

# Context-dependent siderophore exploitability shapes microbial community structure

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## Abstract

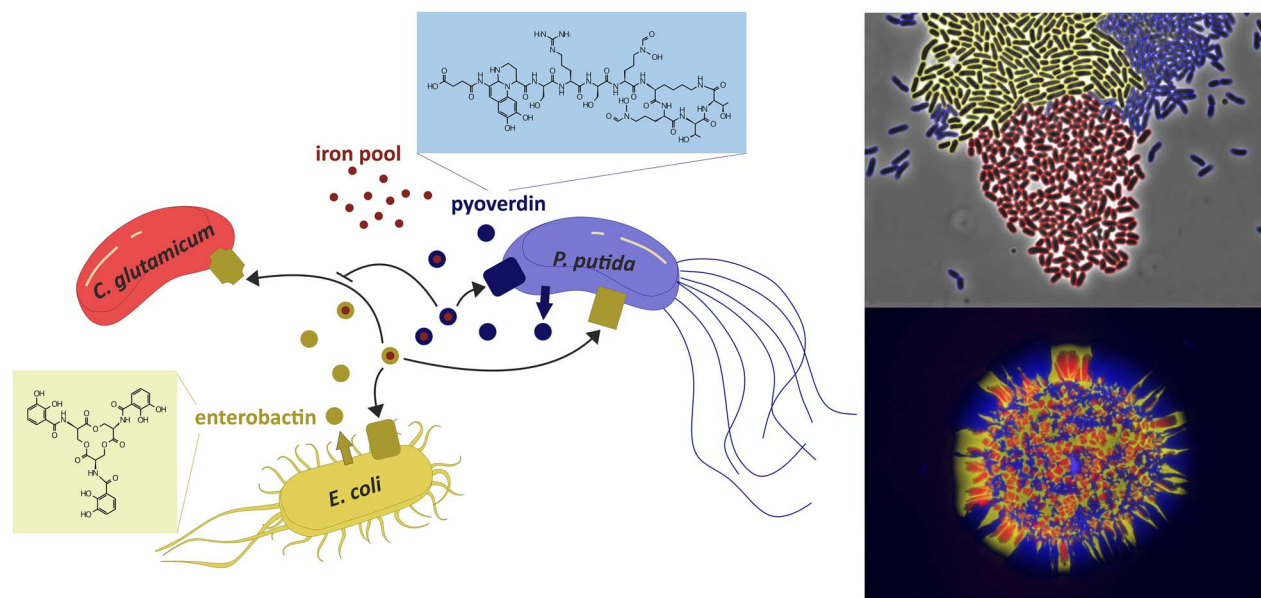
Siderophores are classically viewed as shared iron-scavenging public goods, yet their ecological roles in multispecies communities remain incompletely understood. Here, we establish a synthetic microbial community to dissect how different siderophores, their uptake compatibility and spatial structure shape iron competition. Using *Corynebacterium glutamicum* as a model, we show that this siderophore non-producer accesses diverse xenosiderophores, including enterobactin secreted by *Escherichia coli*. However, exploitation was constrained and co-cultures converged to stable compositions. Dose–response experiments combined with mathematical modelling indicated that the producer retains more effective access to enterobactin than the exploiter. The presence of *Pseudomonas putida* altered this interaction, as it exploited enterobactin while producing pyoverdine, a siderophore inaccessible to the other community members that restricted their iron access. Across different cultivation scales, community dynamics was strongly influenced by spatial organization and initial composition. These findings identify siderophores as context-dependent iron-allocation agents that can promote microbial coexistence or exclusion.

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## Graphical abstract



**Keywords** siderophores, exploitation, *Corynebacterium*, *Pseudomonas*, enterobactin, pyoverdine, microbial communities, single cell analysis, iron, public good

## Introduction

Iron is essential for almost all living organisms because it serves as a cofactor in fundamental cellular processes including respiration, central carbon metabolism, and DNA synthesis [1, 2]. Yet, despite its abundance in many environments, biologically accessible iron is often scarce under aerobic conditions, where iron occurs predominantly as poorly soluble ferric iron [3]. To overcome this limitation, many bacteria, fungi, and plants produce siderophores that are small high-affinity metabolites that chelate ferric iron and deliver it to cells through dedicated uptake systems [4]. Because siderophore biosynthesis imposes a substantial metabolic burden, these compounds are generally produced and secreted only under iron limiting conditions [5, 6]. To date, more than 500 siderophores have been identified, encompassing a wide range of structural classes, including catecholate, carboxylate, hydroxamate, and mixed-type siderophores [7, 8]. Enterobactin, produced by *Escherichia coli* and *Klebsiella pneumoniae*, is considered the archetypal siderophore and exhibits the highest reported affinity for iron [9].

Siderophores are not only iron-scavenging molecules but also mediators of microbial interactions [2]. In bacterial communities, siderophore-bound iron can be accessed only by organisms carrying compatible uptake systems. As a result, siderophores may function as shared resources, privatized goods, or competitive agents [2, 10]. Gram-negative bacteria recognize specific iron-loaded siderophores via matching outer membrane receptors, like FepA of *Escherichia coli* for enterobactin [11], or FpvA in *Pseudomonas putida* for pyoverdine uptake [12]. In contrast, Gram-positive bacteria scavenge them by specific membrane-anchored siderophore-binding lipoproteins and ABC transporters [13]. Many microbes encode receptors and transporters for siderophores that they do not synthesize themselves, enabling them to exploit xenosiderophores produced by neighbors

[14–17]. For example, *E. coli* synthesizes and utilizes enterobactin, but also has the receptor FhuA for ferrichrome, which is a siderophore produced by many fungal species like *Penicillium* spp. or *Ustilago* spp. [18, 19]. Moreover, *Pseudomonas aeruginosa* upregulates the siderophore transport system FemIRA when facing mycobactin produced by *Mycobacterium* spp. [20]. Xenosiderophore uptake and utilization reduces the metabolic cost of iron acquisition, but it can also deprive competing organisms of accessible iron [2, 21, 22]. Conversely, siderophore producers can restrict iron availability to organisms lacking matching receptors [23, 24]. These dynamics make siderophores a powerful determinant of microbial competition and coexistence in iron-limited habitats such as soil, the gut, and further host-associated microbiomes [25]. In these environments, xenosiderophore exploitation is emerging as an important ecological mechanism and a potential target for intervention. For example, the soil bacterium *Pseudomonas fluorescens* pirates desferrioxamines and coelichelin from *Streptomyces ambofaciens* [26], whereas in the nasal microbiome, corynebacterial species consume siderophores produced by *Staphylococcus aureus*, thereby reducing *S. aureus* growth [27]. Likewise, several *Pseudomonas* spp. have been shown to produce pyoverdines with significant antimicrobial properties against several human pathogens due to provoking iron limitation [28]. To date, the molecular basis of siderophore production, regulation, and chemical diversity has been extensively studied, while in recent years their ecological roles in diverse microbial communities are an emerging field of research [2, 29]. Much previous work has focused on individual siderophore systems [30, 31], pairwise producer–cheater interactions [24, 32, 33], or the effects of siderophores on microbial co-cultures [34–36]. However, natural communities often contain multiple phylogenetically diverse species that differ in siderophore production, uptake compatibility, and spatial organization. Under

these conditions, the ecological role of a siderophore may not be fixed: the same molecule could promote growth of one community member while excluding another, depending on receptor compatibility, relative abundance, and the spatial structure of the habitat. A tractable synthetic system comprising model bacteria with well-defined traits is therefore valuable for elucidating how sharing, exploitation, and exclusion emerge and interact.

Here, we established a synthetic community to examine context-dependent siderophore interactions among phylogenetically distant species and the benefits and detriments of heterologous siderophores. We chose *Corynebacterium glutamicum* as a model xenosiderophore exploiter because corynebacteria were recently described as common exploiters in natural microbiomes [27]. *C. glutamicum* is a well-established, genetically tractable model organism that does not produce siderophores on its own [37], but exhibits a broad repertoire of putative xenosiderophore transport proteins for iron acquisition that was not yet studied in detail (Table S1) [38, 39]. We first identified diverse bacterial and fungal siderophores, including enterobactin, that support growth of iron-starved *C. glutamicum*. We then built a tripartite community consisting of the well-established model species *C. glutamicum* as an exploiter, *E. coli* as an enterobactin-producing public-good provider, and *Pseudomonas putida* as both an enterobactin exploiter and a pyoverdine (private good) producer. By analysing community development and composition across scales from single cells to structured communities on plates, we show that siderophore-mediated interactions are strongly context dependent. Enterobactin acts as a public good that supports the growth of each community member, whereas pyoverdine acts as a private good—a non-exploitable good that restricts iron access from *E. coli* and *C. glutamicum* even in the presence of exploitable enterobactin. A mathematical model parameterized with experimental data suggests that exploiters can utilize the siderophore less efficiently than producers, e.g. due to a substantially lower receptor affinity, constraining exploitation despite access to enterobactin. Spatial structure further modulated these interactions by promoting niche formation for exploiters while also facilitating enterobactin privatization by the producer. Together, these findings highlight the context dependency of siderophore-mediated interactions and underscore the central role of spatial organization in shaping microbial community dynamics.

## Material and methods

### Microbial strains and cultivation conditions

All microbial strains used in this study are listed in Table S3. Microbial strains were streaked from frozen 20% glycerol stocks on 1.5% (w/v) agar medium containing complex medium. For *C. glutamicum*, brain heart infusion (BHI) (Difco, BD, Heidelberg, Germany) (37 g/L BHI) medium was used as complete medium. All other microbial strains were cultivated in lysogeny broth (LB) (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) medium, while *Ustilago maydis* was cultivated in LB or yeast extract-peptone-sucrose (YEPS) (10 g/L yeast extract, 10 g/L peptone, 10 g/L sucrose) medium. Strains were cultivated exclusively at 30°C, with exception for *E. coli* in first pre-cultures at 37°C. For liquid cultivations, single colonies from agar plates were picked and incubated in 5 ml complex medium in reaction tubes at 170 rpm or in 1 ml medium in deep-well plates (VWR International, Radnor, PA, United States) at 900 rpm. Upon cultivating for ~8 h in BHI or LB medium, resulting cell pellets were used to inoculate a second preculture in CGXII minimal medium (20 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 g/L K<sub>2</sub>HPO<sub>4</sub>,

1 g/L KH<sub>2</sub>PO<sub>4</sub>, 5 g/L urea, 42 g/L 3-morpholinopropane-1-sulfonic acid (MOPS), 13.3 mg/L CaCl<sub>2</sub>\*2H<sub>2</sub>O, 0.25 g/L MgSO<sub>4</sub>\*7H<sub>2</sub>O, 0.2 mg/L biotin, 0, 1 or 10 mg/L FeSO<sub>4</sub>\*7H<sub>2</sub>O, 10 mg/L MnSO<sub>4</sub>\*H<sub>2</sub>O, 0.1 mg/L ZnSO<sub>4</sub>\*7H<sub>2</sub>O, 0.02 mg/L CuSO<sub>4</sub>\*5H<sub>2</sub>O, 0.002 mg/L NiCl<sub>2</sub>\*6H<sub>2</sub>O; pH was adjusted with KOH to pH of 7.0.) [40] containing 2% (w/v) glucose as carbon source. To starve cells of the second pre-culture from iron prior to experiments, CGXII minimal medium without FeSO<sub>4</sub> was used (referred to “iron-free CGXII”). Protocatechuate (PCA) (3,4-dihydroxybenzoic acid), a ferric chelator, is usually added to the CGXII minimal medium to facilitate iron uptake and was therefore left out in our cultivations [40, 41]. For *E. coli*, 1 μM FeSO<sub>4</sub> was added to the second pre-culture. Second pre-cultures were performed in 20–25 ml minimal medium in shaking flasks at 120 rpm or in 1 ml medium in 96-deep-well plates (VWR International, Radnor, PA, United States) at 900 rpm.

Main cultures were inoculated at an optical density at 600 nm (OD<sub>600</sub>) of 1, if not stated otherwise, and either performed in shaking flasks in respective volumes or for online monitoring of growth in 750 μl in 48-well microtiter Flower Plates in a BioLector I microtiter cultivation system (Beckman Coulter GmbH, Aachen, Germany) [42]. In the microtiter cultivation system, backscatter (a.u.) was measured in 30 min intervals as scattered light intensity at λ: 620 nm (signal gain: 20). In this context, backscatter is the intensity of light scattered by the cells in the culture, serving as a proxy for biomass. *P. putida* cultures were inoculated at an OD<sub>600</sub> of 0.5 in a volume of 1 ml. As standard conditions, CGXII minimal media was used as described above. For analysis of growth in presence of LB-based spent media, cells were inoculated in two-fold concentrated iron-free CGXII medium supplemented with 2\*2% glucose and 2\*150 μM 2,2'-dipyridyl (hereafter referred to as DIP). For CGXII-based spent media, cells were inoculated in two-fold concentrated CGXII medium with 2\*2% glucose, 3.6 μM FeSO<sub>4</sub> and no DIP. Each cell-media suspension was mixed with 50% of respective spent medium. The addition of DIP is a well-established strategy to mimic non-accessible iron pools in siderophore studies [43–46]. Siderophores have a higher affinity for iron than this chelator, thereby enabling iron uptake by those cells, which can use the specific siderophore. When enterobactin was added as pure compound (Sigma-Aldrich, dissolved in DMSO), 3.6 μM FeSO<sub>4</sub> and 150 μM DIP was added in liquid cultivations.

Bacterial strains containing plasmids mediating a kanamycin resistance were cultivated in the presence of appropriate amounts of the antibiotic (*C. glutamicum*: 25 μg/L; *E. coli*: 50 μg/L) to ensure plasmid maintenance. If needed, LacI-P<sub>tac</sub>-derived gene expression was induced by the addition of isopropyl-β-D-1-thiogalactopyranoside (IPTG).

### Production and harvesting of siderophore containing spent medium

Spent medium refers to the siderophore-enriched growth medium remaining after cultivating of a respective siderophore-producing organism, but cells removed.

For screening purposes, LB-based spent medium was used: Cells of siderophore-producing strains from an overnight culture were transferred at an OD<sub>600</sub> of 0.45 to 50 ml LB medium supplemented with 200 μM DIP, while *U. maydis* cells were cultivated in YEPS medium containing 300 μM DIP.

For more specific analyses, CGXII-based spent medium was used: *E. coli* or *P. putida* strains from an overnight culture were transferred

at an OD<sub>600</sub> of 0.45 to 50 mL CGXII media as described above, but containing 0.36  $\mu\text{M}$  FeSO<sub>4</sub> and 360  $\mu\text{M}$  DIP.

After 2 days of cultivation in shake flasks, the culture supernatant was harvested and sterile filtered (0.2  $\mu\text{m}$ ). Harvested spent medium was stored at  $-20^{\circ}\text{C}$  until further use.

### Recombinant DNA work

Standard molecular methods, such as DNA restriction, PCR, and Gibson assembly, were performed according to the manufacturer's instructions or to previously described standard protocols [47, 48]. All plasmids and primers used in this study are listed in Tables S4 and S5. Oligonucleotide synthesis and Sanger DNA sequencing was performed by the company Eurofins Genomics (Ebersberg, Germany).

In this work, all plasmids were constructed using the enzymatic Gibson assembly approach. Plasmids were transferred into competent *C. glutamicum* cells by electroporation as described previously [49]. For genomic deletions and integrations, derivatives of the suicide vector pK19-*mobsacB* were used [50]. Two step homologous recombination and selection was done as described previously [51].

Plasmids were transferred into competent *E. coli* cells by heat shock or TSS transformation [52, 53]. Genomic deletion of the *entC* gene in strain *E. coli* PC 0886  $\Delta\text{entC}$  was achieved with the Lambda red recombinase-based deletion method described previously [54]. The disruption cassette consisted of the *neoR* resistance gene, encoding the neomycin phosphotransferase providing resistance against neomycin and kanamycin. This gene was flanked by FLP recombinase recognition target (frt) sites, which are needed for later elimination of the *neoR* gene. The up- and downstream sequences of *entC* (~200 nt) formed the ends of the disruption cassette long sequences, allowing for replacement of the *entC* gene by the disruption cassette by homologous recombination.

For genomic integrations, the deletion protocol was modified. An integration cassette was used instead of the disruption cassette. The design was comparable to the disruption cassette, but the sequence that should be integrated was inserted between the *neoR*-frt sequence and the right homologous flank.

For the genomic integration of the constitutive mTagBFP (for fluorescent labeling) and the IronSenseR-mCherry, tetra parental patch-mating was performed on 1% LB agar plates overnight at  $30^{\circ}\text{C}$ . Freshly streaked strains of *P. putida* KT2440 WT or *P. putida*  $\Delta\text{pvdD}$  were plated as acceptor strains. Helper strain *E. coli* HB101 pRK2013 and *E. coli* DH5 $\alpha$ pir pTNS1 either helped with pili formation or delivered necessary transposase genes respectively. Lastly a *E. coli* Pir2 carrying the miniTn7 plasmid with the IronSenseR-mCherry or constitutively expressed fluorescent marker gene (*mTagBFP*) was used as a donor strain. After incubation, cells were streaked on LB plates containing gentamycin (25  $\mu\text{g ml}^{-1}$ ) and irgansan (25  $\mu\text{g ml}^{-1}$ ) and cultivated overnight to select for *P. putida* KT2440 WT or *P. putida*  $\Delta\text{pvdD}$  integration cassette. Then single clones were streaked on LB containing kanamycin (25  $\mu\text{g ml}^{-1}$ ) and irgansan (25  $\mu\text{g ml}^{-1}$ ) as well as LB containing gentamycin (25  $\mu\text{g ml}^{-1}$ ) and irgansan (25  $\mu\text{g ml}^{-1}$ ). Clones only growing on LB containing gentamycin and irgansan were likely carrying the Tn7 integration, which was then confirmed *via* colony PCR.

### Siderophore sharing in structural environments

Double layer agar plate assays were performed to study siderophore sharing in structural environments. LB or CGXII plates consisted of a solid 1.5% agar bottom layer and a soft 0.4% agar top layer to facilitate

diffusion. Plates were prepared both with and without DIP to compare the effects of “free iron” availability versus a condition where iron could only be acquired via siderophores. For LB-based agar plates, 200  $\mu\text{M}$  DIP was added, but no additional iron. For CGXII-based agar plates, 3.6  $\mu\text{M}$  FeSO<sub>4</sub> was added and 100  $\mu\text{M}$  DIP was utilized, while for “full-iron conditions” 36  $\mu\text{M}$  FeSO<sub>4</sub> were used without any addition of DIP.

### Xenosiderophore utilization screen (LB-based)

Cells from LB overnight cultures of different putative siderophore producers (Table S3) were transferred into 0.9% NaCl at cell density of  $0.24 \times 10^{10}$  cells/mL. 40  $\mu\text{L}$  cell suspension were streaked on freshly prepared plates as thick lane. After overnight cultivation of producer strains at  $30^{\circ}\text{C}$ , iron-starved *C. glutamicum* cells were transferred into 0.9% NaCl at cell densities of  $10^{10}$ ,  $10^9$ ,  $10^8$ ,  $10^7$ ,  $10^6$ ,  $10^5$  and  $10^4$  cells/mL. 3  $\mu\text{L}$  of these cell suspensions were dropped at different distances to the lane of siderophore producers. Images were taken after 48 h cultivation at  $30^{\circ}\text{C}$ .

### Siderophore-mediated co-cultivations (CGXII-based)

Cells were cultivated in iron-free CGXII as second pre-culture. Subsequently, cells corresponding to an OD<sub>600</sub> of 1 were washed and resuspended in 0.9% NaCl. Each 2  $\mu\text{L}$  of cell suspension was dropped on the CGXII-soft agar layer either as monocultures, or in duo or trio combinations dropped ~5 mm apart. Plates were incubated at  $30^{\circ}\text{C}$  for 3 days and images were taken under a stereomicroscope Zeiss Axio Zoom V16 with respective filter units (GFP:  $\lambda_{\text{Ex}}$ : 489 nm,  $\lambda_{\text{Em}}$ : 509 nm (*gfp*); HE mPlum:  $\lambda_{\text{Ex}}$ : 587 nm,  $\lambda_{\text{Em}}$ : 649 nm (*E2-crimson*); DAPI:  $\lambda_{\text{Ex}}$ : 358 nm,  $\lambda_{\text{Em}}$ : 463 nm (*bfp*)) once after 48 h and 120 h. Analysis of percentage area was performed using the open source program ImageJ/Fiji [55, 56]. Area per fluorescent channel image was extracted using individual background correction thresholds.

### In vivo measurement of the biosensor for iron in *C. glutamicum*

For the *in vivo* online measuring iron levels in *C. glutamicum*, the fluorescent protein LSSmApple of the original IronSenseR [57] was replaced by mCherry, constructing IronSenseR-mCherry plasmid. For *C. glutamicum*, an N-terminal deca-histidine tag followed by a TEV cleavage site was directly fused to the biosensor encoding gene cassette. Electrocompetent *C. glutamicum* were transformed with the plasmid pPREx210xHis-TEV-MDtxR<sub>G149</sub>GC. Single colonies were cultivated in 800  $\mu\text{L}$  ml BHI supplemented with kanamycin in a deep-well plate at  $30^{\circ}\text{C}$  and 900 rpm for 8 h. The second pre-culture was prepared in iron-free CGXII medium containing 2% (w/v) glucose supplemented with 100  $\mu\text{M}$  IPTG. Cultures were incubated overnight (16 h) at  $30^{\circ}\text{C}$  and 900 rpm in deep-well plates. For the main culture, cells were inoculated to an initial OD<sub>600</sub> of 0.5 in 10 mL CGXII minimal medium supplemented with 2% (w/v) glucose, 3.6  $\mu\text{M}$  FeSO<sub>4</sub> as iron source, no PCA and 100  $\mu\text{M}$  IPTG. Where indicated, enterobactin was added to a final concentration of 10  $\mu\text{M}$ . Cultures were incubated in 100 mL shaking flasks at  $30^{\circ}\text{C}$  for 2 h on a rotary shaker. Cells corresponding to an OD<sub>600</sub> of 0.5 in 1 mL culture volume were harvested by centrifugation (5000 rpm,  $4^{\circ}\text{C}$ , 5 min), washed, and resuspended with ice-cold MOPS buffer (20 mM, pH 7.0). A volume of 100  $\mu\text{L}$  was transferred to a microplate and analysed using a multimode microplate reader (Infinite M1000Pro, Tecan). Emission spectra were recorded in 5 nm increments from 490 to 750 nm for GFP upon excitation

at 453 nm, and from 580 to 750 nm for mCherry upon excitation at 550 nm. Fluorescence values were corrected by subtracting the signal obtained from buffer-only controls. To obtain the ratios, the peak of GFP fluorescence ( $\lambda_{em} = 510$  nm) was divided by the peak value of mCherry ( $\lambda_{em} = 610$  nm). For *P. putida* the strain carrying the integration of the IPTG inducible IronSenseR (*P. putida*  $\Delta pvdD::attTn7$  IronSenseR-mCherry), was inoculated in LB at 30°C and 1200 rpm overnight. The second preculture was inoculated at an OD<sub>600</sub> of 0.5 and incubated overnight in LB at 30°C and 1200 rpm with the addition of 500  $\mu$ M IPTG. For the main culture, cells were inoculated to an initial OD<sub>600</sub> of 0.1 in 1 ml LB 500  $\mu$ M IPTG. Where indicated, 10  $\mu$ M enterobactin and 250  $\mu$ M DIP was added. Cultures were incubated for 6 h at 30°C and 1200 rpm. 100  $\mu$ L of the culture was harvested washed and resuspended in 100  $\mu$ L PBS, transferred to a microtiter plate and analysed using a multimode microplate reader (Infinite M1000Pro, Tecan). Two distinct emission spectra of GFP ( $\lambda_{ex} = 480$  nm,  $\lambda_{em} = 500$ –600 nm) and mCherry ( $\lambda_{ex} = 587$  nm,  $\lambda_{em} = 600$ –700 nm) were recorded. To obtain the ratios, the peak of GFP fluorescence ( $\lambda_{em} = 508$  nm) was divided by the peak of mCherry ( $\lambda_{em} = 608$  nm).

### MS-measurements of enterobactin and organic acids

For MS measurements, four replicates of *C. glutamicum* were cultivated as described above in an iron-free CGXII pre-culture. In the main culture, *C. glutamicum* was cultivated at a starting OD<sub>600</sub> of 1 in 50 ml CGXII supplemented with 2% glucose and either (i) 3.6  $\mu$ M FeSO<sub>4</sub>, 150  $\mu$ M DIP, no PCA (iron limited) (ii) 3.6  $\mu$ M FeSO<sub>4</sub>, 10  $\mu$ M enterobactin, 150  $\mu$ M DIP, no PCA or (iii) 36  $\mu$ M FeSO<sub>4</sub>. Samples were taken after 5, 10, 30, 120, 300, and 1440 min.

For measurements of extracellular enterobactin, cells were centrifuged and supernatant filtered (0.22  $\mu$ m). For intracellular measurements of enterobactin and organic acids, cells corresponding to two OD units were washed in 10 ml 0.9% NaCl and cell culture was rapidly filtered through a nylon membrane with a pore size of 0.45  $\mu$ m using a vacuum filtration system (Merck Millipore). The filter was washed twice with 5 ml ice-cold 0.9% NaCl. The membrane was immediately placed into 2 ml tubes preloaded with steel (2 mm) and glass beads (Retsch) and snap-frozen in liquid nitrogen. The entire sampling and quenching procedures were completed within 45 s.

Enterobactin and organic acids were extracted from the cells using a method adapted from [58]. Details can be found in the supplementary material.

### Flow cytometry of trio cultures

*P. putida*, and *E. coli* were precultured in LB, while *C. glutamicum* was precultured in BHIS overnight at 30°C and 1200 rpm. The second preculture was inoculated at an OD<sub>600</sub> in CGXII medium with 2% glucose and 3.6  $\mu$ M FeSO<sub>4</sub>. For the main culture, the three different strains were mixed at similar ratios to a total OD<sub>600</sub> of 1, in CGXII medium with 2% glucose containing either 0.36  $\mu$ M FeSO<sub>4</sub>, 3.6  $\mu$ M FeSO<sub>4</sub>, 0.36  $\mu$ M FeSO<sub>4</sub> + 250  $\mu$ M DIP or 3.6  $\mu$ M FeSO<sub>4</sub> + 250  $\mu$ M DIP and cultivated for 24 h at 30°C and 1200 rpm and then reinoculated at an OD<sub>600</sub> of one in fresh media inoculating further 24 h. During cultivation and at the end 20  $\mu$ L samples were taken washed and diluted to an OD<sub>600</sub> of 0.05 in 200  $\mu$ L of PBS. Afterwards samples were analysed in the flowcytometer (Amnis CellStreamTM System, Merck, now Cytek Biosciences, California, USA). Cell populations were gated for size and complexity via front scatter and side scatter. Afterwards, populations

were further gated for mCherry, GFP, or mTagBFP fluorescence. For analysis of the Flowcytometry data, the CellStream Analysis Software (Merck, now Cytek Biosciences, California, USA) was used.

### Pyoverdine measurements

Precultures were performed in LB at 30°C and 1200 rpm. Main cultures were inoculated at an OD<sub>600</sub> of 0.05 in LB, with 250  $\mu$ M DIP, 250  $\mu$ M DIP and 10  $\mu$ M enterobactin or no addition. To measure pyoverdine production, after cultivation 20  $\mu$ L of culture were transferred to 96-well clear bottom microtiter plates and diluted with 80  $\mu$ L of PBS. Using a plate reader, Infinite M1000 PRO (Tecan Austria GmbH) absorption was measured at 600 nm and pyoverdine fluorescence ( $\lambda_{ex} = 398$  nm,  $\lambda_{em} = 455$  nm) was measured in the same device.

### Single-cell cultivation in microfluidic chips

Microfluidic chips were fabricated by soft lithography using a two-layer SU-8 master on a four-inch silicon wafer. The Clewin5 design comprised cultivation chambers (60 × 100 × 0.9  $\mu$ m) connected via 9.8  $\mu$ m deep channels to two opposing 10  $\mu$ m wide inlets. Each inlet supplied eight rows of thirty chambers. PDMS (Sylgard 184; 10 wt% curing agent) was poured onto the SU-8 master, then cured for 2 h at 80°C. It was then cut and punched ( $\varnothing$  0.5 mm). The PDMS plates were bonded to glass substrates (D263® Bio, 39.5 × 34.5 × 0.175 mm) using O<sub>2</sub> plasma activation (25 s, 510 W, Femto Plasma Cleaner, Diener Electronics, Germany).

All experiments were performed in PCA-free CGXII minimal medium (1% w/v glucose). *C. glutamicum* was pre-cultured at 30°C in three steps: 8 h BHI, followed by 16 h and 4 h iron-limited CGXII. *E. coli* was grown in LB (37°C), then 16 h iron-limited CGXII, and finally 4 h CGXII with 3.6  $\mu$ M FeSO<sub>4</sub>. All cultures were initiated from single colonies on agar plates. No DIP was required to induce iron-restriction in microfluidic conditions.

For microfluidic experiments, the chips were inoculated with monocultures or co- cultures adjusted to an OD<sub>600</sub> of 0.5. Time-lapse imaging was performed every 30 min using a Nikon Eclipse Ti2 microscope (100× oil objective, DS-Qi2 camera, Perfect Focus System) The fluorescence of the genomically encoded E2-Crimson, GFP and mTagBFP were excited using a Sola LED illumination system with the appropriate filter cubes (E2-Crimson:  $\lambda_{Ex} = 562/40$  nm,  $\lambda_{Em} = 641/84$  nm; GFP:  $\lambda_{Ex} = 520/40$  nm,  $\lambda_{Em} = 540/40$  nm; mTagBFP:  $\lambda_{Ex} = 438/24$  nm,  $\lambda_{Em} = 483/32$  nm).

Initial preprocessing of the raw microscopy data was performed in Fiji (Version 1.54p) by cropping to ~100 × 60  $\mu$ m. Time-lapse sequences were exported as TIFF files and uploaded to an open microscopy environment (OMERO) server.

Subsequent image analysis was implemented in custom Jupyter workflows based on Acia, using Omnipose for segmentation [59]. Fluorescence signals enabled cell differentiation, and segmentation artifacts were excluded by intensity, size, and position-based filtering. Cell areas were extracted from segmentation masks and aggregated per frame.

Subsequent analyses focused on the temporal progression of colony area, defined as the cumulative area of all cells per species within a chamber. Growth rates were calculated as the difference quotient between consecutive time points. Relative population dynamics were quantified as the fraction of species-specific area relative to the total cell area per chamber.

## Model and simulation of microfluidic co-culture experiments

We formulated a deterministic, unstructured population model to describe the interaction between *E. coli* and *C. glutamicum* in microfluidic chambers. The system is represented by coupled ordinary differential equation (ODE) based on Monod-type kinetics. We assumed: well-mixed chamber, with no spatial gradients, no explicit glucose limitation, growth-associated metabolite exchange only, and no cell death or maintenance term. The production of enterobactin and its binding to iron is simplified into the production of an abstract product  $P_{Enterobactin}$ . The set of governing reactions read:

$$\frac{dX_{E.coli}}{dt} = \{\mu_{\min-E.coli} + [(\mu_{\max-E.coli} - \mu_{\min-E.coli}) \times \frac{P_{Enterobactin}}{P_{Enterobactin} + k_{S,X2}}]\} \times X_{E.coli} \quad (1)$$

$$\frac{dX_{C.glut}}{dt} = \mu_{\max-C.glut} \times \frac{P_{Enterobactin}}{P_{Enterobactin} + k_{S,X1}} \times X_{C.glut} \quad (2)$$

$$\frac{dP_{Enterobactin}}{dt} = q_{p-1} \times X_{E.coli} - q_{c-1-E.coli} \times X_{E.coli} - q_{c-1-C.glut} \times X_{C.glut} \quad (3)$$

where  $\mu_{\min-E.coli}$  is the minimal growth rate of *E. coli* in absence of  $P_{Enterobactin}$ ,  $\mu_{\max-E.coli}$  is the maximum growth rate of *E. coli*,  $k_{S,X2}$  is the half saturation constant towards  $P_{Enterobactin}$  of *E. coli*,  $k_{S,X1}$  is the half saturation constant towards  $P_{Enterobactin}$  of *C. glutamicum*,  $X_{E.coli}$  and  $X_{C.glut}$  are the concentrations of both organisms,  $\frac{dX_{E.coli}}{dt}$  and  $\frac{dX_{C.glut}}{dt}$  are the change of *E. coli* and *C. glutamicum* concentration and  $\frac{dP_{Enterobactin}}{dt}$  is the change of the concentration of  $P_{Enterobactin}$ .

In this scenario *E. coli* produces a product which itself need to take up again to stimulate growth. The production and the consumption of  $P_{Enterobactin}$  by both organisms is assumed to be growth dependent.

$$q_{p-1} = q_{\max,p-1-E.coli} \times \frac{\mu_{E.coli}}{\mu_{\max-E.coli}} \quad (4)$$

$$q_{c-1-E.coli} = q_{\max,c-1-E.coli} \times \frac{\mu_{E.coli}}{\mu_{\max-E.coli}} \quad (5)$$

$$q_{c-1-C.glut} = q_{\max,c-1-C.glut} \times \frac{\mu_{C.glut}}{\mu_{\max-C.glut}} \quad (6)$$

These proposed rates have been adjusted to fit qualitatively to our observed data (Table S6 and S7).

### Parametrization

Maximum specific growth rates of both organisms were experimentally determined or obtained from literature (Table S6). The half-saturation constant for *E. coli* was taken from literature, whereas the corresponding parameter for *C. glutamicum* was varied to test the hypothesized interaction mechanism.

Production and uptake coefficients were defined relative to maximum growth rates and adjusted to reproduce qualitative experimental trends. All parameter values and units are summarized in Supplementary Tables S6 and S7.

## Varying starting conditions in microfluidic experiments

To demonstrate the effects of the varying starting conditions given in microfluidic cultivation chambers, we performed two parameter sweeps. In the first step we varied the starting concentrations of total cell dry-weight, which corresponds to different numbers of starting cells which are inside microfluidic chambers. The variation of cell dry-weight corresponds with different start cell areas of all cells in a chamber between 10  $\mu\text{m}^2$  and 100  $\mu\text{m}^2$ .

In the second step the simulated starting cell dry weight concentration is simulated as 19 different compositions of *C. glutamicum* and *E. coli*, between 5% and 95% of the entire cell dry weight concentration. The starting concentration of  $P_{Enterobactin}$  was set, so the calculated growth rate for *E. coli* is 95% of its maximum growth rate.

### Varying affinity relationships of co-culture participants

To investigate the impact of potential variants of affinity towards  $P_{Enterobactin}$  we performed another two-dimensional parameter sweep. We varied the affinity of *C. glutamicum* towards the limiting product by scaling its Monod constant  $k_{S-E-C}$  relative to the producers Monod constant  $k_{S-E-E}$  from 0.01 to 100. To evaluate the system's performance during growth, the mean specific growth rates for both species were calculated exclusively for the growth phase up to a total biomass of 76.15 g/L, corresponding to an observed projected cell area of 2500  $\mu\text{m}^2$ .

### Numerical integration

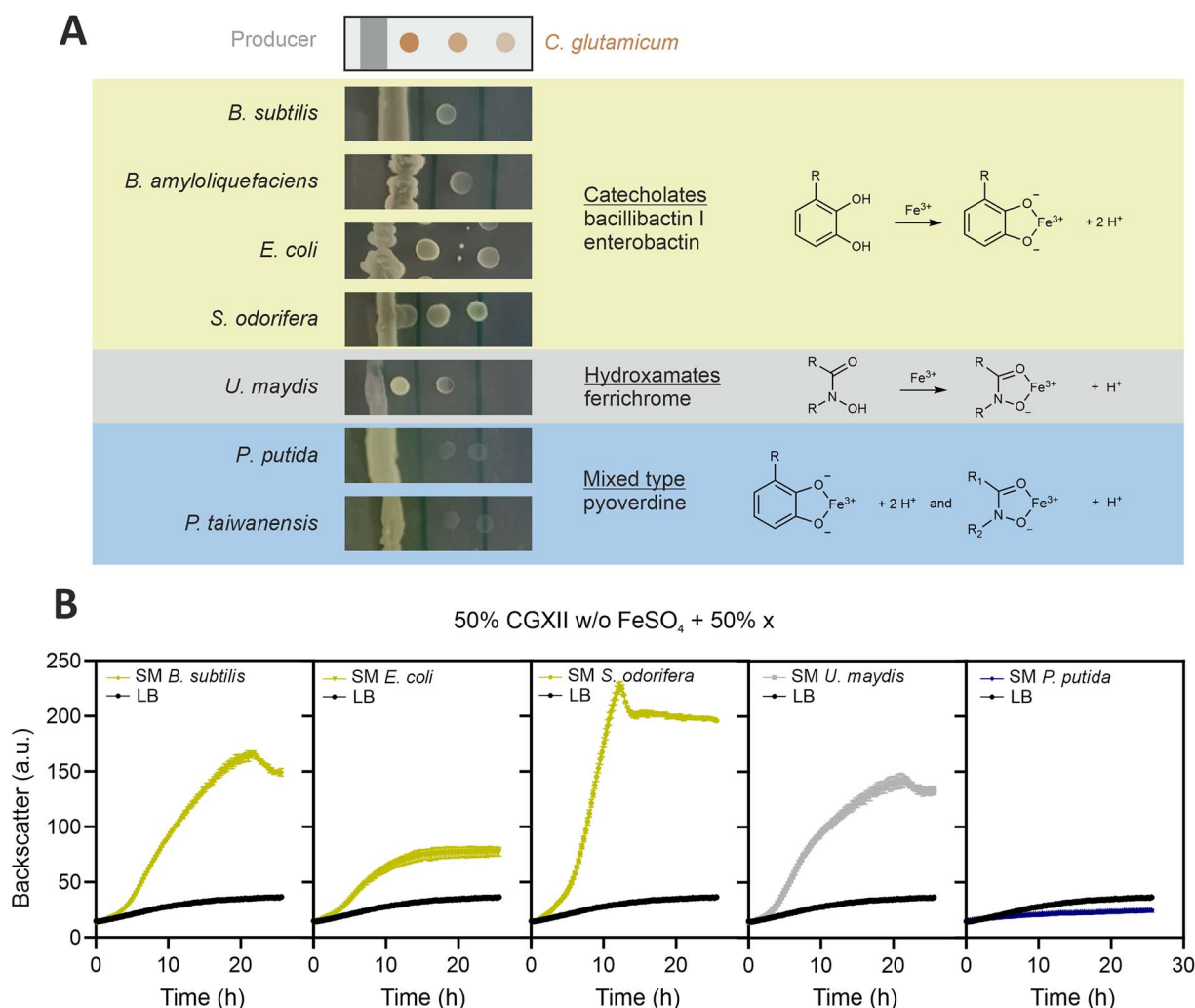
The model was solved numerically using Python (Version 3.8.15) and the `scipy.integrate` library (Version 1.9.1). The LSODA integrator was employed via the `solve_ivp` function. Specific growth rates were recalculated post-simulation based on the resulting concentration profiles and the governing Monod kinetics. Solutions were evaluated at 0.1 h intervals over a total simulation time of 30 h.

## Results

### *Corynebacterium glutamicum* exploits chemically diverse xenosiderophores under iron limitation

Given the predicted capacity of the non-siderophore-producing *C. glutamicum* to exploit xenosiderophores (Table S1), we first determined which siderophores support its growth under iron limitation. To this end, we systematically assessed the growth of iron-starved *C. glutamicum* in proximity to microorganisms producing chemically distinct siderophores, including catecholates, hydroxamates, and mixed-type siderophores (Fig. 1A). As expected, *C. glutamicum* failed to grow on solid iron-restricted media in monoculture (Fig. S1). However, robust growth was observed when growing next to enterobactin producing *E. coli* and *Serratia odorifera*, bacillibactin secreting *Bacillus subtilis* and *Bacillus amyloliquefaciens*, as well as the ferrichrome producing fungus *Ustilago maydis* (Fig. 1A). In contrast, no growth was observed next to *Pseudomonas putida* and *Pseudomonas taiwanensis*, both secreting pyoverdine variants. This xenosiderophore utilization pattern is further supported when analysing growth in liquid cultures supplemented with 50% LB-based spent medium of selected siderophore producing organisms grown under iron depletion (Fig. 1B).

Together, these results demonstrate that *C. glutamicum* can exploit a diverse set of xenosiderophores, spanning multiple iron-chelation



**Figure 1** *C. glutamicum* is able to exploit several chemically diverse xenosiderophores. (A) The potential of seven known siderophore producers to promote growth of neighboring iron-starved *C. glutamicum* on agar-solidified LB medium containing 250  $\mu\text{M}$  DIP (2,2'-dipyridyl) was investigated. *C. glutamicum* cell suspensions were spotted at different distances to the pre-grown lane of siderophore producer. Moieties involved in iron chelation that determine siderophore classification are shown on the right (adapted from [104]). One representative replicate is shown. (B) Liquid cultivation of *C. glutamicum* in 50% two-fold CGXII minimal media without added iron, and 50% spent medium harvested from selected organisms as in (A) grown in LB supplemented with 200  $\mu\text{M}$  DIP. Graph colors represent siderophore types, while LB control is shown in black.  $n = 3$  biological replicates.

chemistries and originating from both Gram-positive and Gram-negative bacteria, and even from fungi, to overcome iron limitation.

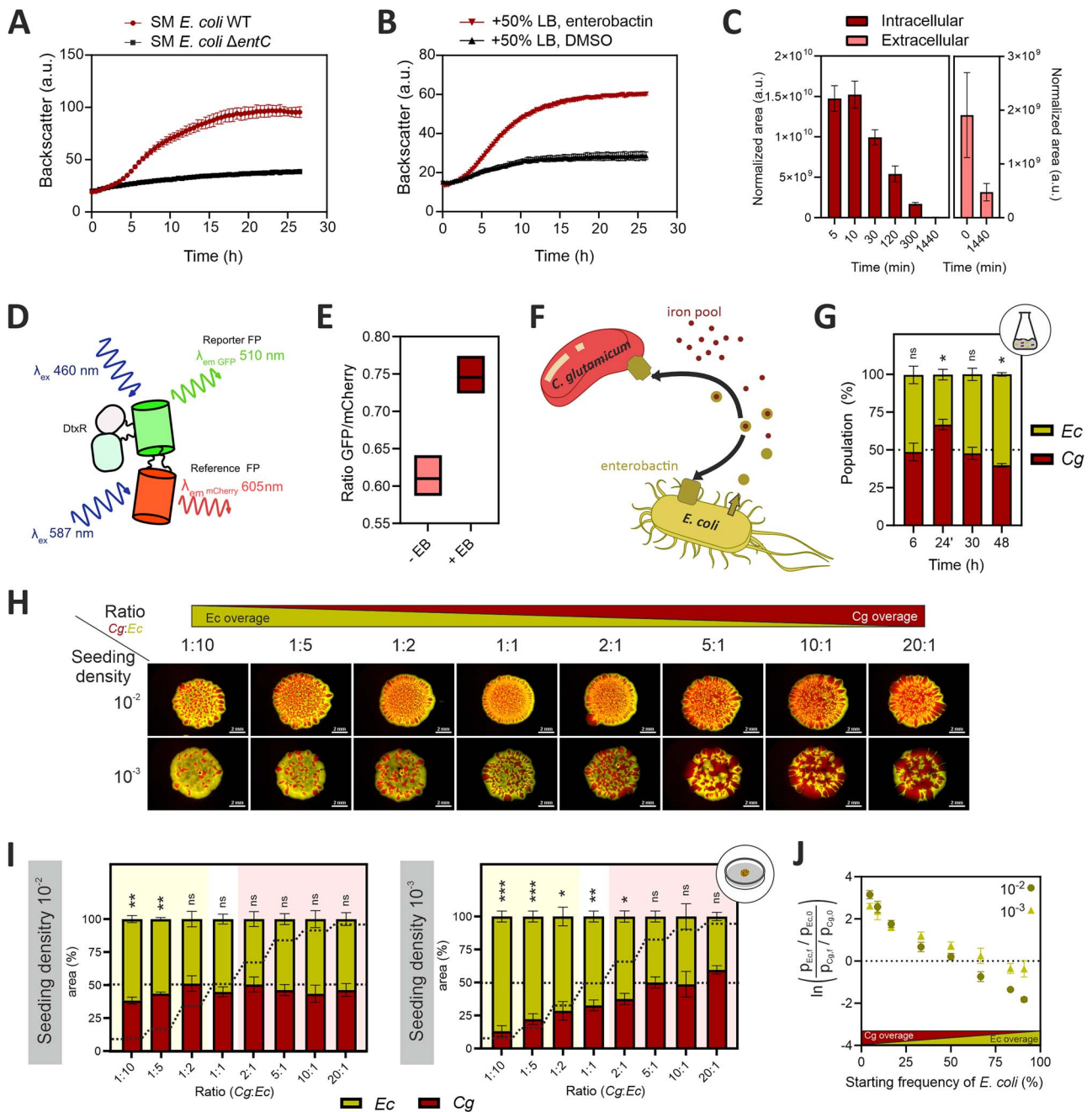
### *C. glutamicum* is an exploiter of enterobactin produced by *E. coli*

Having demonstrated that *C. glutamicum* can exploit different xenosiderophores, we next focused on its interaction with enterobactin and its producer *E. coli*. Based on the dose-dependent growth stimulation of *C. glutamicum* observed upon supplementation of iron-limited medium with LB-based *E. coli* spent medium (SM), a 50% SM dilution supplemented with DIP was used in subsequent experiments (Fig. S2A–B).

To test whether this growth-promoting effect specifically depends on enterobactin, we generated an enterobactin deficient *E. coli* strain ( $\Delta\text{entC}$ ), lacking the gene encoding for the isochorismate synthase EntC that catalyzes the conversion of chorismate to isochorismate in the first step of enterobactin synthesis [60]. In comparison to *E. coli* WT

SM, SM of the  $\Delta\text{entC}$  mutant did not support growth of *C. glutamicum* (Fig. 2A). This effect was also observed for CGXII-based SM, i.e. minimal media (Fig. S2C). Using a plasmid-based LacI- $P_{\text{tac}}$ -controlled *entC* expression, the growth-promoting effect could be restored (Fig. S2D). Supplementation of pure enterobactin also enhanced growth of iron-starved *C. glutamicum* (Fig. 2B).

We next asked whether enterobactin is directly taken up by *C. glutamicum* and thereby relieves intracellular iron limitation. Using liquid chromatography-mass spectrometry (LC-MS) measurements intracellular enterobactin was readily detectable immediately after addition, while declined over time, indicating rapid uptake under iron limitation (Fig. 2C). However, this fraction may also contain cell-associated enterobactin and does by itself not confirm the uptake of the molecule. Relative to iron-replete conditions, intracellular citrate levels showed a reduced log<sub>2</sub>-fold change under enterobactin supplementation compared with the no-iron control, suggesting that enterobactin availability alters citrate-associated iron acquisition in *C. glutamicum* [61] (Fig. S3). This is also reflected in further TCA cycle



**Figure 2** *C. glutamicum* exploits enterobactin produced by *E. coli*. (A) Comparison of the growth of *C. glutamicum* in 50% LB-based spent medium of *E. coli* WT with that in spent medium of non-enterobactin-producing *E. coli*  $\Delta$ entC. (B) *C. glutamicum* growing in 50% LB supplemented with 200  $\mu$ M DIP and either 10  $\mu$ M pure enterobactin (red) or 1  $\mu$ M DMSO as negative control.  $n = 3$  biological replicates. (C) MS-measurement of intra- and extracellular enterobactin content in *C. glutamicum* cultivated with 10  $\mu$ M enterobactin in the presence of 150  $\mu$ M DIP.  $n = 4$  biological replicates. (D) Schematic depiction of ratiometric fluorescent-based modified biosensor “IronSenseR-mCherry” [57]. (E) Comparing iron content by utilizing plasmid-based IronSenseR-mCherry in *C. glutamicum* cultivated in CGXII with or without 10  $\mu$ M enterobactin. (F) Schematic depiction of *C. glutamicum* and *E. coli* making use of a non-accessible iron pool via *E. coli*-produced enterobactin. (G) Flow cytometry measurements of liquid co-cultured *E. coli*-gfp (yellow) and *C. glutamicum*-E2-crimson (red) under iron-restricted (250  $\mu$ M DIP) conditions at a ratio of 1:1. Culture was transferred into fresh media after 24 h (indicated by \*). Samples taken at 6 h and 24 h after cultivation start and each 6 h and 24 h after the fresh transfer. Statistical significance was assessed using a one-sample Student’s *t*-test against the null hypothesis  $\mu = 50\%$ . *P* values were adjusted for multiple comparisons using the Holm method ( $P \leq .05$  (\*),  $P \leq .01$  (\*\*)).  $n = 3$  biological replicates. (H) Representative merged stereomicroscopy images of mixed drop (macrocolony) assay on iron-limited CGXII plates with *C. glutamicum*-E2-crimson (red) and *E. coli*-gfp (yellow) as indicated in varying ratios and two different seeding densities ( $10^{-2}$  and  $10^{-3}$ ). (I) Quantified area of *C. glutamicum* (red) and *E. coli* (yellow) deduced from the microscopy pictures in (H), set in relation to 100%. Statistical significance was assessed using a one-sample Student’s *t*-test against the null hypothesis  $\mu = 50\%$ . *P* values were adjusted for multiple comparisons using the Holm method ( $P \leq .05$  (\*),  $P \leq .01$  (\*\*),  $P \leq .001$  (\*\*\*),  $P \leq .0001$  (\*\*\*\*)). “ns” thereby implies that the outcome balances towards 50%. Horizontal dashed line represents null hypothesis (50%), while diagonal dashed line represents the initial inoculation.  $n = 5$  biological replicates. *Ec* = *E. coli*, *Cg* = *C. glutamicum*. (J) Fitness of *E. coli* in relation to *C. glutamicum* (selection coefficient) vs. starting frequency of *E. coli* (based on area (%)) at two seeding densities  $10^{-2}$  (dark yellow, circles) and  $10^{-3}$  (light yellow, triangles).  $p$  = proportion,  $f$  = final,  $0$  = start.

intermediates like aconitate and isocitric acid. These results additionally indicate relieved intracellular iron limitation in the presence of the siderophore. Extracellular enterobactin levels decreased over time, but significant amounts were still detectable after 24 h, showing that enterobactin is not fully degraded by *C. glutamicum* under the tested conditions (Fig. 2C).

To further assess enterobactin-mediated intracellular iron availability, the recently described DtxR-based, ratiometric fluorescent iron biosensor (IronSenseR) [57], was modified for *in vivo* measurements by exchanging the Large-stokes shift reference domain (LSSmApple) by a more rapidly maturing fluorescent protein (mCherry) (Fig. 2D) and was first validated *in vitro* (Fig. S4). The modified IronSenseR variant indicated binding to ferrous iron in low micromolar range as it was observed for the original IronSenseR previously [57] thus indicating that the modification did not affect the biosensor function nor its specificity for ferrous iron (Fig. S4). Using this biosensor variant (IronSenseR-mCherry), we observed increased intracellular iron levels upon addition of pure enterobactin, as indicated by an increased GFP/mCherry ratio (Fig. 2E). Together, these results demonstrate that enterobactin is directly exploited by *C. glutamicum* and relieves intracellular iron limitation (Fig. 2F).

### Enterobactin exploitation supports stable pairwise coexistence

As we could demonstrate that *C. glutamicum* is a versatile xenosiderophore exploiter, we next asked how *E. coli*-secreted enterobactin exploitation by *C. glutamicum* unfolds in *in vivo* co-cultivations. To address this, *C. glutamicum* and *E. coli* strains were labelled with constitutively expressed fluorescence reporters E2-Crimson and GFP, respectively, and first analysed in liquid microtiter cultivations using flow cytometry (Fig. 2G). Under iron-restricted conditions and a starting ratio of 1:1, the organisms likewise remain at a balanced coexistence after 48 h, with a slight, but significant overrepresentation of *C. glutamicum* after 24 h. Within liquid cultivations, enterobactin availability is equally distributed and sustained cell-to-cell contacts are limited due to shaking. We therefore extended the analysis to spatially structured co-cultures, using macrocolonies in which access to siderophores is likely shaped by cell density, spatial distance, and uptake efficiency. To this end, plate-spot assays were performed under both iron-restricted and iron-replete conditions (Fig. S5).

As controls, *E. coli* WT grew in monoculture under both iron-restricted (3.6  $\mu\text{M}$   $\text{FeSO}_4$  + 100  $\mu\text{M}$  DIP) and iron-replete (36  $\mu\text{M}$   $\text{FeSO}_4$ ) conditions. In contrast, neither *C. glutamicum* nor *E. coli*  $\Delta\text{entC}$  showed growth under iron limitation (Fig. S5A–B). We then co-cultivated *C. glutamicum* with either *E. coli* WT or *E. coli*  $\Delta\text{entC}$  on iron-restricted CGXII agar plates at different starting ratios and two seeding densities ( $10^{-2}$  and  $10^{-3}$ ) (Fig. 2H) as initial cell densities could influence exploitation and therefore final community composition [62]. As expected, there was no growth when *C. glutamicum* and *E. coli*  $\Delta\text{entC}$  were co-cultivated under iron limitation (Fig. S5C). In co-culture with *E. coli* WT, prominent growth of *C. glutamicum* was observed across all inoculation ratios, confirming that *C. glutamicum* depends on producer-derived enterobactin also in spatially structured environments. The two species grew in intricate spatial arrangements within the macrocolonies, leading to the formation of distinct red or yellow fluorescent patches irrespective of the ratio and seeding density. Furthermore, quantification of species-specific colony areas revealed that enterobactin exploitation remained markedly

constrained: At the higher seeding density ( $10^{-2}$ ), which corresponds to closer spatial proximity, co-cultures converged to a balanced (1:1) outcome across most starting ratios, with only a slight shift toward *E. coli* when it was initially overrepresented (one-sample Student's *t*-test against null hypothesis  $\mu = 50\%$ ) (Fig. 2I, S5D). These outcomes indicate a stable coexistence maintained by negative-frequency dependent selection [63, 64], as shown by the selection coefficient crossing zero near an initial *E. coli* frequency of 50% (Fig. 2J). Thus, *E. coli* has a relative fitness advantage when initially rare, but this advantage decreases as its starting frequency increases.

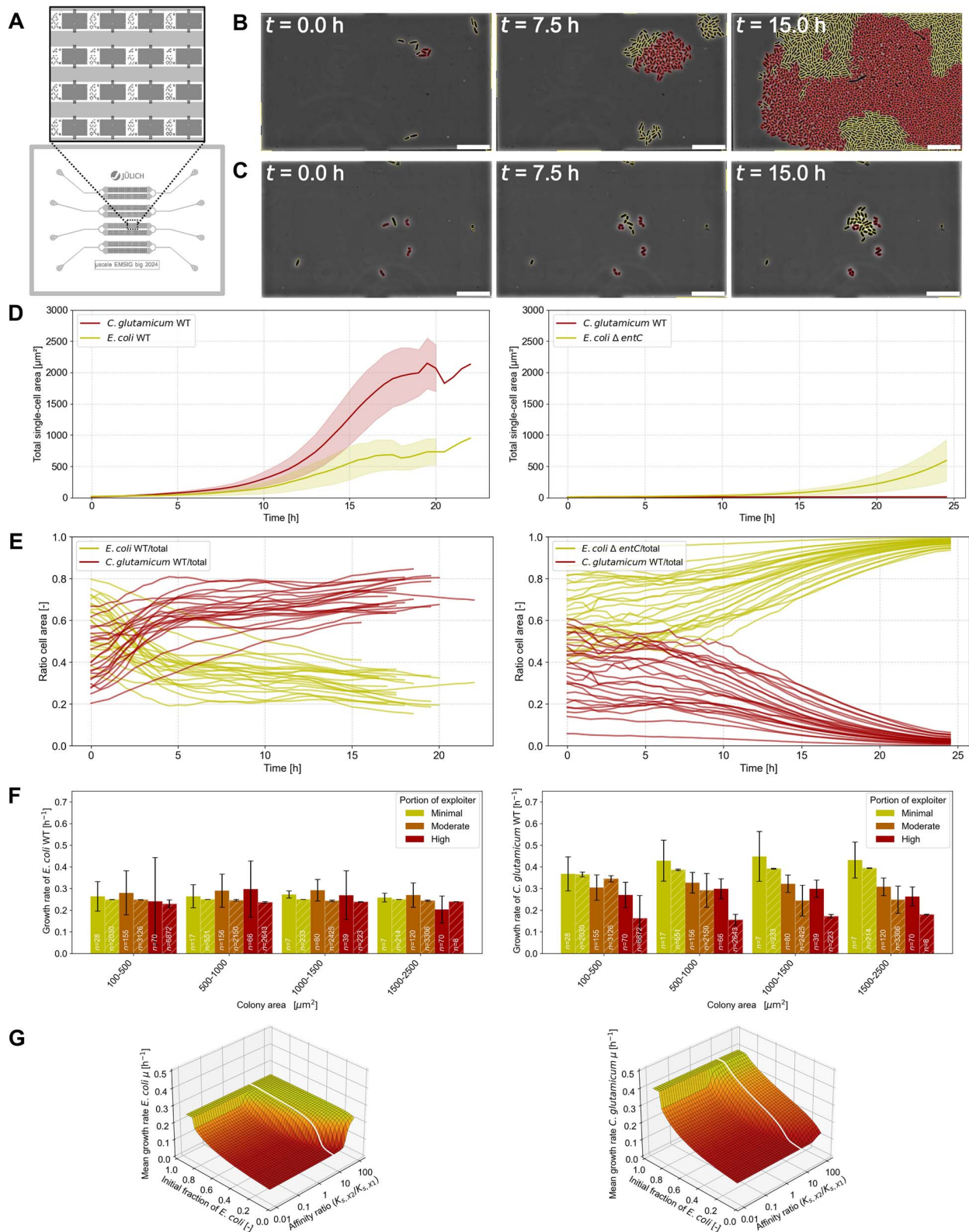
At lower seeding densities ( $10^{-3}$ ), this balance was less robust. When *C. glutamicum* was inoculated at equal or lower initial abundance *E. coli* became dominant, consistent with greater spatial segregation and reduced access to diffusible enterobactin. Even a 5- to 20-fold excess of *C. glutamicum* did not result in its dominance, but instead the community stabilized near a 1:1 ratio (Fig. 2I).

These results suggest that enterobactin is not equally accessible to both partners in macrocolonies like it is the case for the liquid cultivation. Instead, *C. glutamicum* appears to benefit only from the fraction of enterobactin that exceeds recapture by the producer, implying that exploitation depends on the presence of an exploitable surplus. This interpretation is supported by the comparison to *E. coli* WT/*E. coli*  $\Delta\text{entC}$  co-cultures, in which both strains share the same uptake system and grew largely in proportion to their initial inoculation ratio, independent of seeding density (Fig. S5E). However, when the  $\Delta\text{entC}$  mutant is present in excess, a clear difference from *E. coli* WT/*C. glutamicum* macrocolonies could be observed. While the proportion of *C. glutamicum* never exceeded a level of  $\sim 50\%$ , the excess of the siderophore producer (*E. coli* WT). This observation suggests that the efficiency of enterobactin utilization and/or uptake is significantly lower in *C. glutamicum* than in the *E. coli*  $\Delta\text{entC}$  strain.

### Single-cell analysis and modelling reveal siderophore-receptor affinity-constrained exploitation in pairwise co-culture

To resolve the dynamics underlying constrained enterobactin exploitation at the single cell level with higher spatiotemporal resolution, we next analysed co-cultivation of fluorescently labelled *E. coli* and *C. glutamicum* as single-layer microcolonies in microfluidic chambers under iron-restricted conditions (Fig. 3A, Table S2, Video S1–S2). As confirmed previously, *C. glutamicum* grew only in the presence of enterobactin-producing *E. coli* (Fig. 3B), but neither in monoculture nor in co-culture with *E. coli*  $\Delta\text{entC}$  (Fig. 3C). These differences were also reflected in the temporal development of total single-cell area obtained by image-based cell segmentation (Fig. 3D).

Tracking species-specific cell area over time revealed that also at this scale *E. coli* and *C. glutamicum* converged to a stable community composition, independent of the initial inoculation ratio (Fig. 3E). In the presence of WT *E. coli*, *C. glutamicum* accounted for  $\sim 70\%$  of the final population area ( $N = 24$  chambers), whereas its fraction remained minimal when enterobactin was absent and *E. coli*  $\Delta\text{entC}$  dominated through residual growth ( $N = 30$ ). Although this equilibrium differed from the approximately balanced outcome observed in liquid culture and on plates (Fig. 2G–I), all systems consistently showed stable coexistence at a defined community composition rather than unrestricted exploitation.



**Figure 3** Single-cell analysis and modelling reveal affinity-constrained exploitation in pairwise co-culture of *C. glutamicum* and *E. coli*. (A) Schematic layout of the microfluidic device used for co-cultivation. (B) Representative segmented frames of the co-culture of *C. glutamicum* WT and *E. coli* WT at 0, 7.5 and 15 h in CGXII medium under iron limitation (3.6  $\mu\text{M}$   $\text{FeSO}_4$ ) at 30°C. (C) Representative segmented frames of the co-culture of *C. glutamicum* WT and *E. coli*  $\Delta\text{entC}$  at 0, 7.5 and 15 h under identical conditions. (D) Mean total single-cell area ( $\mu\text{m}^2$ ) over time. Left, *C. glutamicum* WT (red) and *E. coli* WT (yellow) with  $N = 24$ ; right, *C. glutamicum* WT and *E. coli*  $\Delta\text{entC}$  with  $N = 30$ . (E) Temporal development of cell area shown separately for each organism. Each line represents an individual chamber. Left, *C. glutamicum* WT and *E. coli* WT with  $N = 24$ ; right, *C. glutamicum* WT and *E. coli*  $\Delta\text{entC}$  with  $N = 30$ . (F) Experimental and model-derived growth-rate dependencies on colony area, fitted with species-specific parameters across colony sizes and exploiter fractions (minimal, 0–0.33; moderate, 0.33–0.66; high, >0.66; color-coded). Dashed lines indicate simulated data, solid lines experimental data. (G) Model-derived growth landscapes illustrating the effects of initial fraction of *E. coli* within the community and relative affinity ratios of exploiter and producer ( $K_{s,x2}$  for *C. glutamicum* /  $K_{s,x1}$  for *E. coli*) for the shared resource. Growth rates represent averages until a total cell area of 2500  $\mu\text{m}^2$  is reached. The white line indicates the baseline model assumption.

To determine whether the population size of *C. glutamicum* does affect *E. coli* growth at the microcolony scale due to enterobactin exploitation, growth rates for each organism were calculated between image frames (0.5 h frame rate) across microcolonies of different sizes and compositions (Fig. 3F). Whereas *E. coli* growth was largely insensitive to colony composition, *C. glutamicum* growth strongly depended on the relative abundance of exploiter cells and declined sharply once *C. glutamicum* exceeded ~70% of the colony. This pattern is consistent with the idea that enterobactin remains more readily available to the producer, whereas exploitation by *C. glutamicum* is less effective.

To conceptually and quantitatively explore the social behavior observed in macrocolonies, we implemented a simplified ODE model that was directly fitted and compared to the experimentally derived single-cell data. To this end, diverging affinities, based on Monod kinetics (K<sub>S</sub>-values) were assumed to test the possibility of different enterobactin-receptor affinities for both organisms (Fig. 3F and G). Although this model does not directly prove the underlying mechanism, it successfully reproduced the experimentally observed interdependence of growth rates when the exploiter was assumed to have 10-fold lower affinity for the shared resource than the producer and consumption and uptake rates were fitted to the given microfluidic data. The model further predicted that increasing enterobactin-receptor affinities of the exploiter promotes collapse of the producer population and can lead to a “tragedy of the commons” [65], whereas coexistence is stabilized when the producer retains superior access to the shared siderophore (Fig. 3G). The model predicts that very high exploiter affinity is also detrimental to the exploiter itself.

To experimentally assess the postulated difference in affinity, we calculated EC<sub>50</sub> (half-maximal effective concentration [66]) values from growth-rate responses to increasing enterobactin concentrations. This analysis indicated a higher apparent affinity for enterobactin in *E. coli* Δ*entC* than in *C. glutamicum* (EC<sub>50-ΔentC</sub> = 0.296 ± 0.039 μM; EC<sub>50-Cg</sub> = 0.744 ± 0.087 μM; Fig. S5F and G), thus supporting the model-based hypothesis. These values do not directly reflect receptor-siderophore binding affinity, but rather the overall efficiency with which enterobactin exploitation is converted into iron uptake and growth. Together, these findings support our hypothesis in which *E. coli* captures enterobactin more efficiently, likely via its dedicated high-affinity receptor FepA, while *C. glutamicum* accesses only a shared and spatially available fraction of the siderophore. This limits exploitation and stabilizes pairwise coexistence, with community dynamics shaped by the interplay of initial composition, siderophore availability, and species-specific affinity.

### Pyoverdine acts as a private good in a three species community by preventing *C. glutamicum* enterobactin exploitation

Siderophore-based microbial interactions in natural contexts are more complex than expressed in one-to-one relationships. To address this in our model system, we extended our public good producer/exploiter system toward a multispecies setting. To this end we chose *Pseudomonas putida* as a third interacting organism, which produces the mixed-type siderophore pyoverdine. As expected, pyoverdine could not be utilized by *C. glutamicum* (Fig. 4A, Fig. S6B, D–E). Likewise, *E. coli* was also unable to utilize pyoverdine, as inferred from co-cultivation assays with the Δ*entC* mutant (Fig. S6C–E). However, *P. putida* efficiently exploited enterobactin produced by *E. coli*—as

shown by using the pyoverdine-deficient *P. putida* Δ*pvdD* mutant (Figs S6 and S7). The Δ*pvdD* strain carries a deletion of the gene of the non-ribosomal peptide synthetase PvdD, which is essential for the assembly of the peptide backbone of pyoverdine [67].

Enterobactin uptake by *P. putida* was further supported by its physiological response. In the presence of pure enterobactin, and likewise in *E. coli* WT spent medium, *P. putida* produced less pyoverdine (Fig. 4B, Fig. S7C and D), consistent with reduced intracellular iron limitation. Furthermore, by using the IronSenseR-mCherry variant in *P. putida*, we observed increased intracellular iron levels in the Δ*pvdD* strain upon addition of enterobactin (Fig. 4C), confirming that *P. putida* can acquire iron from enterobactin. Previous studies already showed homology between the enterobactin receptors FepA of *E. coli* and PfeA of *P. aeruginosa* [68]. Taken together, the described properties establish *P. putida* as a new consortium member capable of acting as a second enterobactin exploiter producing the non-exploitable (private) siderophore pyoverdine.

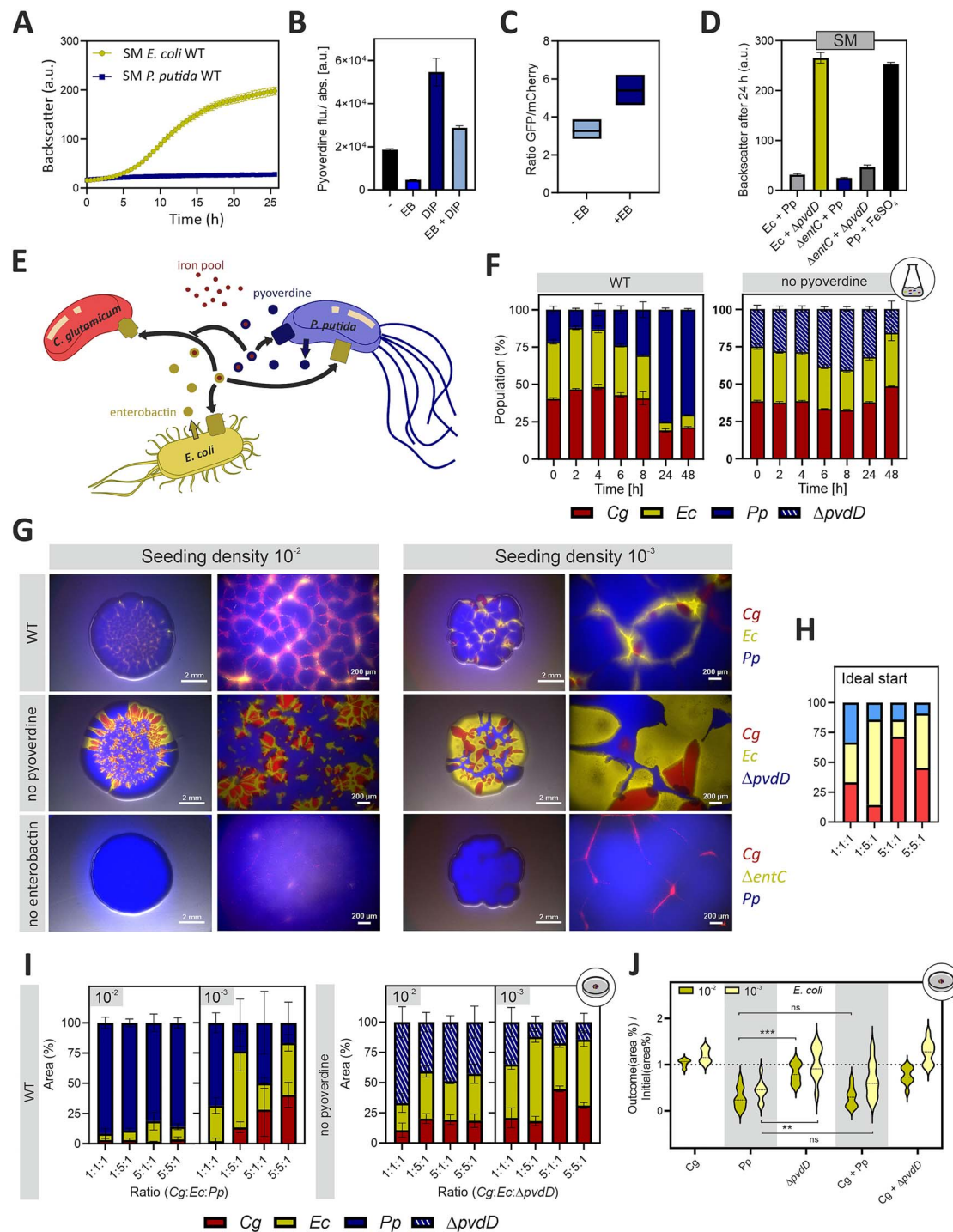
We next tested how simultaneous exposure to enterobactin and pyoverdine affects *C. glutamicum* growth. To mimic a three-species siderophore environment in monoculture, we combined spent media from *E. coli* WT or Δ*entC* with spent media from *P. putida* WT or Δ*pvdD* (30% and 20%, respectively; Fig. 4D). *E. coli* WT spent medium supported growth under iron limitation, but addition of *P. putida* WT spent medium abolished growth despite the presence of enterobactin. Growth was restored with pyoverdine-deficient Δ*pvdD* spent medium as well as by iron supplementation. Thus, pyoverdine can act as a private iron-scavenging good that restricts *C. glutamicum* access to iron, even when exploitable enterobactin is present.

### Spatial context determines community composition in the three-species consortium

Having shown that pyoverdine can override enterobactin-mediated growth promotion under iron limitation, we next examined how this interaction shapes a three-species consortium across liquid and spatially structured environments.

In liquid microtiter cultivations under iron restriction, using a 1:1:1 inoculation ratio, WT *P. putida* became strongly enriched after 24 h (Fig. 4F). *P. putida* initially declined during the first hours, indicating that its dominance was not immediate but emerged over time. This outcome is consistent with *P. putida* combining exploitation of enterobactin with pyoverdine production, which limits iron access for the other community members. Accordingly, when pyoverdine production was abolished, the community remained close to the inoculated ratio after 24 h. Conversely, in the absence of enterobactin, WT *P. putida* prevailed, whereas Δ*pvdD*-containing combinations showed little to no growth (Fig. S8C and D). Under iron-replete conditions without chelator, *P. putida* did not become overrepresented (Fig. S8A and B), confirming that its dominance depends on iron limitation.

We next asked whether spatial structure modifies this interaction by restricting siderophore diffusion and access. On iron-limited CGXII plates, WT *P. putida* again displaced both partners at high seeding density, while *C. glutamicum* and *E. coli* persisted at lower abundance than in liquid culture (10<sup>-2</sup>; Fig. 4G, Fig. S9). However, spatial structure also enabled local niche formation. In the Δ*pvdD* background, *E. coli* and *C. glutamicum* grew in close proximity, whereas *P. putida* Δ*pvdD* was largely confined to peripheral regions. As expected, loss of enterobactin production in *E. coli* enabled *P. putida* to dominate the community. Thus, when diffusion is limited, the balance between



**Figure 4** Siderophore-mediated interaction in the trio-consortium of *C. glutamicum*, *E. coli*, and *P. putida* reveals siderophore characteristics as public and private goods. (A) Growth of *C. glutamicum* with either 50% CGXII-based SM from *E. coli* WT or *P. putida* WT.  $n = 3$  biological replicates. (B) Bar plot of fluorescence measured at  $\lambda_{ex} = 398$  nm and  $\lambda_{em} = 455$  nm from *P. putida* cultivation with and without enterobactin (EB) and/or DIP supply, representing pyoverdine levels. (C) Comparing iron content by utilizing genomically integrated IronSenser-mCherry in *P. putida*  $\Delta pvdD$  cultivated with or without 250  $\mu$ M DIP. (D) Bar plot of backscatter (a.u.) at 24 h of *C. glutamicum* on CGXII-based SM mixes of either 30% *E. coli* WT with 20% *P. putida* WT, 30% *E. coli* WT with 20% *P. putida*  $\Delta pvdD$ , 30% *E. coli*  $\Delta entC$  with 20% *P. putida* WT, 30% *E. coli*  $\Delta entC$  with 20% *P. putida*  $\Delta pvdD$  or 50% *P. putida* supplemented with 3.6  $\mu$ M  $FeSO_4$ .  $n = 3$  biological replicates. (E) Schematic depiction of the siderophore-mediated interactions within a trio consortium consisting of *C. glutamicum* (public good exploiter), *E. coli* (public good provider), and *P. putida* (private good producer and public good exploiter). (F) Flow cytometry measurements of co-cultured *E. coli*-gfp, *C. glutamicum*-E2-crimson, and *P. putida*-bfp (left) or  $\Delta pvdD$  (right) under iron limited conditions.  $n = 3$  biological replicates. (G) Representative stereomicroscopy pictures of mixed drop assay on iron-restricted plates with *C. glutamicum*-E2-crimson (red), *E. coli*-gfp, and *P. putida*-bfp, as well as siderophore-deficient mutants, as indicated in two different seeding densities in a 1:1:1 ratio and 16 $\times$  vs. 80 $\times$  zoom. (H) Schematic overview on ideal start conditions as inoculated for plate assays. (I) Quantified area of *C. glutamicum*, *E. coli*, and *P. putida* deduced from the microscopy pictures as exemplarily shown in (G), set in relation to 100%.  $n = 9$  biological replicates. (J) *E. coli* outcome analysis with a ratio of *E. coli* outcome over initial area in percentage for two seeding densities ( $10^{-2}$  and  $10^{-3}$ ) in all duo and trio co-cultivations at 1:1:1 ratio. Statistical significance by Student's  $t$ -test is highlighted ( $P \leq .05$  (\*),  $P \leq .01$  (\*\*),  $P \leq .001$  (\*\*\*),  $P \leq .0001$  (\*\*\*\*)).

public enterobactin and private pyoverdine becomes locally structured rather than uniformly shared across the consortium.

Varying inoculation ratio and seeding density further revealed strong context dependency (Fig. 4G–I, Fig. S10). Under 1:1:1 WT conditions, *P. putida* became overabundant at the high inoculation density, whereas the lower density and higher initial *E. coli* abundance improved its persistence, consistent with increased spatial separation and reduced siderophore piracy. At  $10^{-3}$  seeding density, *C. glutamicum* grew in distinct patches surrounded by *E. coli*, separating it from *P. putida*. Without pyoverdine, *E. coli* and *C. glutamicum* benefited from high seeding density, whereas lower density maintained compositions closer to the inoculated ratios. Without enterobactin, *P. putida* remained dominant across conditions, whereas under iron-replete conditions *C. glutamicum* outcompeted both partners (Fig. S10).

We finally asked the question of what is the impact of exploitation on the producer. We therefore quantified final-to-initial area ratios and demonstrated that *P. putida* significantly impaired *E. coli* growth despite the higher affinity of enterobactin according to literature values [9] (Fig. 4J). This effect was strongly reduced in the  $\Delta pvdD$  background, although its magnitude varied with initial conditions. Overall, *P. putida* restricts *E. coli* growth while consistently benefiting from co-cultivation with *E. coli*, particularly when *C. glutamicum* is also present (Fig. S10F). By contrast, *C. glutamicum* exploitation did not affect *E. coli* growth, consistent with our previous results (Fig. 3F).

To further resolve how initial community composition influences community dynamics, we performed analogous experiments in microfluidic chambers and followed microcolony development, composition and architecture by time-lapse microscopy (Table S2, Fig. 5). Ternary plots revealed that high initial abundances of either *C. glutamicum* or *P. putida* resulted in a pronounced shift towards *P. putida* dominance, often reaching 70%–100% of the final community. By contrast, when *E. coli* initially represented more than 50% of the community, it persisted at high abundance, ~80%–90%, while also supporting *C. glutamicum* growth (Fig. 5A, Videos S3–S5). This pattern may reflect spatial segregation that enables *E. coli* to limit its exposure to *P. putida*. Replacing WT *P. putida* with the  $\Delta pvdD$  mutant reduced *P. putida* overabundance, with its final proportion stabilizing at ~50% (Fig. 5B, Videos S6–S8). *C. glutamicum* also grew in some chambers in the presence of WT *P. putida*, but only when the initial spatial arrangement allowed *C. glutamicum* patches to form within surrounding *E. coli* cells, thereby locally separating it from *P. putida* (Video S3).

Collectively, these findings show that siderophore-mediated interactions in multispecies communities are highly context dependent. In this three-species community, enterobactin produced by *E. coli* functions as a public good for both *C. glutamicum* and *P. putida*, whereas pyoverdine acts as a private good by reducing iron availability for competitors lacking compatible uptake systems. Spatial organization and initial community composition strongly modulate these interactions by determining the extent to which diffusible siderophores are shared, recaptured, or rendered inaccessible.

## Discussion

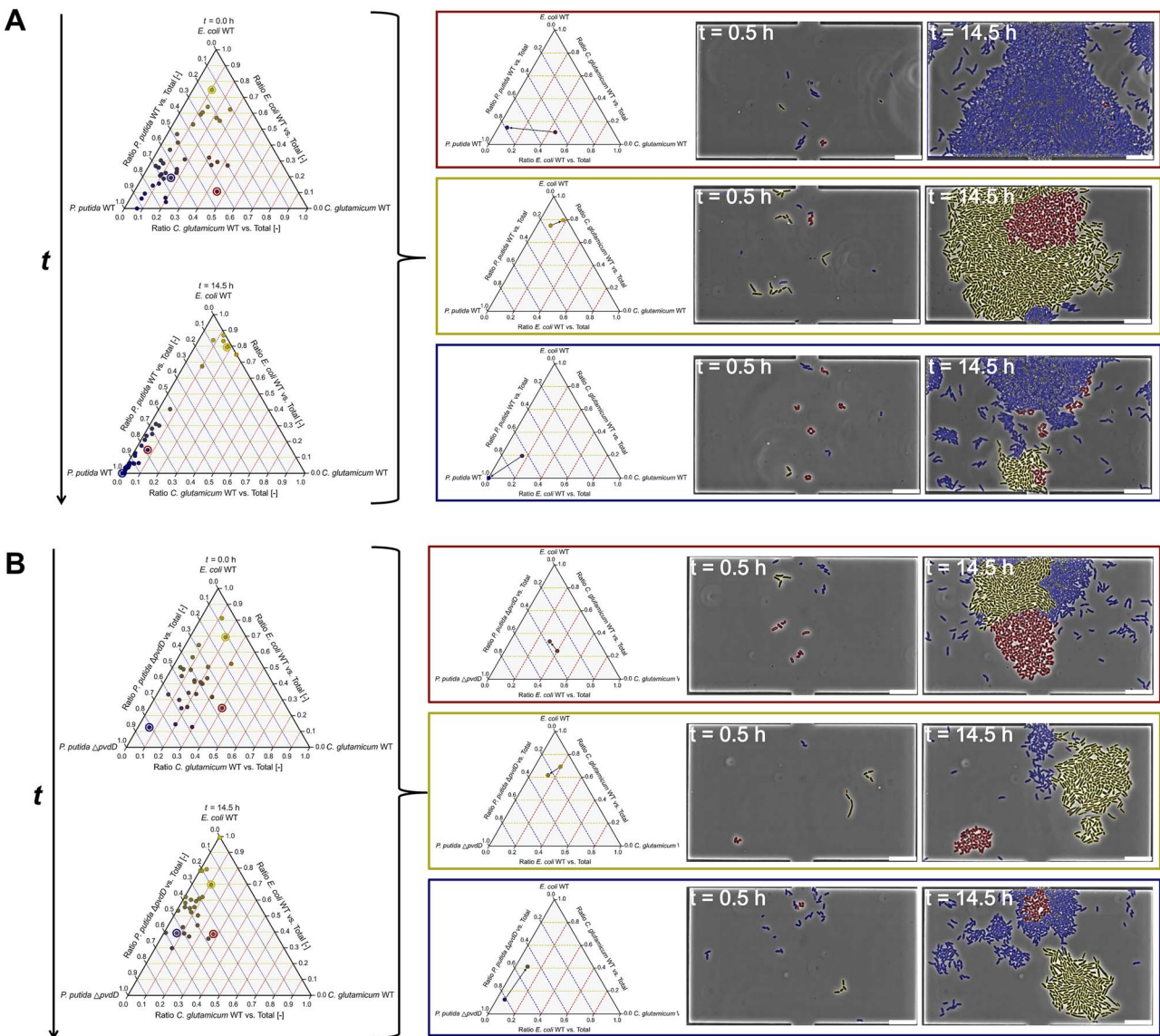
Over the past two decades, many studies of siderophore-mediated social interactions have used relatively simple systems, including interactions among strains of the same species and pairwise interactions between species. These studies have revealed that bacteria can adopt distinct ecological roles as producers, cheaters, or exploiters.

Producers synthesize and secrete siderophores, cheaters benefit from siderophores produced by clonemates, and exploiters access xenosiderophores released by other species [2, 23, 69–71]. However, natural microbial communities are not built from isolated pairwise interactions. They contain multiple, phylogenetically diverse species with different siderophore repertoires, uptake systems, affinities, and spatial arrangements. Consequently, the ecological function of a siderophore is not fixed, but emerges from the community context in which it is produced and used [23, 24, 28, 72]. In this study, we deciphered the impact of heterologous siderophores in a microbial community. We demonstrate that enterobactin from *E. coli* acts as a public good that can support the growth of the exploiters *C. glutamicum* and *P. putida* under iron limitation. In contrast, pyoverdine produced by *P. putida* acts as a private good by inhibiting growth of the competing community members. These results support a broader view of siderophores as context-dependent iron-allocation agents that can either promote coexistence or enforce exclusion, depending on the surrounding community and physical environment.

Corynebacteria are increasingly recognized as common siderophore exploiters in natural microbiomes, yet the extent and ecological consequences of this strategy have remained difficult to dissect mechanistically [73]. By using *C. glutamicum*, we demonstrate that a non-producing bacterium can exploit chemically diverse xenosiderophores from bacteria and fungi. Such diversity reflects adaptation to iron-limited, siderophore-rich environments and is consistent with the Black Queen hypothesis, whereby organisms can benefit from losing costly functions, if these are provided by other members of the community [74, 75]. Related siderophore-mediated interactions have previously been studied in *Pseudomonas*, including interactions among isolates differing in siderophore production and exploitation [10, 24, 76]. These studies established important principles of siderophore-mediated competition and coexistence within closely related taxa. Here, we extend this framework to a synthetic community of phylogenetically distant species with distinct siderophore production and uptake repertoires, allowing us to examine how heterologous siderophores simultaneously mediate sharing, exploitation, and exclusion.

Siderophore systems have long served as model systems for studying microbial cooperation [77], as also reflected in the interaction between *E. coli* and *C. glutamicum* observed here. Across structured plates and microfluidic chambers, exploitation never resulted in a collapse of the producer population. Instead, *C. glutamicum*–*E. coli* co-cultivation reproducibly converged to stable community compositions, suggesting that enterobactin exploitation by *C. glutamicum* is constrained. This dependency resulted in negative frequency-dependent selection, as typical for siderophore mediated interactions, and thereby promoted stable coexistence between producer and exploiter [2, 78].

Modelling and EC<sub>50</sub> analyses further suggest that this stability may be related to asymmetric access to enterobactin. The producer *E. coli*, carrying the cognate FepA receptor for uptake, may capture enterobactin more efficiently than the exploiter *C. glutamicum*. In this scenario, the exploiter accesses only the fraction of enterobactin that escapes producer recapture. This is consistent with the established ecological principle that stable exploitation of a diffusible public good can be promoted by asymmetric access, such that the producer retains privileged uptake [79, 80]. With limited dispersal and dependent on distances, public goods tend to stay in the vicinity of the producer, allowing a benefit from own production [79]. Previous models have



**Figure 5** Microfluidic single-cell analyses of community composition dynamics in three-species consortia. (A) Ternary plots showing the relative abundance of *C. glutamicum* WT, *E. coli* WT, and *P. putida* WT. Each point represents a single chamber, positioned according to the fractional cell area of each species. The plots show the initial composition at 0.5 h (top) and the composition after 14.5 h (bottom). Chambers with strong dominance of one species are highlighted by colored outlines and corresponding representative chambers are shown to the right, including their temporal development and segmented images at  $t = 0.5$  h and 14.5 h. (B) As in (A), but with *P. Putida*  $\Delta pvdD$  instead of WT. All experiments were performed in CGXII medium with  $3.6 \mu\text{M}$   $\text{FeSO}_4$  at  $30^\circ\text{C}$ . All scale bars  $10 \mu\text{m}$ .

described a “public good dilemma”: for a successful cooperation the overall sum of the benefit for the producer plus the benefit received by the exploiter must be bigger than the overall cost of production [80]. This interpretation is consistent with previous work, showing that enterobactin can exhibit density-dependent privatization, remaining partially cell-associated at low cell densities and becoming increasingly shared as extracellular enterobactin accumulates [81]. If exploiter public good access becomes too efficient, the shared resource may no longer stabilize coexistence but instead drives a “tragedy of the commons” scenario as is often observed during interactions between producers and cheaters. In this scenario, cheaters can still take up the corresponding siderophore-iron complexes via the same receptors and thus with the same efficiency as the producers [2, 65, 72]. Although this is not observed during interaction between

*C. glutamicum* and *E. coli*, a related stabilizing principle has been proposed for cross-feeding mutualisms. When exploiter and producer have similar uptake affinities, stable coexistence may still be maintained if the exploiter reciprocates by providing a public good or another trait of comparable ecological value [82–84]. Exemplarily, this was recently observed in *Burkholderia contaminans* making use of the siderophore bacillibactin produced by *Bacillus velezensis*, while providing branched-chain amino acids [36].

Finally, the three-species community revealed an additional layer of ecological complexity beyond the stable coexistence. Although *P. putida* can exploit enterobactin, its own pyoverdine was inaccessible to both *C. glutamicum* and *E. coli*. As a result, pyoverdine abolished the growth benefit that enterobactin otherwise provided to the non-producing exploiter. This is especially interesting considering

enterobactin has been reported to possess a higher binding affinity to iron ( $pFe \approx 34.3$ ) [85, 86] than pyoverdine ( $pFe \approx 27$ ) [87]. Thus, our observation underlines that the ecological outcome of multispecies communities cannot be predicted solely from siderophore binding affinities. Instead, additional factors must be considered, such as siderophore production rates, since the equilibrium of iron binding can shift toward a siderophore with lower binding affinity if produced at sufficiently high quantities [88]. It further depends on how efficient the iron-siderophore complex can be accessed by individual species. This mechanism is certainly relevant beyond the here described model consortium, particularly in communities containing pyoverdine-producing pseudomonads and taxa with only partially overlapping siderophore networks [24, 89]. In addition, differences in the fate of siderophores after uptake may influence community dynamics: In *E. coli*, ferric enterobactin is transported into the cytoplasm of the cell and hydrolysed via an esterase to facilitate iron release. In contrast, pyoverdine remains in the periplasm after uptake and can again be secreted after iron delivery [90, 91]. However, whether and how enterobactin is degraded, modified, or otherwise processed after exploitation by *C. glutamicum* and *P. putida* remains unknown and will require further investigation. Overall, our observations in the tripartite community show that the same siderophore network can produce different ecological outcomes depending on spatial arrangement and starting community composition [92]. The molecules remain the same, but the accessibility landscape changes. Well-mixed environments may favor broader sharing of diffusible siderophores, whereas structured habitats can promote local producer recapture, exploiter niche formation, or exclusion by non-exploitable siderophores such as pyoverdine [79, 93, 94].

Beyond that, siderophore-mediated interactions are only one part of the underlying community structure. Identity effects, i.e. strain-specific traits like baseline growth differences, resource-use efficiency or even general iron demand, further shape the functioning of the community [95]. Although recent work investigated that strain identity effects account for even more variation in microbial communities than interaction effects do, it is also claimed that this gap reduces rapidly in heterogeneous communities including public goods like siderophores [96].

Elucidating interspecific siderophore-mediated interactions in complex microbial communities is highly relevant, as previous studies already highlighted competition for iron as a driver for eco-evolutionary dynamics in natural environments [94, 97, 98]. For example, studies of plant root-associated microbiomes in tomato rhizospheres demonstrated that siderophores can alter microbiome-pathogen interactions resulting in either infection facilitation or pathogen suppression depending on the siderophore types [99]. Moreover, it was shown that higher diversity communities yield in favored siderophore production [100].

Although the three species of this study were not co-isolated from one habitat, they represent ecologically relevant siderophore interaction types that may occur in soil-, rhizosphere-, or host-influenced microbial communities. We therefore use them as a tractable synthetic consortium, rather than as a direct reconstruction of a natural community, to facilitate deciphering distinct modes of siderophore-mediated interactions in spatially structured environments in a bottom-up approach. More broadly, our findings show that siderophore chemistry, uptake compatibility, relative affinity, and spatial structure together determine, which species benefit

from (heterologous) iron-siderophore complexes and which are excluded. This framework provides a basis for understanding iron competition in natural microbiomes and for designing siderophore-shaped synthetic communities in biotechnology, agriculture [101, 102], and host-associated systems [103].

## Declaration of generative AI and AI-assisted technologies in the writing

During the preparation of this work the authors used ChatGPT in order to improve language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the manuscript.

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## Supplementary material

Supplementary material is available at *The ISME Journal* online.

## Conflicts of interest

We declare no conflict of interest.

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## Data availability

The datasets generated and/or analysed during the current study are included in this published article and its supplementary information files. Further, full raw data sets can be found in the ARC Data Hub <https://archive.nfdi4plants.org/records/b6rne-tnd39>. It is available under the DOI: 10.60534/yt353-ycp92. The code for modelling has been deposited in a public repository (<https://jugit.fz-juelich.de/m.pesch/race-for-iron-model-code.git>), ensuring full reproducibility of all results presented in this study. Additionally, the model has been deployed in a browser and can be interactively interrogated

under [computational-biology-aachen.github.io/mxl-web/dynamic-enterobactin](https://computational-biology-aachen.github.io/mxl-web/dynamic-enterobactin).

## References

- Andrews SC, Robinson AK, Rodríguez-Quinones F. Bacterial iron homeostasis. *FEMS Microbiol Rev* 2003;**27**:215–37. [https://doi.org/10.1016/s0168-6445\(03\)00055-x](https://doi.org/10.1016/s0168-6445(03)00055-x)
- Kramer J, Özkaya Ö, Kümmerli R. Bacterial siderophores in community and host interactions. *Nat Rev Microbiol* 2020;**18**:152–63. <https://doi.org/10.1038/s41579-019-0284-4>
- Chipperfield JR, Ratledge C. Salicylic acid is not a bacterial siderophore: a theoretical study. *Biometals* 2000;**13**:165–8. <https://doi.org/10.1023/a:1009227206890>
- Miethke M, Marahiel MA. Siderophore-based iron acquisition and pathogen control. *Microbiol Mol Biol Rev* 2007;**71**:413–51. <https://doi.org/10.1128/mmb.00012-07>
- Neilands JB. Iron absorption and transport in microorganisms. *Annu Rev Nutr* 1981;**1**:27–46. <https://doi.org/10.1146/annurev.nu.01.070181.000331>
- Raymond KN, Allred BE, Sia AK. Coordination chemistry of microbial iron transport. *Acc Chem Res* 2015;**48**:2496–505. <https://doi.org/10.1021/acs.accounts.5b00301>
- Ahmed E, Holmström SJ. Siderophores in environmental research: roles and applications. *Microb Biotechnol* 2014;**7**:196–208. <https://doi.org/10.1111/1751-7915.12117>
- Hider RC, Kong X. Chemistry and biology of siderophores. *Nat Prod Rep* 2010;**27**:637–57. <https://doi.org/10.1039/b906679a>
- Harris WR, Carrano CJ, Cooper SR, et al. Coordination chemistry of microbial iron transport compounds. 19. Stability constants and electrochemical behavior of ferric enterobactin and model complexes. *ChemInform* 1979;**11**:11. <https://doi.org/10.1002/chin.198003104>
- Laffont C, Kümmerli R. Microbial primer: bacterial metallophores – their role for metal homeostasis and social interactions. *Microbiology* 2026;**172**. <https://doi.org/10.1099/mic.0.001688>
- Armstrong SK, Francis CL, McIntosh MA. Molecular analysis of the *Escherichia coli* ferric enterobactin receptor FepA. *J Biol Chem* 1990;**265**:14536–43. [https://doi.org/10.1016/S0021-9258\(18\)77336-5](https://doi.org/10.1016/S0021-9258(18)77336-5)
- Thupvong T, Wiideman A, Dunn D, et al. Sequence heterogeneity of the ferripyoverdine uptake (*fpuA*), but not the ferric uptake regulator (*fur*), genes among strains of the fluorescent pseudomonads *Pseudomonas aeruginosa*, *pseudomonas aureofaciens*, *Pseudomonas fluorescens* and *pseudomonas putida*. *Biometals* 1999;**12**:265–75. <https://doi.org/10.1023/a:1009270302536>
- Gaballa A, Helmann JD. Substrate induction of siderophore transport in *Bacillus subtilis* mediated by a novel one-component regulator. *Mol Microbiol* 2007;**66**:164–73. <https://doi.org/10.1111/j.1365-2958.2007.05905.x>
- Miethke M, Kraushaar T, Marahiel MA. Uptake of xenosiderophores in *Bacillus subtilis* occurs with high affinity and enhances the folding stabilities of substrate binding proteins. *FEBS Lett* 2013;**587**:206–13. <https://doi.org/10.1016/j.febslet.2012.11.027>
- Cornelis P, Bodilis J. A survey of TonB-dependent receptors in fluorescent pseudomonads. *Environ Microbiol Rep* 2009;**1**:256–62. <https://doi.org/10.1111/j.1758-2229.2009.00041.x>
- Cornelis P, Dingemans J. *Pseudomonas aeruginosa* adapts its iron uptake strategies in function of the type of infections. *Front Cell Infect Microbiol* 2013;**3**:75. <https://doi.org/10.3389/fcimb.2013.00075>
- D'Onofrio A, Crawford JM, Stewart EJ, et al. Siderophores from neighboring organisms promote the growth of uncultured bacteria. *Chem Biol* 2010;**17**:254–64. <https://doi.org/10.1016/j.chembiol.2010.02.010>
- Neilands JB. Microbial envelope proteins related to iron. *Ann Rev Microbiol* 1982;**36**:285–309. <https://doi.org/10.1146/annurev.mi.36.100182.001441>
- Luckey M, Wayne R, Neilands JB. In vitro competition between ferriochrome and phage for the outer membrane T5 receptor complex of *Escherichia coli*. *Biochem Biophys Res Commun* 1975;**64**:687–93. [https://doi.org/10.1016/0006-291x\(75\)90375-7](https://doi.org/10.1016/0006-291x(75)90375-7)
- Llamas MA, Mooij MJ, Sparrius M, et al. Characterization of five novel *Pseudomonas aeruginosa* cell-surface signalling systems. *Mol Microbiol* 2008;**67**:458–72. <https://doi.org/10.1111/j.1365-2958.2007.06061.x>
- Griffin AS, West SA, Buckling A. Cooperation and competition in pathogenic bacteria. *Nature* 2004;**430**:1024–7. <https://doi.org/10.1038/nature02744>
- West SA, Buckling A. Cooperation, virulence and siderophore production in bacterial parasites. *Proc Biol Sci* 2003;**270**:37–44. <https://doi.org/10.1098/rspb.2002.2209>
- Niehues R, Picot A, Oliveira NM, et al. The evolution of siderophore production as a competitive trait. *Evolution* 2017;**71**:1443–55. <https://doi.org/10.1111/evo.13230>
- Butaite E, Baumgartner M, Wyder S, et al. Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater pseudomonas communities. *Nat Commun* 2017;**8**:414. <https://doi.org/10.1038/s41467-017-00509-4>
- Kumar R, Singh A, Srivastava A. Xenosiderophores: bridging the gap in microbial iron acquisition strategies. *World J Microbiol Biotechnol* 2025;**41**:69. <https://doi.org/10.1007/s11274-025-04287-w>
- Galet J, Deveau A, Hôtel L, et al. *Pseudomonas fluorescens* pirates both ferrioxamine and ferricoelichelin siderophores from *Streptomyces ambifaciens*. *Appl Environ Microbiol* 2015;**81**:3132–41. <https://doi.org/10.1128/aem.03520-14>
- Zhao Y, Bitzer A, Power JJ, et al. Nasal commensals reduce *Staphylococcus aureus* proliferation by restricting siderophore availability. *ISME J* 2024;**18**:wrae123. <https://doi.org/10.1093/ismejo/wrae123>
- Vollenweider V, Rehm K, Chepkirui C, et al. Antimicrobial activity of iron-depriving pyoverdines against human opportunistic pathogens. *elife* 2024;**13**:92493. <https://doi.org/10.7554/eLife.92493>
- Schalk IJ. Bacterial siderophores: diversity, uptake pathways and applications. *Nat Rev Microbiol* 2025;**23**:24–40. <https://doi.org/10.1038/s41579-024-01090-6>
- Fisk MB, Barrera Ramirez J, Merrick CE, et al. Identification and characterization of the biosynthesis of the hybrid NRPS-NIS siderophore Nocardichelin. *ACS Chem Biol* 2025;**20**:1435–46. <https://doi.org/10.1021/acscchembio.5c00286>
- Goswami D, Halder SK, Mondal KC. Characterization of siderophore from probiotic bacillus spp. strain isolated from traditional fermented food of the Himalaya. *Syst Microbiol Biomanuf* 2024;**4**:1150–61. <https://doi.org/10.1007/s43393-024-00257-9>
- Rajapitamahuni S, Lyou ES, Kang BR, et al. Microbial interaction-induced siderophore dynamics lead to phenotypic differentiation of *Staphylococcus aureus*. Original research. *Front Cell Infect Microbiol* 2023;**13**:1277176. <https://doi.org/10.3389/fcimb.2023.1277176>
- Rosenstein R, Torres Salazar BO, Sauer C, et al. The *Staphylococcus aureus*-antagonizing human nasal commensal *staphylococcus*

- lugdunensis* depends on siderophore piracy. *Microbiome* 2024; **12**:213. <https://doi.org/10.1186/s40168-024-01913-x>
34. Lyng M, Jørgensen JPB, Schostag MD, et al. Competition for iron shapes metabolic antagonism between *Bacillus subtilis* and *Pseudomonas marginalis*. *ISME J* 2024;**18**:1–13. <https://doi.org/10.1093/ismejo/wrad001>
  35. Pham HT, Kim W, Jang J, et al. Siderophores can alter the population dynamics of fungal-bacterial communities by inhibiting specialized metabolism. *bioRxiv*. 2025;2025.05.12.653243. <https://doi.org/10.1101/2025.05.12.653243>
  36. Xie J, Sun X, Hesselberg-Thomsen V, et al. Metabolic exchange and siderophore sharing underlie emergent biofilm synergism. *bioRxiv*. 2026;2026.02.24.707706. <https://doi.org/10.64898/2026.02.24.707706>
  37. Dertz EA, Stintzi A, Raymond KN. Siderophore-mediated iron transport in *Bacillus subtilis* and *Corynebacterium glutamicum*. *J Biol Inorg Chem* 2006;**11**:1087–97. <https://doi.org/10.1007/s00775-006-0151-4>
  38. Baumgart M, Unthan S, Kloss R, et al. *Corynebacterium glutamicum* chassis C1\*: building and testing a novel platform host for synthetic biology and industrial biotechnology. *ACS Synth Biol* 2018;**7**:132–44. <https://doi.org/10.1021/acssynbio.7b00261>
  39. Küberl A, Mengus-Kaya A, Polen T, et al. The iron deficiency response of *Corynebacterium glutamicum* and a link to thiamine biosynthesis. *Appl Environ Microbiol* 2020;**86**:e00065–20. <https://doi.org/10.1128/aem.00065-20>
  40. Keilhauer C, Eggeling L, Sahn H. Isoleucine synthesis in *Corynebacterium glutamicum*: molecular analysis of the *ilvB-ilvN-ilvC* operon. *J Bacteriol* 1993;**175**:5595–603. <https://doi.org/10.1128/jb.175.17.5595-5603.1993>
  41. Liebl W, Klammer R, Schleifer K-H. Requirement of chelating compounds for the growth of *Corynebacterium glutamicum* in synthetic media. *Appl Microbiol Biotechnol* 1989;**32**:205–10. <https://doi.org/10.1007/BF00165889>
  42. Kensy F, Zang E, Faulhammer C, et al. Validation of a high-throughput fermentation system based on online monitoring of biomass and fluorescence in continuously shaken microtiter plates. *Microb Cell Factories* 2009;**8**:31. <https://doi.org/10.1186/1475-2859-8-31>
  43. Ouyang Z, Isaacson R. Identification and characterization of a novel ABC iron transport system, *fit*, *Escherichia coli*. *Infect Immun* 2006;**74**:6949–56. <https://doi.org/10.1128/iai.00866-06>
  44. Nakouti I, Hobbs G. Incorporation of L-lysine and 2,2'-dipyridyl in the growth media promotes desferrioxamine E production by an actinobacterium. *World J Microbiol Biotechnol* 2014;**30**:331–4. <https://doi.org/10.1007/s11274-013-1425-y>
  45. Grosse C, Brandt N, Van Antwerpen P, et al. Two new siderophores produced by *pseudomonas* sp. NCIMB 10586: the anti-oomycete non-ribosomal peptide synthetase-dependent mupirochelin and the NRPS-independent triabactin. *Front Microbiol* 2023; **14**:1143861. <https://doi.org/10.3389/fmicb.2023.1143861>
  46. Mridha S, Kümmerli R. Coordination of siderophore gene expression among clonal cells of the bacterium *Pseudomonas aeruginosa*. *Commun Biol* 2022;**5**:545. <https://doi.org/10.1038/s42003-022-03493-8>
  47. Green MR, Sambrook J. *Molecular Cloning: A Laboratory Manual*, 4th edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2012.
  48. Gibson DG, Young L, Chuang RY, et al. Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Methods* 2009;**6**:343–5. <https://doi.org/10.1038/nmeth.1318>
  49. van der Rest ME, Lange C, Molenaar D. A heat shock following electroporation induces highly efficient transformation of *Corynebacterium glutamicum* with xenogeneic plasmid DNA. *Appl Microbiol Biotechnol* 1999;**52**:541–5. <https://doi.org/10.1007/s002530051557>
  50. Schäfer A, Tauch A, Jäger W, et al. Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene* 1994;**145**:69–73. [https://doi.org/10.1016/0378-1119\(94\)90324-7](https://doi.org/10.1016/0378-1119(94)90324-7)
  51. Niebisch A, Bott M. Molecular analysis of the cytochrome bc1-aa3 branch of the *Corynebacterium glutamicum* respiratory chain containing an unusual diheme cytochrome c1. *Arch Microbiol* 2001;**175**:282–94. <https://doi.org/10.1007/s002030100262>
  52. Chung CT, Niemela SL, Miller RH. One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. *Proc Natl Acad Sci USA* 1989;**86**:2172–5. <https://doi.org/10.1073/pnas.86.7.2172>
  53. Hanahan D, Jessee J, Bloom FR. Plasmid transformation of *Escherichia coli* and other bacteria. *Methods Enzymol* 1991; **204**:63–113. [https://doi.org/10.1016/0076-6879\(91\)04006-a](https://doi.org/10.1016/0076-6879(91)04006-a)
  54. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci* 2000;**97**:6640–5. <https://doi.org/10.1073/pnas.120163297>
  55. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods* 2012;**9**:676–82. <https://doi.org/10.1038/nmeth.2019>
  56. Schindelin J, Rueden CT, Hiner MC, et al. The ImageJ ecosystem: an open platform for biomedical image analysis. *Mol Reprod Dev* 2015;**82**:518–29. <https://doi.org/10.1002/mrd.22489>
  57. Papadopoulos A, Anlauf MT, Reiners J, et al. A novel biosensor for ferrous iron developed via CoBiSe: a computational method for rapid biosensor design. *ACS Sens* 2026;**11**:119–35. <https://doi.org/10.1021/acssensors.5c02481>
  58. Rabinowitz JD, Kimball E. Acidic acetonitrile for cellular metabolome extraction from *Escherichia coli*. *Anal Chem* 2007;**79**: 6167–73. <https://doi.org/10.1021/ac070470c>
  59. Seiffarth J, Kasahara K, Bund M, et al. Acia-workflows: automated single-cell imaging analysis for scalable and deep learning-based live-cell imaging analysis workflows. *arXiv Computer Science*. 2025. <https://doi.org/arXiv:2510.05886v2>
  60. Dahm C, Müller R, Schulte G, et al. The role of isochorismate hydroxymutase genes *entC* and *menF* in enterobactin and menaquinone biosynthesis in *Escherichia coli*. *Biochim Biophys Acta* 1998;**1425**:377–86. [https://doi.org/10.1016/s0304-4165\(98\)00089-0](https://doi.org/10.1016/s0304-4165(98)00089-0)
  61. von der Osten CH, Gioannetti C, Sinskey AJ. Design of a defined medium for growth of *Corynebacterium glutamicum* in which citrate facilitates iron uptake. *Biotechnol Lett* 1989;**11**:11–6. <https://doi.org/10.1007/BF01026778>
  62. van Gestel J, Weissing FJ, Kuipers OP, et al. Density of founder cells affects spatial pattern formation and cooperation in *Bacillus subtilis* biofilms. *ISME J* 2014;**8**:2069–79. <https://doi.org/10.1038/ismej.2014.52>
  63. Fraser BA, Hughes KA. Exploring negative frequency-dependent selection across levels: from genetics to ecology and back again. *Philos Trans R Soc B: Biol Sci* 2026;**381**:20250170. <https://doi.org/10.1098/rstb.2025.0169>
  64. Ayala FJ, Campbell CA. Frequency-dependent selection. *Annu Rev Ecol Evol Syst* 1974;**5**:115–38. <https://doi.org/10.1146/annurev.es.05.110174.000555>
  65. Hardin G. The tragedy of the commons. *Science* 1968;**162**:1243–8. <https://doi.org/10.1126/science.162.3859.1243>

66. Sebaugh JL. Guidelines for accurate EC50/IC50 estimation. *Pharm Stat* 2011;**10**:128–34. <https://doi.org/10.1002/pst.426>
67. Merriman TR, Merriman ME, Lamont IL. Nucleotide sequence of *pvdD*, a pyoverdine biosynthetic gene from *Pseudomonas aeruginosa*: PvdD has similarity to peptide synthetases. *J Bacteriol* 1995;**177**:252–8. <https://doi.org/10.1128/jb.177.1.252-258.1995>
68. Moynié L, Milenkovic S, Mislin GLA, *et al.* The complex of ferric-enterobactin with its transporter from *Pseudomonas aeruginosa* suggests a two-site model. *Nat Commun* 2019;**10**:3673. <https://doi.org/10.1038/s41467-019-11508-y>
69. Buckling A, Harrison F, Vos M, *et al.* Siderophore-mediated cooperation and virulence in *Pseudomonas aeruginosa*. *FEMS Microbiol Ecol* 2007;**62**:135–41. <https://doi.org/10.1111/j.1574-6941.2007.00388.x>
70. Smith P, Schuster M. Public goods and cheating in microbes. *Curr Biol* 2019;**29**:R442–7. <https://doi.org/doi:10.1016/j.cub.2019.03.001>
71. Harrison F, Paul J, Massey RC, *et al.* Interspecific competition and siderophore-mediated cooperation in *Pseudomonas aeruginosa*. *ISME J* 2008;**2**:49–55. <https://doi.org/10.1038/ismej.2007.96>
72. Jin Z, Li J, Ni L, *et al.* Conditional privatization of a public siderophore enables *Pseudomonas aeruginosa* to resist cheater invasion. *Nat Commun* 2018;**9**:1383. <https://doi.org/10.1038/s41467-018-03791-y>
73. Fukushima T, Allred BE, Raymond KN. Direct evidence of iron uptake by the gram-positive siderophore-shuttle mechanism without iron reduction. *ACS Chem Biol* 2014;**9**:2092–100. <https://doi.org/10.1021/cb500319n>
74. Morris JJ, Lenski RE, Zinser ER. The black queen hypothesis: evolution of dependencies through adaptive gene loss. *MBio* 2012;**3**:e00036-12. <https://doi.org/10.1128/mbio.00036-12>
75. Mas A, Jamshidi S, Lagadeuc Y, *et al.* Beyond the black queen hypothesis. *ISME J* 2016;**10**:2085–91. <https://doi.org/10.1038/ismej.2016.22>
76. Leinweber A, Fredrik Inglis R, Kümmerli R. Cheating fosters species co-existence in well-mixed bacterial communities. *ISME J* 2017;**11**:1179–88. <https://doi.org/10.1038/ismej.2016.195>
77. West SA, Griffin AS, Gardner A. Evolutionary explanations for cooperation. *Curr Biol* 2007;**17**:R661–72. <https://doi.org/10.1016/j.cub.2007.06.004>
78. Ross-Gillespie A, Gardner A, West Stuart A, *et al.* Frequency dependence and cooperation: theory and a test with bacteria. *Am Nat* 2007;**170**:331–42. <https://doi.org/10.1086/519860>
79. Julou T, Mora T, Guillon L, *et al.* Cell-cell contacts confine public goods diffusion inside *Pseudomonas aeruginosa* clonal microcolonies. *Proc Natl Acad Sci* 2013;**110**:12577–82. <https://doi.org/10.1073/pnas.1301428110>
80. Allen B, Gore J, Nowak MA. Spatial dilemmas of diffusible public goods. *elife* 2013;**2**:e01169. <https://doi.org/10.7554/eLife.01169>
81. Scholz RL, Greenberg EP. Sociality in *Escherichia coli*: enterochelin is a private good at low cell density and can be shared at high cell density. *J Bacteriol* 2015;**197**:2122–8. <https://doi.org/10.1128/jb.02596-14>
82. Harcombe W. Novel cooperation experimentally evolved between species. *Evolution* 2010;**64**:2166–72. <https://doi.org/10.1111/j.1558-5646.2010.00959.x>
83. Culp EJ, Goodman AL. Cross-feeding in the gut microbiome: ecology and mechanisms. *Cell Host Microbe* 2023;**31**:485–99. <https://doi.org/doi:10.1016/j.chom.2023.03.016>
84. LaSarre B, McCully AL, Lennon JT, *et al.* Microbial mutualism dynamics governed by dose-dependent toxicity of cross-fed nutrients. *ISME J* 2017;**11**:337–48. <https://doi.org/10.1038/ismej.2016.141>
85. Loomis LD, Raymond KN. Solution equilibria of enterobactin and metal-enterobactin complexes. *Inorg Chem* 1991;**30**:906–11. <https://doi.org/10.1021/ic00005a008>
86. Johnstone TC, Nolan EM. Beyond iron: non-classical biological functions of bacterial siderophores. *Dalton Trans* 2015;**44**:6320–39. <https://doi.org/10.1039/C4DT03559C>
87. Albrecht-Gary A-M, Blanc S, Rochel N, *et al.* Bacterial iron transport: coordination properties of Pyoverdine PaA, a peptidic siderophore of *Pseudomonas aeruginosa*. *Inorg Chem* 1994;**33**:6391–402. <https://doi.org/10.1021/ic00104a059>
88. Kraemer SM. Iron oxide dissolution and solubility in the presence of siderophores. *Aquat Sci* 2004;**66**:3–18. <https://doi.org/10.1007/s00027-003-0690-5>
89. Figueiredo ART, Özkaya Ö, Kümmerli R, *et al.* Siderophores drive invasion dynamics in bacterial communities through their dual role as public good versus public bad. *Ecol Lett* 2022;**25**:138–50. <https://doi.org/10.1111/ele.13912>
90. Imperi F, Tiburzi F, Visca P. Molecular basis of pyoverdine siderophore recycling in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci* 2009;**106**:20440–5. <https://doi.org/10.1073/pnas.0908760106>
91. Winkelmann G, Cansier A, Beck W, *et al.* HPLC separation of enterobactin and linear 2,3-dihydroxybenzoylserine derivatives: a study on mutants of *Escherichia coli* defective in regulation (*fur*), esterase (*Fes*) and transport (*fepA*). *Biometals* 1994;**7**:149–54. <https://doi.org/10.1007/BF00140485>
92. Bäcker M, Doekes HM, Garza DR, *et al.* Spatial structure: shaping the ecology and evolution of microbial communities. *FEMS Microbiol Rev* 2026;**50**:fuaf067. <https://doi.org/10.1093/femsre/fuaf067>
93. Chen L, Li Z-Y, Wang G-Y. Siderophores produced by marine microorganisms. *Antonie Van Leeuwenhoek* 2025;**118**:91. <https://doi.org/10.1007/s10482-025-02098-5>
94. Kümmerli R, Schiessl KT, Waldvogel T, *et al.* Habitat structure and the evolution of diffusible siderophores in bacteria. *Ecol Lett* 2014;**17**:1536–44. <https://doi.org/doi:10.1111/ele.12371>
95. Bell T, Newman JA, Silverman BW, *et al.* The contribution of species richness and composition to bacterial services. *Nature* 2005;**436**:1157–60. <https://doi.org/10.1038/nature03891>
96. Kramer J, Maréchal S, Figueiredo ART, *et al.* Strain identity effects contribute more to *pseudomonas* community functioning than strain interactions. *ISME J* 2025;**19**:wraf025. <https://doi.org/10.1093/ismej/wraf025>
97. Barber MF, Elde NC. Buried treasure: evolutionary perspectives on microbial iron piracy. *Trends Genet* 2015;**31**:627–36. <https://doi.org/10.1016/j.tig.2015.09.001>
98. Cordero OX, Ventouras LA, DeLong EF, *et al.* Public good dynamics drive evolution of iron acquisition strategies in natural bacterioplankton populations. *Proc Natl Acad Sci USA* 2012;**109**:20059–64. <https://doi.org/10.1073/pnas.1213344109>
99. Gu S, Wei Z, Shao Z, *et al.* Competition for iron drives phytopathogen control by natural rhizosphere microbiomes. *Nat Microbiol* 2020;**5**:1002–10. <https://doi.org/10.1038/s41564-020-0719-8>
100. O'Brien S, Culbert CT, Barraclough TG. Community composition drives siderophore dynamics in multispecies bacterial communities. *BMC Ecol Evol* 2023;**23**:45. <https://doi.org/10.1186/s12862-023-02152-8>
101. Singh P, Chauhan PK, Upadhyay SK, *et al.* Mechanistic insights and potential use of Siderophores producing microbes in rhizosphere for mitigation of stress in plants grown in degraded land. *Front Microbiol* 2022;**13**:898979. <https://doi.org/10.3389/fmicb.2022.898979>

102. Deb CR, Tatung M. Siderophore producing bacteria as biocontrol agent against phytopathogens for a better environment: a review. *S Afr J Bot* 2024;**165**:153–62. <https://doi.org/doi:10.1016/j.sajb.2023.12.031>
103. Ellermann M, Arthur JC. Siderophore-mediated iron acquisition and modulation of host-bacterial interactions. *Free Radic Biol Med* 2017;**105**:68–78. <https://doi.org/doi:10.1016/j.freeradbiomed.2016.10.489>
104. Górska A, Sloderbach A, Marszał MP. Siderophore-drug complexes: potential medicinal applications of the 'Trojan horse' strategy. *Trends Pharmacol Sci* 2014;**35**:442–9. <https://doi.org/10.1016/j.tips.2014.06.007>