



Mingang Zhu (Autor)

**Salmochelin, a new siderophore of *E. coli*:
biosynthesis, transport and utilization**

Salmochelin, a new siderophore of
E. coli: biosynthesis, transport and
utilization

Mingang Zhu



Cuvillier Verlag Göttingen

<https://cuvillier.de/de/shop/publications/2552>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,
Germany

Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: <https://cuvillier.de>

Index

1.Introduction	1
1.1 The cell wall of bacteria	1
1.2 The outer membrane of Gram-negative bacteria	1
1.2.1 Diffusion	1
1.2.2 Facilitated diffusion	2
1.2.3 Active transport	2
1.3 Iron and bacterial growth	2
1.3.1 Bacterial growth is iron dependent	2
1.3.2 Iron supply systems contribute to the virulence of bacteria	3
1.3.3 The catecholate siderophore of <i>Escherichia. coli</i> and <i>Salmonella. Enterica</i>	3
1.3.4 Siderophore receptors in the outer membrane and transport systems of <i>E. coli</i> and <i>S. Enterica</i>	4
1.3.5 The uptake pathway of catecholate siderophores in <i>E. coli</i> and <i>S. Enterica</i>	6
1.3.6 The molecular structure of salmochelin S4, S2, S1, Sx, DHBS and the pathway of biosynthesis and degradation	6
1.4 The aims of this dissertation	7
2.Material and methods	8
2.1 Material	8
2.1.1 Chemicals and instruments	8
2.1.2 Media	10
2.1.3 Solid media	10
2.1.4 Solutions and buffers	11
2.1.4.1 Gel electrophoresis buffer	11
2.1.4.2 Solutions for SDS-PAGE	12
2.1.4.3 Western blotting	13
2.1.4.4 Silver staining	13
2.1.4.5 Solution for rapid affinity purification with PET His-tag systems	13
2.1.4.6 Solution for pMal fusion-protein purification	14
2.1.4.7 Solution for renaturation	14
2.1.4.8 Solution for transport assays	14
2.1.4.9 Solution for β -galactosidase measurement	14
2.1.4.10 Solution for preparation of competent cells	14
2.1.4.11 Solution for preparation of competent cells by electroporation	15
2.1.5 Strains	15
2.1.6 Plasmid vectors and constructed plasmids	16
2.1.6.1 Vectors for cloning	16
2.1.6.2 Plasmids	16
2.1.6.3 Constructed plasmids	16
2.1.7 Synthetic primers	17
2.1.8 Sequencing primers	18
2.2 Methods	18
2.2.1 Microbiological methods	18
2.2.1.1 Growth conditions	18
2.2.1.2 Analysis of salmochelin production	18
2.2.1.3 Growth stimulation and inhibition tests	19
2.2.1.4 Conjugation	19

2.2.1.5 P1 transduction	19
2.2.2 DNA methods	19
2.2.2.1 Isolation of chromosomal DNA	19
2.2.2.2 Isolation of plasmid DNA	20
2.2.2.3 Preparation of competent cells and transformation	20
2.2.2.4 Molecular cloning technology	20
2.2.2.5 Transformation by electroporation	20
2.2.2.6 Polymerase chain reaction (PCR)	21
2.2.2.7 DNA sequencing	21
2.2.3 Protein technology	21
2.2.3.1 Polyacrylamide electrophoresis (SDS-PAGE)	21
2.2.3.2 Silver staining of the gel	22
2.2.3.3 Determination of protein concentration	22
2.2.3.4 Overexpression of cytoplasmic proteins	22
2.2.3.5 Purification of protein under denaturing conditions	23
2.2.3.5.1 Preparation of cell extract	23
2.2.3.5.2 Resin preparation for protein purification	23
2.2.3.5.3 Protein purification	23
2.2.3.6 Overexpression of periplasm protein	23
2.2.3.6.1 Purification of MalE fusion protein	24
2.2.3.7 Western blotting	24
2.2.3.7.1 Transfer	24
2.2.3.7.2 Staining of the blot	24
2.2.3.8 Determination of IroD and IroE enzyme kinetics	24
2.2.3.9 Preparation of a crude extract for a Fes esterase assay	25
2.2.4 Transport assays	25
2.2.5 Renaturation of IroD and McmK protein	25
2.2.6 Selection of the enzyme assay buffer	25
2.2.7 Assays of IroD, IroE and PfeE enzymes	26
2.2.8 HPLC analysis	26
2.2.9 Mass spectroscopy	26
2.2.10 Study of <i>iro</i> promoters	26
2.2.10.1 The cloning of the <i>iroN</i> promoter into the vector pRS414	26
2.2.10.2 Regulation of the <i>iro</i> promoter by iron	27
2.2.10.3 Construction of single copy promoter- <i>lacZ</i> fusions in strain H1443 and SK22 <i>lac::Tn10</i>	27
2.2.10.4 Assay of β -galactosidase activity	28
3. Results	29
3.1 Salmochelin production by <i>E. coli</i> complemented with the <i>iro</i> gene cluster	29
3.1.1 <i>E. coli</i> $\chi 7122$ produced salmochelin	29
3.1.2 <i>iroBCD</i> is enough for the production of salmochelin	29
3.1.3 <i>E. coli</i> K-12 complemented by the <i>iroA</i> gene cluster	31
3.2 The <i>iroD</i> gene encodes a Fes-like protein	32
3.2.1 Cloning and purification of IroD	33
3.2.2 The esterase activity of renatured IroD in vitro	34
3.2.3 IroD did not cleave Salmochelin S0	37
3.2.4 IroD cleaved enterobactin in vitro	38
3.2.5 <i>iroD</i> complemented a <i>fes</i> mutant	40
3.2.6 In vitro IroD could not split Fe^{3+} -S4 complex or Ga^{3+} -S4 complex	40
3.2.7 The activity of IroD esterase was not inhibited by EGTA or EDTA in vitro	42

3.2.8 <i>Km</i> determination of IroD with iron free S4 at the room temperature	44
3.3 Do other Fes-like proteins also split esterolytic salmochelin?	44
3.3.1 Overexpression and purification of McmK in vitro was very difficult	45
3.3.2 McmK splits S2 in vitro	47
3.3.3 <i>mcmK</i> complements a <i>fes</i> mutant in vivo	48
3.3.4 Purification of Fes was very difficult	49
3.4 Characterization of the IroE protein	50
3.4.1 IroE splits salmochelin S4 into S2	50
3.4.2 IroE did not split iron free salmochelin S2	52
3.4.3 IroE did not split Ga ³⁺ -Salmochelin S4	53
3.4.4 IroE splits iron-Salmochelin S4	53
3.4.5 IroE splits enterobactin into a linear trimer	54
3.4.6 The activity of IroE was not inhibited by EDTA	55
3.4.7 <i>Km</i> determination of IroE on iron free S4 at the room temperature	57
3.4.8 The construction of an <i>iroE</i> mutant	57
3.4.9 Influence of IroE on the growth of <i>E.coli</i> K-12	57
3.4.10 Siderophore production of <i>E.coli</i> K-12 transformed with the whole <i>iroBCDEN</i> gene cluster and with an <i>iroE</i> mutant plasmid	58
3.4.11 Uptake of salmochelin from the periplasm into cytoplasm is independent of the FepBCDG system	58
3.4.12 IroE is not a binding protein into the periplasm	59
3.5 IroC dependent uptake of Iron-salmochelin S2 into the cytoplasm	60
3.6 IroE-like proteins are also found in other bacteria	61
3.6.1 Purification of the PfeE (IroE-like) protein from <i>P. aeruginosa</i>	62
3.6.2 MalE-PfeE splits salmochelin S4 in vitro	63
3.6.3 MalE-PfeE splits enterobactin in vitro	63
3.6.4 MalE-PfeE did not split gallium-salmochelin in vitro, but it splits iron-salmochelin in vitro	65
3.7 The difficulty to clone <i>iroN</i>	65
3.8 Transport experiments	66
3.9 Study of <i>iroB</i> and <i>iroN</i> promoter fusions	67
3.9.1 Regulation of <i>iroN</i> and <i>iroB</i> promoter by dipyrityl	67
4. Discussion	69
4.1 <i>iroBCDEN</i> gene cluster in <i>E.coli</i>	69
4.1.1 <i>E.coli</i> K-12 is able to produce salmochelin with the <i>iro</i> gene cluster	69
4.1.2 <i>iroN</i> and <i>iroE</i> are not required for salmochelin production	69
4.2 IroD is a Fes-like protein	70
4.3 <i>iroD</i> and other <i>fes</i> -like genes could complement a <i>fes</i> mutant in vitro and vivo	70
4.4 Comparison of Fes-like proteins	71
4.5 The IroE hydrolase	73
4.5.1 IroE is another esterase located in the periplasm	73
4.5.2 The salmochelin uptake system is independent of the FepBCDG permease of the enterobactin uptake systems	73
4.5.3 IroE is not a binding protein in the periplasm	74
4.6 Are there any other regulators of the salmochelin transport and utilization system?	74

4.7 PfeE is an IroE-like protein	75
4.8 Transport of $^{55}\text{Fe}^{3+}$ -salmochelin	76
4.9 Character of <i>iroN</i>	76
4.10 The <i>iroN</i> and <i>iroB</i> promoter were repressed by FeSO_4	76
4.11 A model for the uptake of salmochelin S4 by <i>E. coli</i> and <i>S. enterica</i>	77
5. Summary	78
6. Reference	79
7. Abbreviations	88
8. Appendix	90