its Fe-complex isolated from the yeast *Rhodotorula pilimanae* [28].

Details of the transport mechanisms

Iron transport and metal regulation in *E. coli* was the main topic of the group of Braun and Hantke [29] in Tuebingen identifying the transport proteins of the outer membrane, the cytosolic membrane and periplasmic transport proteins for ferric hydroxamates, like FhuA, FhuE, FoxA, the periplasmic FhuBCD and the Fec system. A major breakthrough was the identification of beta-barrel structure of the outer membrane siderophore transporting channels. The crystal structure of FhuA with bound lipopolysaccharide was solved in 1998 by Ferguson *et al.* [30] and the structural basis of gating by FecA was also achieved by Ferguson *et al.* [31]. A detailed description of the structures of siderophore receptors was presented by van der Helm and Chakraborty in the book Microbial Transport Systems [32]. Based on crystallographic data Hans Vogel presented a complete structure of the FhuD-bound coprogen and antibiotic albomycin [33]. A beta-barrel finder (BBF) program allowing identification of outer membrane beta-barrel proteins encoded within prokaryotic genomes was published by Zhai and Saier [34].

Siderophores as a treatment for iron overload

Looking at old siderophore structures a group of chemists and biologists at the ETH Zürich in Switzerland elucidated many new hydroxamates of the ferrichrome-type, like, ferricrocin, ferrichrysin, ferrirhodin and ferrirubin. Most interesting was the finding of the ferrioxamines by studying the group of the Streptomycetes [35] by Bickel *et al.* which finally resulted in the production of desferrioxamine B (DFOB). By making it more water soluble as a methane sulfonate it was named Desferal^{R} which was highly suitable for the treatment of iron overload in patients suffering with Hemochromatosis, Thalassemia or transfusional overload of iron and aluminum. Desferal turned out to be more specific for ferric iron than ethylenediaminotetracetic acid (EDTA) and was highly tolerable in the body. After intravenous injection of Desferal most of the depot iron could be excreted *via* the urine.

The group in Tuebingen started with the fungus *Neurospora crassa* finding a mutant deficient in

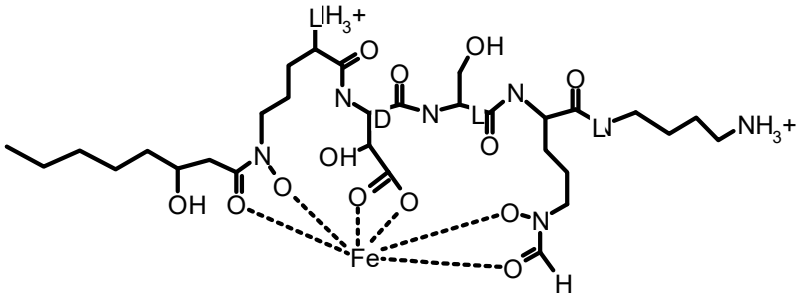


Fig. 1. Fe-Ornibactin C8.

ornithine transaminase that was unable to synthesize the siderophore coprogen [36]. Besides coprogen [37] several other siderophores, like ferrichrome, ferricrocin and ferrichrysin were taken up by this fungus. However, a synthetic ferrichrome having an enantio configuration around the metal center was not recognized when compared to the natural compound [38]. This led to the view that a correct lambda-cis configuration is required for recognition during uptake or subsequent enzymatic utilization in fungi. Most of the known siderophores could be separated at that time by high pressure liquid chromatography (HPLC) [39].

Siderophores from the Tuebingen lab

This review will not summarize all the transport studies of new siderophores in various laboratories, but will focus on some recent novel structures from the Tuebingen Lab, like ornibactin (Fig. 1), salmochelin (Fig. 2), yersinabactin, (Fig. 3) as well as rhizoferrin and glomuferrin (Fig. 4) which inspired many follow up publications. As shown in Fig. 2 low-iron cultures of *Salmonella enterica* generally produce a set of different salmochelin compounds that besides salmochelin (S4) contains di-glucosyl (S2) and mono-glucosyl (S1) derivatives. Although enterobactin was initially regarded as the main catecholate siderophore of *E. coli*, its pathogenicity was reduced due to high lipophilicity and binding by the innate siderocalins like lipocalin (Lan-2) produced by macrophages and epithelial cells. The structure of the salmochelins [40, 41] were solved, which are produced by *Salmonella enterica* species and *E. coli* UPEC strains carrying the iro A gene. Thus salmochelins are glucosylated enterobactins containing two C-glucosyl residues. The production of salmochelin is dependent on the presence of the outer membrane receptor IroN, encoded by a iroBCDEN gene cluster. IroD and IroE have been identified as siderophore esterases [42]. A glcosyltransferase is located on iroB and the export through the cytosolic membrane is encoded by iroC [43]. The high water solubility prevents from binding by siderocalins and serum albumin. This makes Salmonella species more pathogenic and enables UPEC strains to provoke urinary tract infections. Many publications recently appeared reporting on the occurence and functions of iron-free and iron-containing salmochelins [44, 45]. The breakdown products of salmochelin, the

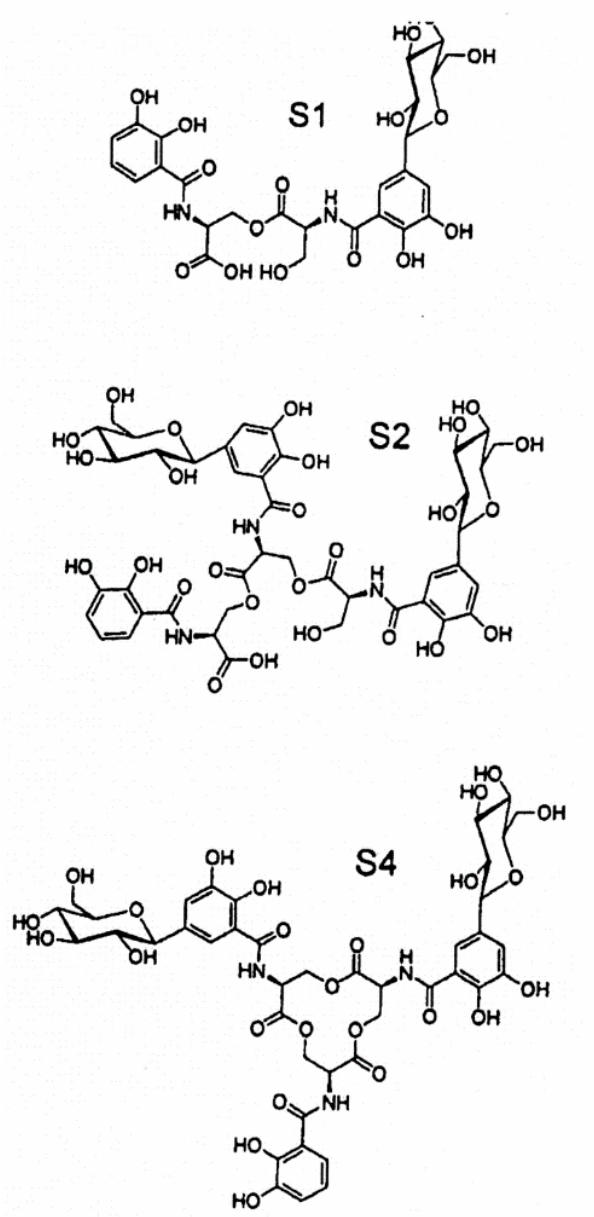


Fig. 2. Salmochelin S4 and its hydrolysis products Salmochelin S1 and S2.

mono-glycosylated or diglycosylated forms, may also bind to iron and behave as further iron carriers in egg white, as shown by the group of Anrews [46]. A further novel siderophore identified by the Tuebingen group was Yersiniabactin (Fig. 3) isolated from *Yersinia enterolytica* [47]. The structure elucidation was made from a genetic construct with *E. coli* that turned out to be a thiazoline derivative. Yersiniabactin was recently also identified in *Klebsiella* [48]. Rhizoferrin (Fig. 4), a novel

siderophore originally isolated from the fungus *Rhizopus microsporus* [49, 50], was also found in most Mucorales (Zygomycetes) where no hydroxamates are synthesized [51]. The coordination chemistry has been described by Carrano *et al*. [52]. Rhizoferrin was subsequently also detected in a bacterial strain, *Ralstonia picckettii*, containing an *S,S* configuration [53] which differed from the *R,R*–configuration of the fungi. Rhizoferrin was subsequently found in a pathogenic species, *Legionella pneumophila*, named Legiobactin [54]. The pathogen *Francisella tularensis* also produces rhizoferrin [55]. Staphyloferrin A, produced by Staphylococcus strains has a similar structure, where the diamino backbone is replaced by ornithine [56].

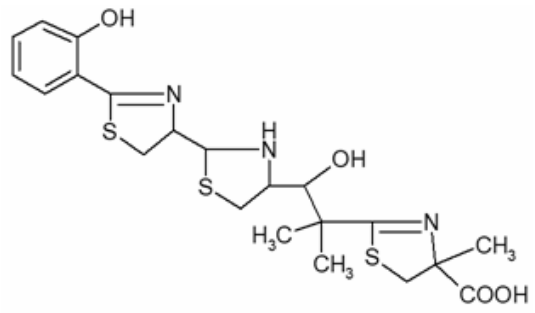
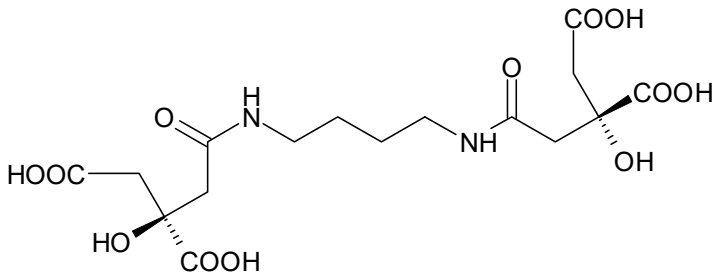


Fig. 3. Yersiniabactin.

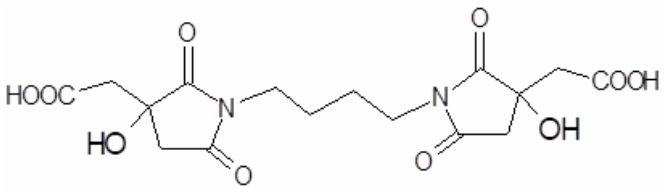
As shown previously, the fungal group of Zygomycetes is so far the only fungal group that is able to synthesize rhizoferrin for iron nutrition, while most other fungi like Ascomycetes and Basidiomycetes use hydroxamate siderophores [57, 58]. However, in recent times the lab in Tuebingen focussed on the occurence of rhizoferrin in plant roots and detected rhizoferrin and one of its dehydration products, glomuferrin [59, 60], in plant roots of *Tagetes nana*. As shown in Fig. 4 rhizoferrin may occur together with two dehydration products, imido-rhizoferrin and bis.imido-rhizoferrin. The latter was named Glomuferrin due to its detection in the plant roots after inoculation with *Glomus* spores. Thus, arbuscular mycorrhizal fungi synthesize rhizoferrin and its dehydration products during intracellular symbiosis. Aproximately 70% of vascular plants live in symbiosis with arbuscular mycorrhizal fungi. Although the wide-spread hydroxamate siderophores of Ascomycetes and Basidiomycetes possess higher formation constants with ferric iron, they are not produced by arbuscular mycorrhizal fungi.

CONCLUSION

This short history of siderophores could not list all novel siderophore structures that have appeared in recent times and could not mention all laboratories and scientists that are involved, but represents a



Rhizoferrin



Glomuferrin (= Bis-imido-rhizoferrin)

Fig. 4. Rhizoferrin which may change to Glomuferrin after dehydration.

very personal overview of the development and impact of small molecules that control life and growth of microorganisms. In the future many new siderophores will be found which will help to understand their basic role in various ecosystems.

ACKNOWLEDGEMENTS

The author thanks Klaus Hantke for reading the manuscript and supporting with strain and suggestions.

CONFLICT OF INTEREST STATEMENT

There exist no conflict of interest.

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