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The specificity of bacterial siderophore receptors probed by bioassays

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Summary. The ability to utilize siderophores of bacterial and fungal origin has been studied in wild-type and mutant strains of the enterobacterial genera *Salmonella, Escherichia, Shigella, Moellerella, Klebsiella, Enterobacter, Hafnia, Pantoea, Ewingella, Tatumella, Yersinia,* and in the non-enterics *Aeromonas, Pseudomonas* and *Aureobacterium*. Although only a few representative strains were tested, the results show characteristic genus-specific differences in the utilization of hydroxamate and catecholate siderophores. Moreover, the different response to structural alterations of certain siderophore classes by some wild-type and mutant strains points to variable interacting receptor domains.

Key words: Siderophores - Bacterial iron transport - Siderophore receptors

Introduction

Bioassays for the detection of trace amounts of siderophores had already been used long before the chemical structures of the various siderophores were known and they are still the most sensitive analytical methods. One of the widely used indicator strains for the detection of hydroxamate siderophores is the siderophore-auxotrophic soil bacterium *Arthrobacter flavescens* JG9 (Lochhead and Burton 1956) which is now called *Aureobacterium flavescens* JG9. This strain grows in the presence of hydroxamate siderophores but does not respond to catecholate siderophores produced by enteric bacteria. Therefore, this strain has been primarily used for the detection of fungal siderophores (Moore and Emery 1976; Powell et al. 1980; Bossier and Verstraete 1986a, b). The type of hydroxamate (mono-, di-, or trihydroxamate) and the overall structure of the ligand seems to be of minor importance for siderophore recognition in this strain.

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Uptake of siderophores and utilization of siderophore-bound iron has been shown to be dependent on outer membrane receptors and on binding-protein-dependent transport systems in the cytoplasmic membrane of Gram-negative bacteria (Braun et al. 1991; Köster 1991). Every siderophore utilized by *Escherichia coli* has its corresponding outer membrane receptor: ferric enterobactin (FepA), ferric citrate (FecA), ferrichrome (FhuA), coprogen (FhuE), aerobactin (Iut) and other catecholates (Cir and Fiu) (Hantke 1990). A FoxA receptor protein for the transport of ferrioxamines in strains of *Enterobacter agglomerans* (*Erwinia herbicola*), now reclassified as *Pantoea agglomerans* (Gavini et al. 1989), has recently been detected (Berner and Winkelmann 1990). *Pseudomonas aeruginosa* has been shown to take up pyoverdins (Hohnadel and Meyer 1988) and, after induction, also enterobactin (Poole et al. 1990). A ferric-pseudobactin receptor (PupA) has been identified in *Pseudomonas putida* WCS358 which shows considerable similarity to the FhuE receptor of *E. coli* (Bitter et al. 1991). Although Gram-positive bacteria lack an outer membrane, transport of siderophores has been well documented in species of several genera. For example, Gram-positive bacteria are known to take up schizokinen in *Bacillus* (Mullis et al. 1971), mycobactins and exochelins in *Mycobacterium* (Hall and Ratledge 1987), ferrioxamines in *Streptomyces* (Müller and Raymond 1984), and the recently described staphyloferrins in *Staphylococcus* (Konetschny-Rapp et al. 1990). However, only in the case of *Mycobacteria* have specific envelope proteins been associated with siderophore iron uptake (Sritharan and Ratledge 1990; Hall et al. 1987).

Materials and methods

Strains used. Most mutant strains of *Salmonella* and *Escherichia* represent *ent* or *aroB* strains which are unable to synthesize enterobactin. The mutants *Salmonella typhimurium* LT2 *enb-7* and *S. typhimurium* TA 2700 were kindly provided by J. B. Neilands (Berkeley, CA). *S. typhimurium* HR15 is a mutant derived from the parent SL1027/23 (Braun et al. 1977). *E. coli* strains H1728,

H1774, H1875, H1876, H1877, and H1882 were from K. Hantke (Tübingen). *E. coli* AB2847 and the derived *fhuA* mutant P8, was provided by V. Braun (Tübingen). *E. coli* LG1522 was a gift from P. Williams (Leicester). *Aeromonas hydrophila* SB22 was from the laboratory of R. Byers, provided by S. Barghouthi (Jackson, MS), *Shigella flexneri* SA 255 was a gift from S. Payne (Austin, TX) and *Yersinia enterocolitica* 96C, a plasmid-cured 0:9 strain, was obtained from J. Heesemann (Würzburg). *Pseudomonas aeruginosa* PAO 6609 was from J.-M. Meyer (Strasbourg, France). All other strains mentioned originated either from the CDC Atlanta, kindly provided by J. J. Framer III, or were from the stock of our institutes.

Chemicals and siderophores. Ferrirhodin was isolated from *Botrytis cinerea* (Konetschny et al. 1988). Ferrirubin was isolated from a strain of *Penicillium variabile*. Ferrichrome A was isolated from *Ustilago sphaerogena*. Propionyl-, and butyryl-ferrichrome were kindly provided by W. Keller-Schierlein (Zürich). Des(diserylglycyl)ferrirhodin was a gift from D. van der Helm (Norman, OK). Ferrioxamines E and G_1 were isolated from *Pantoea* and *Hafnia* strains respectively (Berner et al. 1988; Reissbrodt et al. 1990). Coprogen was isolated from *Neurospora crassa* as described earlier (Wong et al. 1983). Arthrobactin was from H. Diekmann (Hannover). Schizokinen and amonabactin (T+P) were obtained from R. Byers (Barghouthi et al. 1989). Aerobactin was prepared according to Braun (1981). Nannochelin B and myxochelin A (Kunze et al. 1989) were kindly provided by H. Reichenbach (GBF, Braunschweig). Structural formulaes of the relevant siderophores are shown below. The reader is also referred to two recent comprehensive books which describe structures and functions of siderophores in greater detail (Winkelmann et al. 1987; Winkelmann 1991).

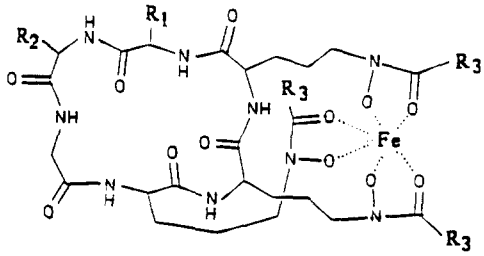
Bioassays. Siderophore assays were performed on Tris succinate agar (Reissbrodt and Rabsch 1988), or on Tris-M9-agar, containing per litre: 12.1 g Tris, 0.3 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl, 1 mM $MgSO_4 \cdot 7H_2O$ (autoclaved separately), 0.1 mM $CaCl_2 \cdot 2H_2O$ (autoclaved separately), 4.0 g glucose (autoclaved separately), 1 mM ethylenediamine-*N,N'*-bis(2-hydroxyphenylacetic acid) (EDDHA) and 4.0 g agar, pH 7.0 (Berner and Winkelmann 1990). Bioassay medium (10 ml) was inoculated with 0.3 ml of an overnight culture grown in nutrient broth. The bioassay with *Aureobacterium flavescens* JG9 was performed without EDDHA at 27° C in a medium containing per litre: 2 g K_2HPO_4, 0.5 g $(NH_4)_2HPO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 1.0 g yeast extract, 4 g glucose (autoclaved separately). Liquid precultures need 0.1 µg of a hydroxamate siderophore. Filter discs (6 mm diameter) containing 10 µl methanolic or aqueous solutions of siderophores were used. Concentrations of siderophores were adjusted to $\Delta A_{436} = 0.1$ (hydroxamates) and to $\Delta A_{578} = 0.1$ (catecholates).

Results and Discussion

A collection of wild-type and mutant strains (Table 1) was used in the present investigation to determine the utilization of hydroxamate and catecholate sidero-

Table 1. Strains used for siderophore bioassays

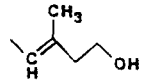
Strain	Parent	Relevant genotype biosynthesis	receptor	Reference or source
Salmonella				
S. typhimurium LT2 enb-7	LT2	*ent-7*		Pollack et al. (1970)
S. typhimurium TA2700	LT2 enb-7	*ent-1,*	*sidA1*	Luckey et al. (1972)
S. typhimurium SR1001	LT2 enb-7	*ent-7,*	*tonB*	Rabsch et al. (1991)
S. typhimurium HR 15	SL1027/23	*fhuA*		this paper
S. stanleyville GRR32	207/81	*fepA*		Rabsch et al. (1991)
S. stanleyville GRR17	207/81			this paper
Escherichia, Moellerella, Shigella				
E. coli LG 1522	AN263	ColV-K30	*fepA, fhuA*	Carbonetti & Williams (1984)
E. coli AB2847		*aroB*		Winkelmann & Braun (1981)
E. coli VR 42/B9	AB2847	*aroB*	*fepA, cir*	Hancock et al. (1977)
E. coli P8	AB2847	*aroB*	*fhuA*	Hartmann & Braun (1979)
E. coli H1728	H1443	*aroB*	*fiu, cir*	Hantke (1990)
E. coli H1774	H1443	*aroB*	*fhuE*	Hantke, unpublished
E. coli H1875	H1443	*aroB*	*fepA, cir*	Hantke (1990)
E. coli H1876	H1443	*aroB*	*fepA, fiu, cir*	Hantke (1990)
E. coli H1877	H1443	*aroB*	*fepA, fiu*	Hantke (1990)
E. coli H1882	MC4100	Δ (*ent*)	Δ *fep*	Hantke, unpublished
S. flexneri SA255	SA 100	*iuc*	*iut*	Lawlor and Payne (1984)
M. wisconsensis		wild type		CDC 289-78
Klebsiella				
K. pneumoniae KN4401	DL44	*ent, iuc*	*iut*	Williams et al. (1989)
K. ozeanae		wild type		CDC 404-68
K. rhinoskleromatis		wild type		CDC 417-68
Aeromonas hydrophila SB22	495A2	*amoA*		Barghouti et al. (1990)
Aureobacterium flavescens JG9		hydroxamate auxotroph		ATCC 29091
Pantoea agglomerans (*E. herbicola*) K4		wild type		Berner & Winkelmann (1990)
Pantoea agglomerans (*E. herbicola*) FM13		*foxA*		Berner & Winkelmann (1990)
Ewingella americana		wild type		CDC 1468-78
Hafnia alvei 7473		wild type		Reissbrodt et al. (1990)
Pseudomonas aeruginosa PAO 6609		pyoverdine-negative		Meyer unpubl.
Tatumella ptyseos		wild type		CDC 9556-78
Yersinia enterocolitica Y-96C				Heesemann (1987)

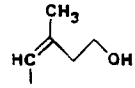


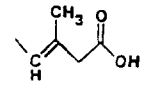
Ferrichrome: $R_1 = R_2 = H$ $R_3 = CH_3$

Propionylferrichrome: $R_1 = R_2 = H$ $R_3 = CH_2\text{-}CH_3$

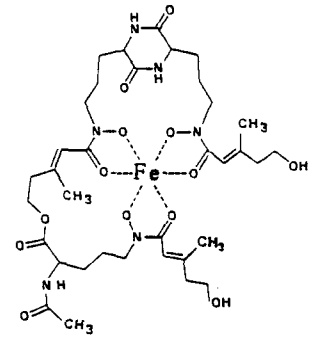
Butyrylferrichrome: $R_1 = R_2 = H$ $R_3 = CH_2\text{-}CH_2\text{-}CH_3$

Ferrirubin: $R_1 = R_2 = CH_2OH$ $R_3 =$ 

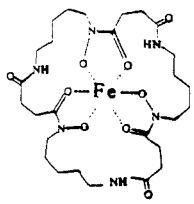
Ferrirhodin: $R_1 = R_2 = CH_2OH$ $R_3 =$ 

Ferrichrome A: $R_1 = R_2 = CH_2OH$ $R_3 =$ 

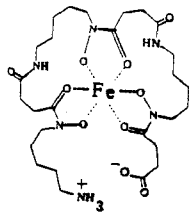
Des(diserylglycyl)ferrirhodin: lacking the amino acid sequence ser-ser-gly



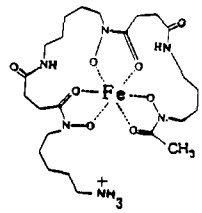
Coprogen



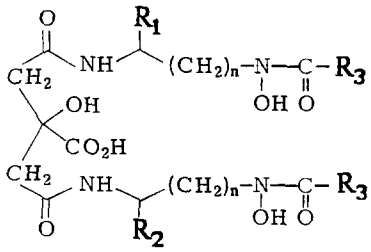
Ferrioxamine E

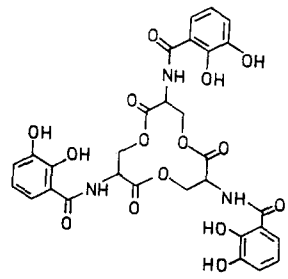


Ferrioxamine G_1

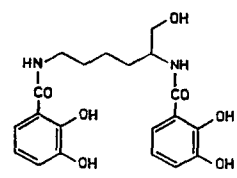


Ferrioxamine B



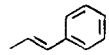


Enterobactin



Myxochelin A

Aerobactin: $R_1 = R_2 = COOH$ $R_3 = CH_3$ $n = 4$

Nannochelin B: $R_1 = COOH$ $R_2 = COOCH_3$ $R_3 =$  $n = 4$

Arthrobactin: $R_1 = R_2 = H$ $R_3 = CH_3$ $n = 4$

Schizokinen: $R_1 = R_2 = H$ $R_3 = CH_3$ $n = 2$

phores. Most of the strains tested belong to the family of Enterobacteriaceae which are known to take up siderophores via outer membrane receptors. Thus, strains of the genera *Salmonella, Escherichia, Shigella, Moellerella, Klebsiella, Ewingella, Hafnia, Yersinia* and *Pantoea* (*Enterobacter/Erwinia*) have been investigated in this study. In addition, one strain each of the non-enterics *Aeromonas* and *Pseudomonas,* as well as the Gram-positive *Aureobacter flavescens* JG9, were included. Of particular value are those strains which are blocked in their own siderophore biosynthesis. Strains possessing an *ent* or *aroB* mutation are unable to synthesize enterobactin unless a precursor is added. These strains allow a clear interpretation of exogenous siderophore-iron uptake as inter-ligand exchange of iron with its own siderophore is excluded. However, even in wild-type strains, a dense growth zone often indicates utilization of exogenously supplied siderophores. This is because inter-ligand iron exchange is a very slow event at neutral pH (Tuffano and Raymond 1981; Emery 1986). In cases where poor growth is observed and long incubation periods are required, one has to consider inter-ligand exchange events. Some of the strains used are receptor-deficient. Some strains which have been screened for that purpose earlier have been included as a proof for the presence of a receptor-mediated route. For example, strains exhibiting *fhuA* and *fepA* mutations are unable to take up ferrichrome and enterobactin siderophores, respectively. Thus, *S. typhimurium* TA2700 is unable to take up any hydroxamate siderophore but still utilizes the catecholates amonabactin, enterobactin and myxochelin A. While ferrichrome and enterobactin have been studied earlier most extensively, some analogues and derivatives of ferrichrome, as well as some novel catecholates such as myxochelin A and amonabactin, have not been tested so far. These siderophores may therefore be suited to characterize further the already known uptake systems or even point to new receptors within the membrane.

As shown in Table 2, the various enterobacterial genera show certain common characteristics regarding the utilization of siderophores. The *Salmonella typhimurium* strains tested in this study are generally able to utilize ferrichromes, ferrioxamines and catecholates. This is in contrast to *Escherichia, Shigella* and *Moellerella* which are generally unable to use ferrioxamines. Although earlier reports have indicated some utilization of ferrioxamine B in *E. coli*, this did not hold true when the concentration of the ferrioxamine solution on filter dics was lowered to 0.03 μmol/ml ($\Delta A = 0.1$) or

Table 2. Siderophore utilization by selected bacterial wild type and mutant strains

Strain	F	PF	BF	Fr	Fh	FA	HA	DF	FE	FG	FB	Co	Ar	Sk	NB	Ae	Eb	MA	Am	Db
S. enb-7	+	+	+	+	(+)	−	−	−	+	+	+	+	−	−	−	−	+	+	+	+
S. TA2700	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
S. SR1001	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
S. GRR32	+	+	+	+	(+)	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
S. GRR17	+	+	+	+	(+)	−	−	−	+	+	+	(−)	−	−	−	−	+	+	−	−
S. HR15	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	(−)	−	−	−
E. coli AB2847	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	+	+	+	+
E. coli H1882	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
E. coli H1774	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
E. coli VR42/B9	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+
E. coli P8	(−)	−	+	+	−	−	−	−	−	−	−	+	−	−	−	−	+	+	+	+
E. coli LG1522	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−
Shig. SA255	+	+	+	+	(+)	−	−	−	−	−	−	−	−	−	−	(−)	+	−	−	−
M. wiscons.	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
K. KN4401	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
K. ozeanae	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+
K. rhinoskl.	+	+	+	+	+	+	+	+	+	+	+	−	(+)	−	+	+	−	(+)	−	−
E. americana	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	−	−	−
T. ptyseos	+	+	+	+	+	+	+	−	+	(+)	+	−	−	−	−	−	+	−	+	−
H. alvei 7473	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	+	+	−	−
P. agglommerans K4	+	+	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	+	+	−
P. agglommerans FM13	+	+	+	+	+	−	−	−	(−)	(−)	(−)	+	−	−	−	−	+	+	+	−
Y. enter. 96C	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	+	+	+	−
A. hydr. SB22	+	+	+	−	−	−	−	−	+	(+)	+	−	−	−	−	−	(+)	+	+	−
Ps. aeruginosa	+	+	+	+	+	−	−	+	+	(+)	(+)	+	+	+	+	+	+	(+)	+	−
A. flav. JG9	+	+	+	(+)	(+)	(+)	+	−	+	+	+	+	+	+	−	−	−	−	−	−

Siderophore utilization was determined in agar plates as described in Materials and methods. Growth promotion was recorded after 24 h of growth at 27° C or 37° C. Symbols: − no visible growth, (+) poor growth (7–8 mm), + good growth (12–20 mm), (−) selected mutation.

Siderophores: F = ferrichrome, PF = propionic acid derivative of ferrichrome, BF = butyric acid derivative of ferrichrome, Fr = ferrirubin, Fh = ferrirhodin, HA = hexahydroferrichrome A, FA = ferrichrome A, DF = des(diserylglycyl)ferrirhodin, FE = ferrioxamine E, FG = ferrioxamine G, FB = ferrioxamine B, Co = coprogen, Ar = arthrobactin, Sk = schizokinen, NB = nannochelin B, Ae = aerobactin, Am = amonabactin (P + T), Db = dihydroxybenzoic acid, Eb = enterobactin, MA = myxochelin A

less, as was routinely used in the present investigation. Most strains of *Salmonella, Escherichia* and *Shigella* failed to utilize the neutral citrate-based hydroxamates, such as arthrobactin, schizokinen and nannochelin B. However, the plasmid- and chromosomally-encoded utilization of the negatively charged citrate-based aerobactin in these strains has been well documented (Payne 1988). *Klebsiella* strains can be characterized by their ability to utilize most of the supplied siderophores (ferrichromes, ferrioxamines and enterobactin) but were unable to use amonabactin. This applies also to *Hafnia* and *Ewingella*. A ferrioxamine receptor-deficient *foxA* mutant of *P. agglomerans* (*Erwinia herbicola* FM13), has been included to confirm the specificity of ferrioxamine uptake (Berner and Winkelmann 1990). *Yersinia enterocolitica* 96C, grown in a bioassay containing nutrient broth, was able to utilize a variety of siderophores, such as enterobactin, myxochelin A, the ferrichromes and ferrioxamines but cannot grow with coprogen as an iron source. The Gram-positive *A. flavescens* JG9 revealed the expected positive results with most hydroxamates, with the exception of ferrirubin, and des(diserylglycl)ferrirhodin. Catecholates are generally not recognized by this strain. It is interesting to note that ferrichrome A and its hydrogenated form hexhydroferrichrome A are excluded from utilization in the genera *Salmonella* and *Escherichia* but are well utilized by *Klebsiella, Ewingella* and *Tatumella*. On the other hand, the ferrichrome derivatives propionyl-ferrichrome and butyryl-ferrichrome seem to be good substrates for all ferrichrome-utilizing bacteria.

Apart from these general characteristics of siderophore utilization in different genera, we observed some more specific behaviour in certain strains. For example, the mutant *E. coli* P8, a *fhuA* mutant derived from the parent AB2847 (Hartmann and Braun 1979) is unable to utilize ferrichromes (ferrichrome, ferricrocin). This mutant is still able to utilize butyryl-ferrichrome and ferrirubin, suggesting a residual recognition capacity for certain ferrichrome-type siderophores. The better utilization of ferrirubin compared to ferrirhodin by most *S. typhimurium* strains corresponds well with an earlier observation of Luckey et al. (1972). Although *M. wisconsensis* utilizes ferrichrome and its propionyl and butyryl derivatives, the more bulky ferrichromes (ferrirubin, ferrirhodin, ferrichrome A) as well as ferrioxamines or coprogen were not accepted. It is also noteworthy that several enterobacterial strains, such as *Shigella, Moellerella, Tatumella, Hafnia* and *Yersinia* were found to be unable to utilize coprogen. *Pseudomonas aeruginosa* has been reported earlier to utilize exogenous siderophores, e.g. aerobactin and enterobactin (Liu and Shokrani 1978; Poole et al. 1990). As shown here, *P. aeruginosa* PAO 6609, although unable to produce ferrioxamines, was still able to utilize various ferrioxamines. It has been shown earlier that *P. stutzeri* is able to produce and utilize ferrioxamine E (Meyer and Abdallah 1980). The monomeric hydrolysis product of enterobactin, dihydroxybenzoylserine, functions as a siderophore in *E. coli* by using the Cir and Fiu outer membrane receptor proteins (Hantke 1990). We have shown here that amonabactins (P+T) are also transported via Cir and Fiu receptors in *E. coli* (Table 3). The only *E. coli* mutants (H1728, H1876) which failed to show a growth response are defective in both Cir and Fiu receptors. Deletion in one of these receptors, as shown by the mutants H1877 and H1875, was insufficient to prevent utilization. In most enterobacterial genera concomitant utilization of both amonabactin via Cir or Fiu and enterobactin via Fep A was observed. This was also found with *Aeromonas hydrophila*, the producer of amonabactin, in which Cir- and Fiu-like receptors and an enterobactin receptor (FepA) seem to be present. Myxochelin A utilization seems likewise to be closely correlated with the presence of an enterobactin receptor although we noted some exceptions, e.g. *Klebsiella, Ewingella, Shigella, Hafnia* and *Tatumella*.

Table 3. Identification of the outer membrane receptor for amonabactin

E. coli strain	Relevant genotype	Growth response
AB2847	*aroB*	+
H1774	*fhuE*	+
H1728	*fiu, cir*	−
H1876	*fiu, cir, fepA*	−
H1877	*fiu, fepA*	+
H1875	*cir, fepA*	+

Growth response was measured as described in Materials and methods: 5 µl of an amonabactin (P+T) solution in methanol (1 mg/ml) was pipetted on filter paper discs and growth response was read after a 24-h incubation at 37° C

The present investigation is a comparative study on siderophore utilization in bacteria. It clearly shows the enormous potential of siderophore utilization and the specificity of siderophore recognition in bacteria. Thus, some of the enterobacterial genera can be differentiated by their property to utilize certain siderophore classes. For example, the genera *Salmonella, Escherichia/Shigella* and *Klebsiella* can be distinguished by a simple siderophore-utilization test containing ferrichrome A, ferrioxamine E and ferrichrome as a positive control. Ferrioxamine E is utilized by *Salmonella* and *Klebsiella* species, but not by *Escherichia* and *Shigella*. On the other hand, ferrichrome A is denied by *Salmonella* and *Escherichia/Shigella* but is accepted as an iron source by *Klebsiella*. Further studies will be required to confirm if this holds true for all species within these genera.

Some mutant strains within these genera may deviate from this scheme which might indicate a kind of recognition variability of siderophore receptor domains. For example, receptors which have been mutated and selected by albomycin resistance may still retain the ability to recognize certain ferrichrome-type siderophores, as has been shown with butyryl-ferrichrome or ferrirubin in *E. coli* P8 and *E. coli* LG1522. This opens further opportunities to map receptors with synthetic siderophore derivatives which will possibly help to discover how siderophore molecules are attached to or interact with membrane siderophore recep-

tors. Thus we have recently shown that chiral linear analogues of ferrioxamines may utilize both FoxA and FhuA receptors (Berner et al. 1991). It therefore appears that different genera and species may contain analogous but not completely homologous receptor domains for siderophore recognition.

Conclusion

Although only a small collection of strains have been analyzed in the present investigation, certain characteristic features seem to be obvious. For example, within the faimily of Enterobacteriaceae, the three genera *Salmonella, Escherichia/Shigella* and *Klebsiella* can be separated by a simple three-siderophore-utilization test (e.g. ferrichrome, ferrioxamine E, ferrichrome A), provided that low siderophore concentrations (<0.03 μmol/disc) are used. Whether further siderophore utilization tests can be established for the differentiation of non-enterics has to be elaborated with a larger collection of strains. The nature of siderophore receptor domains involved in siderophore recognition and their interaction with specifically designed siderophores are currently under investigation.

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