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Research Article

RESEARCH ARTICLE

Growth of desferrioxamine-deficient *Streptomyces* mutants through xenosiderophore piracy of airborne fungal contaminations

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One sentence summary: Uncultivable desferrioxamine non-producers of *Streptomyces* recover growth by acquiring the siderophores produced by airborne neighboring filamentous fungi.
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ABSTRACT

Due to the necessity of iron for housekeeping functions, nutrition, morphogenesis and secondary metabolite production, siderophore piracy could be a key strategy in soil and substrate colonization by microorganisms. Here we report that mutants of bacterium *Streptomyces coelicolor* unable to produce desferrioxamine siderophores could recover growth when the plates were contaminated by indoor air spores of a *Penicillium* species and *Engyodontium album*. UPLC-ESI-MS analysis revealed that the HPLC fractions with the extracellular 'resuscitation' factors of the *Penicillium* isolate were only those that contained siderophores, i.e. Fe-dimerum acid, ferrichrome, fusarinine C and coprogen. The restored growth of the *Streptomyces* mutants devoid of desferrioxamine is most likely mediated through xenosiderophore uptake as the cultivability depends on the gene encoding the ABC-transporter-associated DesE siderophore-binding protein. That a filamentous fungus allows the growth of desferrioxamine non-producing *Streptomyces* in cocultures confirms that xenosiderophore piracy plays a vital role in nutritional interactions between these taxonomically unrelated filamentous microorganisms.

Keywords: fungi–bacteria interactions; interkingdom interactions; siderophore piracy; commensalism; iron utilization; secondary metabolism

INTRODUCTION

Soil-dwelling, sporulating, filamentous fungi and filamentous bacteria (i.e. actinomycetes, which include *Streptomyces* spp.) display similar saprophytic lifestyles and morphology, making them ideal models to illustrate convergent evolution (Wosten and Willey 2000). An important common property is their genetic adaptation for carbohydrate utilization and, in particular, their ability to degrade recalcitrant organic polymers such as lignocellulose and chitin thanks to an extremely diversified

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enzymatic arsenal (Hodgson 2000; Bertram *et al.* 2004; Sanchez 2009; Bugg *et al.* 2011). The filamentous growth of fungi and actinomycetes appears to possess the appropriate morphology for the penetration of plant residues, mainly consisting of cellulose and hemicellulose surrounded by a dense network of lignin (Boer *et al.* 2005). Hyphal growth and functionally similar lignocellulolytic secretomes suggest that filamentous fungi and actinomycetes would compete for nutrients when they occupy the same environmental niche (Zeltins and Schrempf 1995; Boer *et al.* 2005; Siemieniewicz and Schrempf 2007; Chater *et al.* 2010; Frey-Klett *et al.* 2011; Seipke *et al.* 2011; Seipke, Kaltenpoth and Hutchings2012). However, some studies have reported mutualistic and commensal associations between actinomycetes and fungi (Boer *et al.* 2005; Tarkka, Sarniguet and Frey-Klett 2009; Frey-Klett *et al.* 2011; Moree *et al.* 2012; Seipke, Kaltenpoth and Hutchings 2012).

The battle for iron resources could be an important aspect to interactions during confrontations between fungi and actinomycetes. Indeed, iron is not only essential for housekeeping functions such as respiration but is also believed to mechanically contribute to the destruction of the cellulosic material through the generation of reactive oxygen species (Hammel *et al.* 2002; Jung *et al.* 2015). Furthermore, siderophores are involved in triggering morphogenesis and antibiotic biosynthesis in different *Streptomyces* species (Yamanaka *et al.* 2005; Traxler *et al.* 2012; Eto *et al.* 2013; Traxler *et al.* 2013; Lambert *et al.* 2014), and thus possibly in the production of antifungal agents. In many different respects, actinomycetes could thus have adapted the onset of their siderophore biosynthetic and uptake systems to the presence of filamentous fungi. This hypothesis is supported by previous studies that suggest possible fungal siderophore piracy by streptomycetes, as the exogenous supply of pure ferrichrome, as well as pure coprogen, could partially restore the growth of otherwise uncultivable desferrioxamine (DFO)-null mutants of *Streptomyces coelicolor* grown in an iron-limited medium (Barona-Gomez *et al.* 2006). Prior to this, Müller, Matzanke and Raymond (1984) had monitored high-affinity xenosiderophore transport by *S. pilosus*. However, whether fungal siderophore utilization by *Streptomyces* really occurs in coculture and/or in a natural environment is yet to be shown. Indeed, the exogenous supply of pure fungal siderophore in a sterile medium inoculated with a dense *Streptomyces* spore suspension is very different than an ordinary growth context where cohabiting streptomycetes and fungi compete for nutrients, and could also impair each other's growth through the secretion of cell wall lytic enzymes (peptidoglycan hydrolases and chitinases), and/or by producing antimicrobial (antifungal and antibacterial) metabolites.

Here we show that under challenging coculture conditions for *Streptomyces*, i.e. when it is rendered incapable of accessing the iron resources of the culture medium, DFO-non-producing mutants of *S. coelicolor* were able to recover growth by acquiring all types of siderophores produced by a neighbouring filamentous fungus. Our findings provide additional evidence of the importance of xenosiderophore utilization in niche colonization between taxonomically unrelated microorganisms.

MATERIALS AND METHODS

Bacterial strains and culture conditions

The *S. coelicolor* Δ*desA* mutant strain in which the open reading frame SCO2782 is replaced by an apramycin resistance cassette was provided by Prof. Juan Martin from the University of Leon, Spain (strain JMG1, Juan Martin Gift, Tunca *et al.* 2007). *Streptomyces coelicolor* strains W3, W13 and W23 were provided by Prof. Gregory Challis (Barona-Gomez *et al.* 2006). The LM1 mutant strain is a spontaneous mutant of strain W3 (Δ*cchH*) that has also lost the ability to produce DFO. *Streptomyces coelicolor* M145 was used as wild-type strain. The R2YE medium was used for siderophore production assays and growth studies, and prepared according to the 'Practical *Streptomyces* Genetics' manual (Kieser *et al.* 2000).

Fungal species identification

Species identification was confirmed by sequencing two regions of the fungal ribosomal RNA genes: the internal transcribed spacer 2 (ITS2) and the D1/D2 region of the large rRNA subunit (Ferrer *et al.* 2001). Before sequencing, both fungal strains were grown in Sabouraud liquid medium (Sigma-Aldrich, Belgium) and incubated at 30°C for 5–7 days. Afterwards, fungal DNA was extracted from a large mycelial mass by using the MOBIO Ultraclean microbial DNA isolation kit (bioMérieux, France). The region targeted by ITS2 was amplified using the ITS86 forward primer 5′-GTGAATCATCGAATCTTTGAA-3′ and ITS4 reverse primer 5′-TCCTCCGCTTATTGATATGC-3′. The D1/D2 region was amplified by using NL1 forward primer (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 reverse primer (5′-GGTCCGTGTTTCAAGACGG-3′) (Leaw *et al.* 2006).

The purification of PCR and sequenced products was performed on the Sciclone G3 Automated Handling Work Station (Perkin Elmer, USA) using the Exosap IT technique (Amersham, GE Healthcare Europe GmbH, Belgium) and the Agencourt CleanSeq Kit® (Agencourt Bioscience, USA), respectively. Sequencing was performed on the 3130xl DNA sequencer (Applied Biosystems, USA). Sequences were edited using the ABI Sequence Scanner V.1.0 software (Applied Biosystems) and were compared to the CBS database for filamentous fungi, which collates several databases including Genbank (http://www.cbs.knaw.nl).

Siderophore production, extraction and purification

Siderophore extraction was performed as described previously (Craig *et al.* 2012). After 72 h of incubation on R2YE medium at 28°C, siderophores were extracted from the spent agar with an equal volume of MilliQ water, lyophilized and finally resuspended in MilliQ water. The purification of fungal siderophores by reversed-phase high-performance liquid chromatography (RP-HPLC) was performed as previously described (Craig *et al.* 2012). Briefly, 0.4 μM $FeCl_3$ were first added to concentrated extracts in order to convert the siderophores into their ferric form. A Waters Alliance e2695 HPLC system equipped with a 2998 PDA detector (Waters) was used for HPLC analysis. Samples were analysed on a C18ec column (250/4,6 Nucleodur 100–5 C18ec, Macherey-Nagel) and eluted with MilliQ water + 0.1% TFA as solvent A, and acetonitrile + 0.1% TFA as solvent B. Elution started with solvent B at 0% and increased gradually to 35% over 18 min. A second gradient bringing solvent B to 100% over 2 min was then applied. Solvent B remained at 100% for 3 min followed by a plateau at solvent A 100% for 4 min at a flow rate of 1 ml min^{-1}. Ferric-hydroxamate siderophores were detected by measuring absorbance at 435 nm as reported previously (Barona-Gomez *et al.* 2006).

Fractionation of the supernatant was performed by HPLC and with the elution programme described above. An amount of 100 μl of the crude extract of a 3-day-old R2YE *Penicillium chrysogenum* culture was injected ($n = 3$). Fractions of 250 μl were

collected between 7 and 20 min using a Waters Fraction Collector III. Fractions collected at the same retention times from the three different injections were then pooled, concentrated by lyophilization and resuspended in 200 μl of water. An amount of 5 μl of each fraction were applied onto 4-mm-diameter paper discs in order to identify which fractions contained compounds able to restore growth in the LM1 mutant.

UPLC-ESI-MS analysis of siderophores

Penicillium.chrysogenum extracts were analysed mainly as described previously (Maciejewska *et al.* 2015) with UPLC (Acquity H-class, Waters s.a., Zellik, Belgium) coupled to a single quadrupole mass spectrometer (Waters SQD mass analyser) on an ACQUITY UPLC® BEH C_{18} 1.7 μm column. An amount of 10 μl were injected and elution was performed at 40°C with a constant flow rate of 0.6 ml min^{-1} using a gradient of acetonitrile in water (acidified with 0.1% formic acid) as follows: 0.5 min at 0%, from 0 to 35% over 2.5 min, from 35 to 100% over 0.2 min, and maintained at 100% for 1.8 min. Compounds were identified as protonated molecular ions using an electrospray in positive ion mode (scan in the mass range *m/z* 200–1300) by setting SQD parameters as follows: source temperature 130°C, desolvation temperature 300°C and desolvation/cone nitrogen flow 1000/50 L h^{-1}. For optimal detection, the cone voltage was set at 80 V.

Confocal laser microscopy

Laser confocal microscopy was mainly performed as described previously (Tenconi *et al.* 2012, 2013). Staining of viable filaments was performed with SYTO 9 green fluorescent nucleic acid, and according to the manufacturer's instructions (LIVE/DEAD BacLight Bacterial Viability Kit, Molecular Probes, L-13 152). Samples were then examined under a Leica TCS-SP2 confocal laser-scanning microscope at a wavelength of 488 nm for excitation and 530 nm (green) for emission. Quantification of germinating spores at a distance from the fungus was performed using the 3D object counter tool of the ImageJ-Fiji software (Schindelin *et al.* 2012).

RESULTS

Fungal contaminations resuscitate *S. coelicolor* DFO-non-producing mutants

DFO siderophores are essential for the growth of *S. coelicolor* on the iron-limited medium R2YE (Lambert *et al.* 2014). Indeed, when this medium is inoculated with ~10^6 spores of the *desA* null mutant of *S. coelicolor* (strain Δ*desA*, JMG1; DFO-null mutant, Tunca *et al.* 2007) or the LM1 mutant (coelichelin- [CCLN] and DFO-null mutant), no growth is observed (Fig. 1a). The inability of both mutants to grow under iron-limited conditions on R2YE medium is due only to their failure to produce DFO siderophores (Fig. S1, Supporting Information) as, for an unknown reason, the production of CCLN by the *desA* mutant is not sufficient to sustain the growth of *S. coelicolor* (Lambert *et al.* 2014). It is possible to grow these *S. coelicolor* mutants on the R2YE medium by supplying 0.1 μM of desferrioxamine B or about 30 μM of $FeCl_3$ (Fig. 1a), i.e. the iron concentration at which siderophore production on the R2YE medium is fully inhibited in the wild-type strain *S. coelicolor* M145 and, therefore, the iron concentration at which DFOs are no longer required to sustain growth on this medium.

Interestingly, we observed that indoor air fungal contaminations (FCs) on R2YE plates previously inoculated with either the Δ*desA* or LM1 mutants were able to restore the growth of both *S. coelicolor* DFO-non-producing mutants (Fig. 1b). Figure 1b shows the original R2YE plates contaminated with fungi where we first observed abundant growth in the *S. coelicolor* strain LM1 around the first fungal contamination ($FC^{\#1}$), and where three colonies of the *desA* null mutant were observed to grow next to a second type of fungal contamination ($FC^{\#2}$). In order to establish that the restored growth of *S. coelicolor* DFO-non-producing mutants was indeed due to the FCs and not due to the occurrence of spontaneous revertants that frequently appear when the inoculum of *desA* or LM1 mutants is more dense (see Fig. S2, Supporting Information and Fig. 3), we picked up filaments of $FC^{\#1}$ and $FC^{\#2}$ and spotted them in the middle or at the border of R2YE plates freshly inoculated with either mutant (Fig. 1c). We observed that the growth of mutant strains Δ*desA* and LM1 started where the fungal hyphae were developing in the Petri dish, confirming that the FCs were responsible for restoring growth in the mutants.

DNA extraction and sequencing of PCR amplicons of the internal transcribed spacer 2 (ITS2) region using ITS universal primers (Ferrer *et al.* 2001) revealed that the $FC^{\#1}$ which allowed the LM1 mutant to grow belongs to the *Penicillium* subgenus group with *Penicillium chrysogenum* as the closest match (GenBank KP216986; 99.7% identity (296/297, 0 gap; *E*-value 4e-151; see Fig. S3, Supporting Information). For the $FC^{\#2}$, overlapping PCR amplicons of the ITS2 region and the D1/D2 region of the large rRNA subunit suggested *Engyodontium album* as closest match with *E. album* isolate A1S6–6 (GenBank KJ767115; 99.7% identity) for the ITS86/ITS4 region, and *E. album* isolate AHF (GenBank KC311469; 99.7% identity) for the D1/D2 region (Fig. S4, Supporting Information). These two species belong to the Ascomycetes division and are common environmental contaminants. Crude extracts from the spent agar of a 3-day R2YE culture of the *P. chrysogenum* species were able to restore the growth of both *desA* and LM1 mutants while extracts from *E. album* were only able to restore growth in the *desA* mutant (Fig. 1d). The incapability of extracts of *E. album* to restore growth in the LM1 mutant could be attributed to the absence of several types of siderophores (CCLN and DFOs) in the LM1 strain which results in a more severe iron-dependent phenotype than observed for the Δ*desA* mutant.

It is interesting to note that production of the blue-pigmented antibiotic actinorhodin by the *S. coelicolor* mutants was higher in the vicinity of the fungi while production of the red-pigmented prodiginines was visible at the periphery of the *S. coelicolor* growing zone (Fig. 1c). As actinorhodin is produced later than prodiginines in the course of the lifecycle of *S. coelicolor* (Huang *et al.* 2001), this suggests that the growth of LM1 and *desA* mutants is accelerated in the vicinity of the fungi. To confirm this hypothesis, we quantified by confocal microscopy the germinating spores of the *S. coelicolor* LM1 mutant at 24 h post-inoculation and prior to *Streptomyces* macroscopic visualization (Fig. 2a). Five different growing stages post-emergence of the germination tube were determined and quantified (Fig. 2b). At 24 h, we observed that the germination of the spores and the hyphal growth of the LM1 mutant were proportionally weaker with increasing fungi–bacteria distance, with spores remaining dormant at an approximate distance of 1 mm from the *Penicillium* species (zone 15 in Fig. 2b). Although a weak inhibition of spore germination was observed at the periphery of the fungus (zone 1 in Fig. 2b), we also observed early germinating spores of the *S. coelicolor* LM1 mutant thoroughly embedded in the dense filamentous network of the *Penicillium* species (Fig. 2c) suggesting that *Streptomyces* could possibly access the growth factors at their source, i.e. directly from the secreting fungal hyphae.

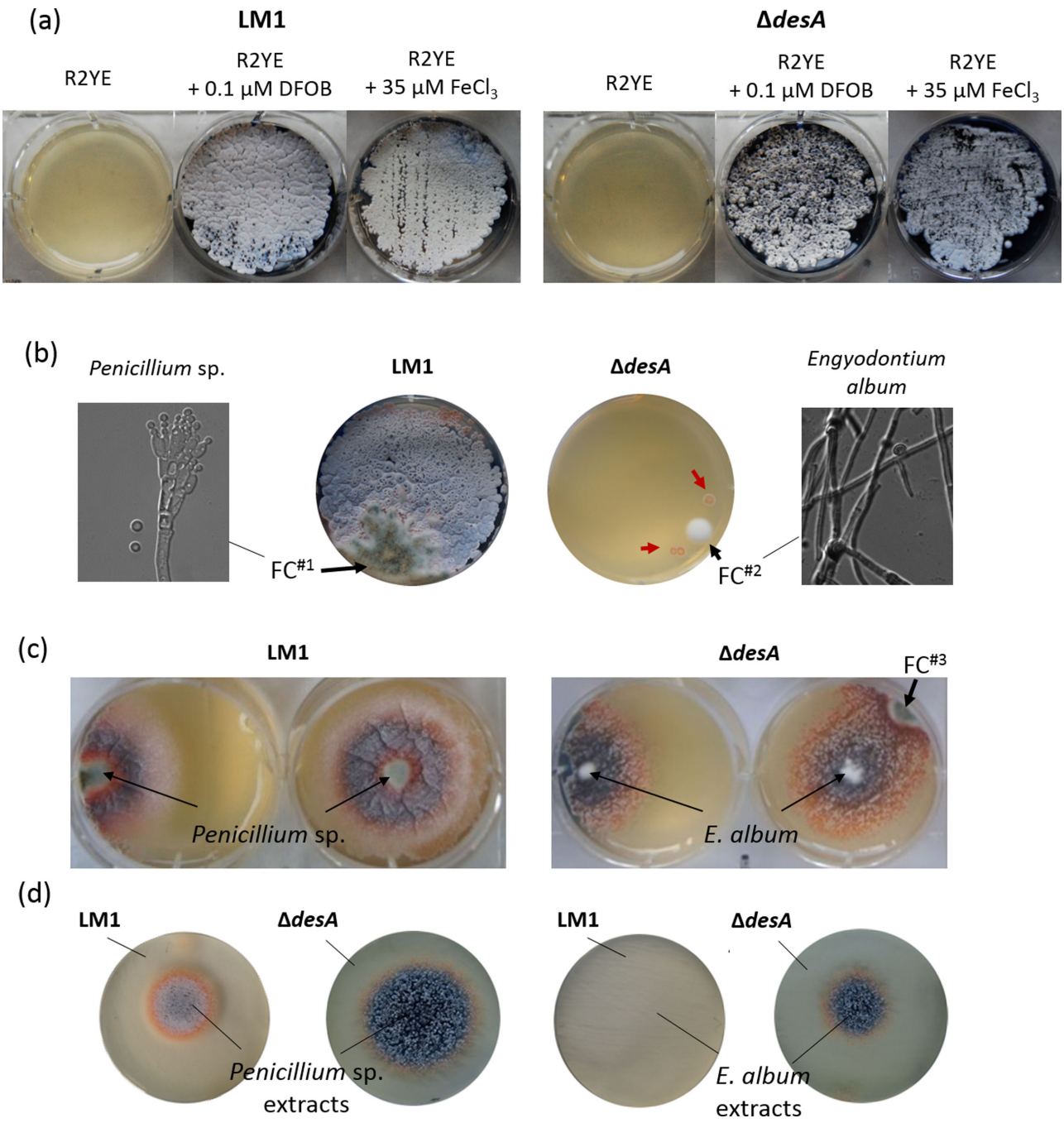

Figure 1. Fungal contaminations 'resuscitate' *S. coelicolor* DFO-non-producing mutants. (**a**) Impaired growth of *S. coelicolor* DFO-non-producing mutants LM1 and Δ*desA* on R2YE medium. Mutant strains Δ*desA* and LM1 (~10^6 spores) start growth when desferrioxamine B (0.1 μM DFOB) or iron (35 μM of $FeCl_3$) is added to the R2YE medium. (**b**) Original R2YE plates contaminated with *P. chrysogenum* (left panels) and *E. album* (right panels). FC = fungal contamination. Red arrows point to the three developing colonies of the *desA* mutant next to *E. album*. (**c**) Confirmation that the restored growth of *S. coelicolor* DFO-null mutants is indeed due to the FCs. (**d**). Effect of compounds extracted from the spent agar of a 3-day-old R2YE culture of *P. chrysogenum* (left panels) and *E. album* (right panels) on the growth of *S. coelicolor* DFO-non-producing mutants. An amount of 30 μl of the extracellular crude extracts was transferred to the centre of the R2YE plates previously inoculated with *S. coelicolor* mutant strains Δ*desA* or LM1.

Penicillium resuscitation factors are iron-chelating molecules

The conditional growth of DFO-non-producing mutants of *S. coelicolor* illustrated here suggests that strains Δ*desA* and LM1 can use growth factors, most likely siderophores, secreted by fungi which enable them to grow on the iron-limited R2YE medium. The lower capacity of *E. album* to grow on the R2YE medium compared to the *Pencillium* species and, therefore, the relative lower ability of *E. album* to secrete *S. coelicolor* mutants' resuscitation factors (Fig. 1d), prompted us to focus our further analyses on our *Penicillium* isolate. Moreover, *E. album* is a fungus for which information on secreted iron-chelating compounds is not available while *Pencillium* species are one of the most common fungal contaminations from indoor air (Fox *et al.* 1990; Scott *et al.* 2004), and one of the best-studied filamentous

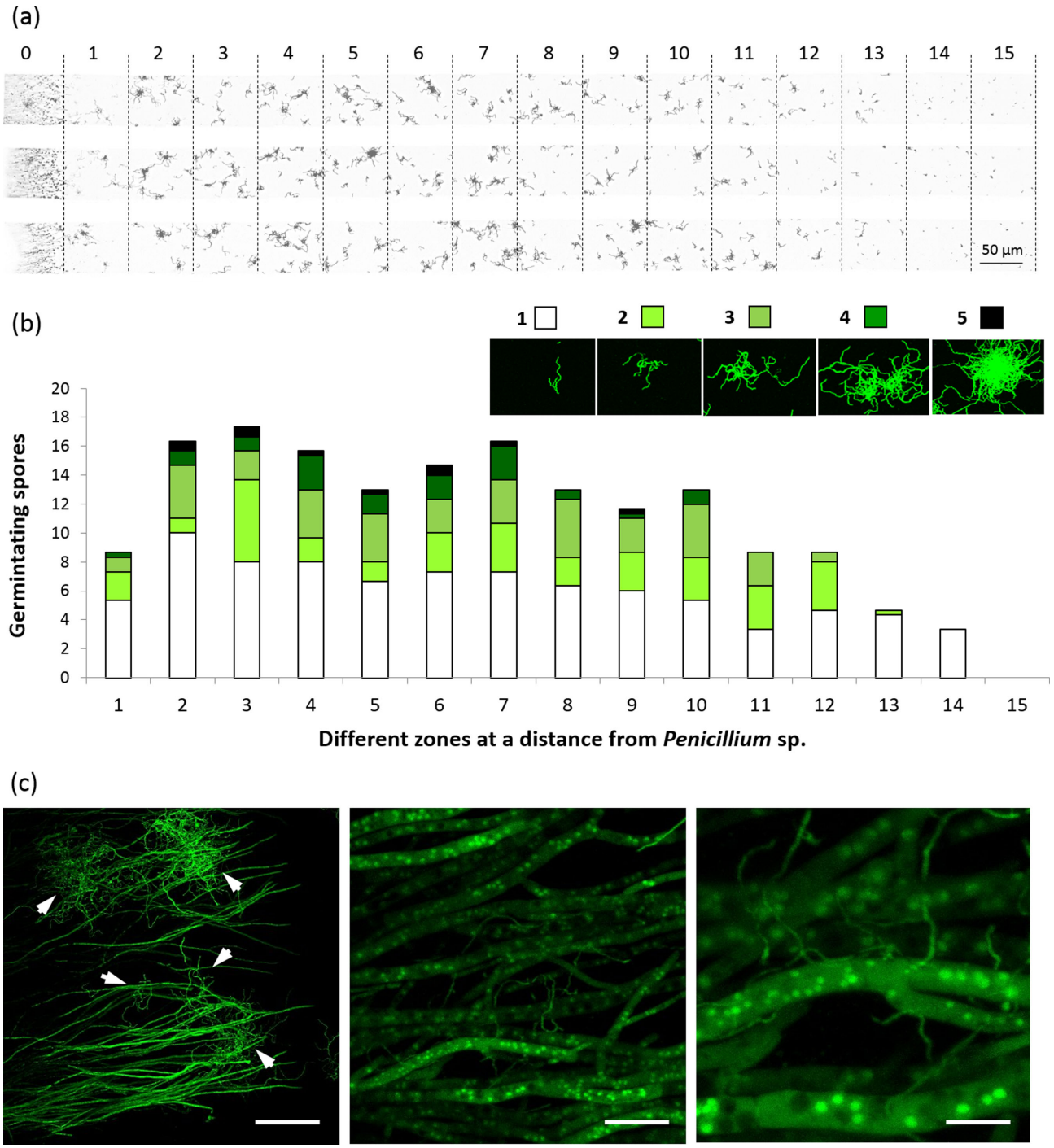

Figure 2. Microscopic visualization of the *P. chrysogenum*-dependent induced spore germination and growth of the *S. coelicolor* LM1 mutant. Micrographs were taken 24 h after *P. chrysogenum* inoculation and prior to *Streptomyces* LM1 growth visualization. (**a**) Visualization and (**b**) quantification of the *Penicillium*-dependent germination of spores of the *S. coelicolor* LM1 mutant. Values of the stacked histograms are the averages of counts of the different germination stages displayed in the three panels in (a). The border of *Penicillium* culture is shown in zone 0. (**c**) Evidence of physical contact between the *Penicillium* species and *S. coelicolor* LM1. *Streptomyces* and *Penicillium* SYTO9-stained filaments are around 1 and 5 μm in width, respectively, allowing for the differentiation between the species. Bars are equal to 80 μM (left image), 20 μM (centre) and 8 μM (right). White arrows in (c) point to LM1 germinating spores centres within the *P. chrysogenum* filamentous network. Quantification of germinating spores at a distance from the fungus was performed using the 3D object counter tool of ImageJ-Fiji software.

fungi with endogenous siderophores identified and described (Charlang *et al.* 1981; Hordt, Romheld and Winkelmann 2000).

In order to identify the metabolites associated with the restored growth of the DFO-non-producing mutants of *S. coelicolor*, a MilliQ water extraction from the spent agar of a 3-day-old solid R2YE culture of our *Penicillium* isolate was subjected to HPLC to separate the different metabolites of the complex mixture. Fractions were collected and assessed for their ability to restore growth in the *S. coelicolor* LM1 mutant (Fig. 3). The results displayed in Fig. 3 reveal that fractions #9, #13, #19–#23,

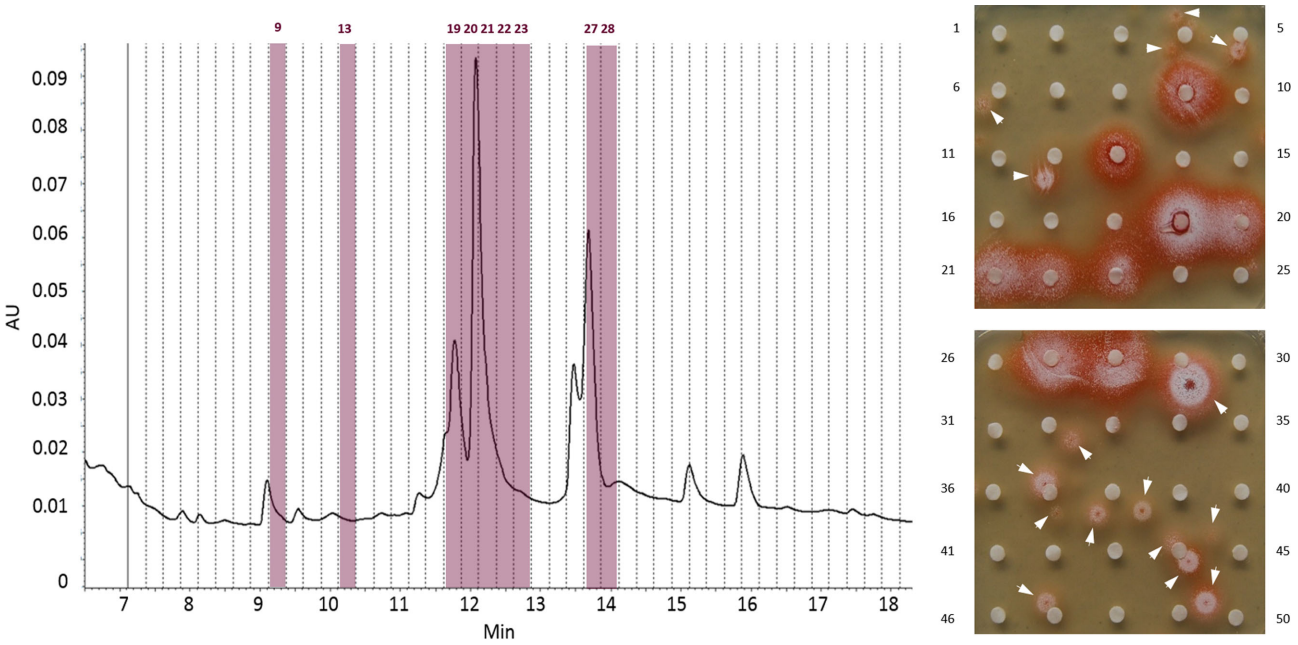

Figure 3. Identification of HPLC fractions from the *P. chrysogenum* supernatant that restore growth of the *S. coelicolor* LM1 mutant. HPLC chromatogram (Abs 435 nm) of extracellular compounds secreted by *P. chrysogenum* (left) and the effect of HPLC collected fractions on the growth of the *S. coelicolor* LM1 mutant (right panel). Fractions in violet in the HPLC chromatogram contain extracellular compounds of *P. chrysogenum* that restore growth of the LM1 mutant. Arrows point to the spontaneous revertants of LM1 (see Fig. S2, Supporting Information, for details).

#27 and #28 were able to promote growth in the *S. coelicolor* LM1 mutant. These HPLC fractions were subjected to UPLC-ESI–MS analyses to identify the mass of the major constituents which revealed that they all contained iron-chelating molecules previously identified in *P. chrysogenum* (Fig. 4). More precisely, Fe-dimerum acid was detected as major compound with a molecular ion species $[M\text{-}2H+Fe]^+$ at m/z 538 in fractions #9 and #13. Ferrichrome (expected $m/z = 741$) was detected in fractions #19 and #20. Fusarinine C ($m/z = 780$) was detected in fractions #20 (together with ferrichrome), #21, #22 and #23. Finally, coprogen ($m/z = 822$) was detected in fractions #27 and #28.

Although siderophores were by far the most abundant molecules in the 'resuscitating' HPLC fractions, we cannot exclude at this stage that other metabolites secreted by our fungus isolate could also contribute to the restored growth of the *S. coelicolor* LM1 mutant. To guarantee that the growth-restoring molecules are indeed iron-chelating molecules and that acquisition of iron by *S. coelicolor* DFO-null mutants is mediated by xenosiderophore transport, we used strain *S. coelicolor* W13 (Barona-Gomez *et al.* 2006) which is a CCLN- and DFO-null mutant which is also devoid of the *desE* gene. DesE is an ATP-binding cassette (ABC) transporter-like iron-siderophore binding lipoprotein adjacent to the DFO biosynthetic cluster. DesE is thought to be the major xenosiderophore uptake system in the *Streptomyces* species (Bunet *et al.* 2006; Patel, Song and Challis 2010). The *S. coelicolor* W13 mutant was not able to recover growth on the R2YE medium around paper discs impregnated with HPLC fractions containing ferrichrome, fusarinine C and coprogen (Fig. 5). In contrast, the *S. coelicolor* W23 mutant (*cch* cluster null mutant and Δ*desD*) that has an intact copy of *desE* was able to recover growth around paper discs with HPLC fractions containing ferrichrome, fusarinine C and coprogen. Results with Fe-dimerum acid were less distinct as partial grow of the *S. coelicolor* W13 mutant was observed (Fig. 5) suggesting that uptake of this iron-chelating molecule is not fully DesE dependent and could possibly involve genes of the tri-cistronic operon *cdtCBA* encoding a putative ABC transporter for ferric siderophore in *Streptomyces* species (Bunet *et al.* 2006; Patel, Song and Challis 2010). That growth of the DFO-null mutant is dependent on the ABC transporter iron-siderophore-binding lipoprotein DesE definitely supports the importance of iron acquisition via xenosiderophores of our *Penicillium* isolate as causal factor for the restored growth.

DISCUSSION

Our work provides evidence for the ability of *S. coelicolor* to utilize the siderophores produced by a neighbouring fungus. We came to this conclusion through the observation of fungal contaminations which enabled growth of siderophore-negative mutants of *S. coelicolor* via the secretion of iron-chelating metabolites in an iron-limited agar medium. Combined HPLC fractionation and UPLC-ESI-MS analyses revealed that the 'resuscitation' factors were all siderophores of our *Penicillium* isolate (Charlang *et al.* 1981; Hordt, Romheld and Winkelmann 2000), demonstrating that *S. coelicolor* can utilize fungal siderophores in cocultures. Previous *in vitro* studies have shown the ability of *S. coelicolor* and *S. pilosus* to take up pure hydroxamate-containing fungal siderophores (Müller, Matzanke and Raymond 1984; Barona-Gomez *et al.* 2006). DesE, the iron-siderophore binding lipoprotein adjacent to the DFO biosynthetic cluster, and to a lesser extent genes of the tri-cistronic operon *cdtCBA* encoding a putative ABC transporter for ferric siderophores, have been indicated as xenosiderophore uptake systems in the *Streptomyces* species (Bunet *et al.* 2006; Patel, Song and Challis 2010). Our investigation confirms that DesE is indeed essential for fungal siderophore utilization by streptomycetes as the *S. coelicolor* W13 mutant that lacks the complete DFO cluster (Barona-Gomez *et al.* 2006), including *desE*, was not able to recover growth on the R2YE medium around

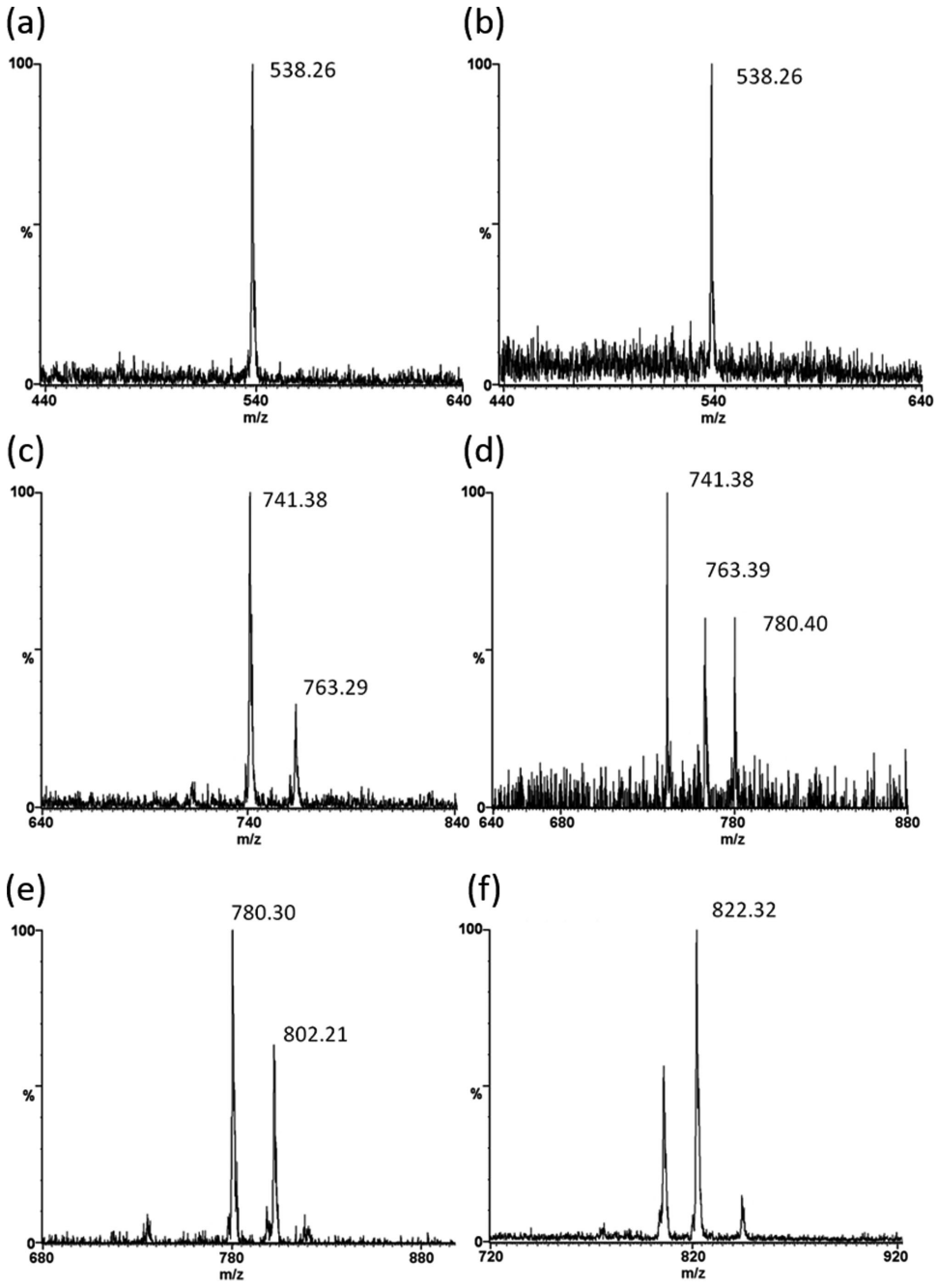

Figure 4. Electrospray ionisation (ESI) positive mass spectrum of HPLC fractions that restore growth of LM1 mutant. Fe-dimerum acid ($m/z = 538$) was identified in HPLC fractions #9 and #13 (**a** and **b**). Ferrichrome ($m/z = 741$) was detected in fractions #19 and #20 (**c** and **d**). Fusarinine C ($m/z = 780$) was detected in fractions #20 (d), #21, #22 and #23 (**e**). Coprogen ($m/z = 822$) was detected in fractions #27 and #28 (**f**).

paper discs impregnated with HPLC fractions containing ferrichrome, fusarinine C and coprogen (Fig. 5). ^{55}Fe-siderophore uptake assays should definitively demonstrate that transport of fungal siderophore by streptomycetes is directly mediated via a DesE-dependent ABC-transporter system.

The growth dependence of *S. coelicolor* DFO-non-producing mutants on 'stealing' the siderophores produced by the fungal contaminations could be analogous to a 'cheating' behaviour. Siderophore piracy between bacteria has been extensively documented (Challis and Hopwood 2003; Traxler *et al.* 2012; Cornelis and Dingemans 2013). Metabolite stealing implies that the interaction is only beneficial to one of the two interacting microbes, and indeed, we observed that the growth of the *Penicillium* species as monoculture is much more abundant than when the fungus is inoculated with the *S. coelicolor* LM1 and Δ*desA* siderophore non-producing strains (Fig. S5, Supporting Information). The assumption of antagonistic interactions between filamentous fungi and filamentous bacteria is supported by the fact that many streptomycetes produce compounds with fungicidal activities (Challis and Hopwood 2003; Hopwood 2007; Seipke *et al.* 2011) which make them good biological control agents of plant diseases (Macagnan *et al.* 2008; Palaniyandi *et al.* 2013). A battle for iron between actinomycetes and fungi would also provide a rationale for the observed negative control of

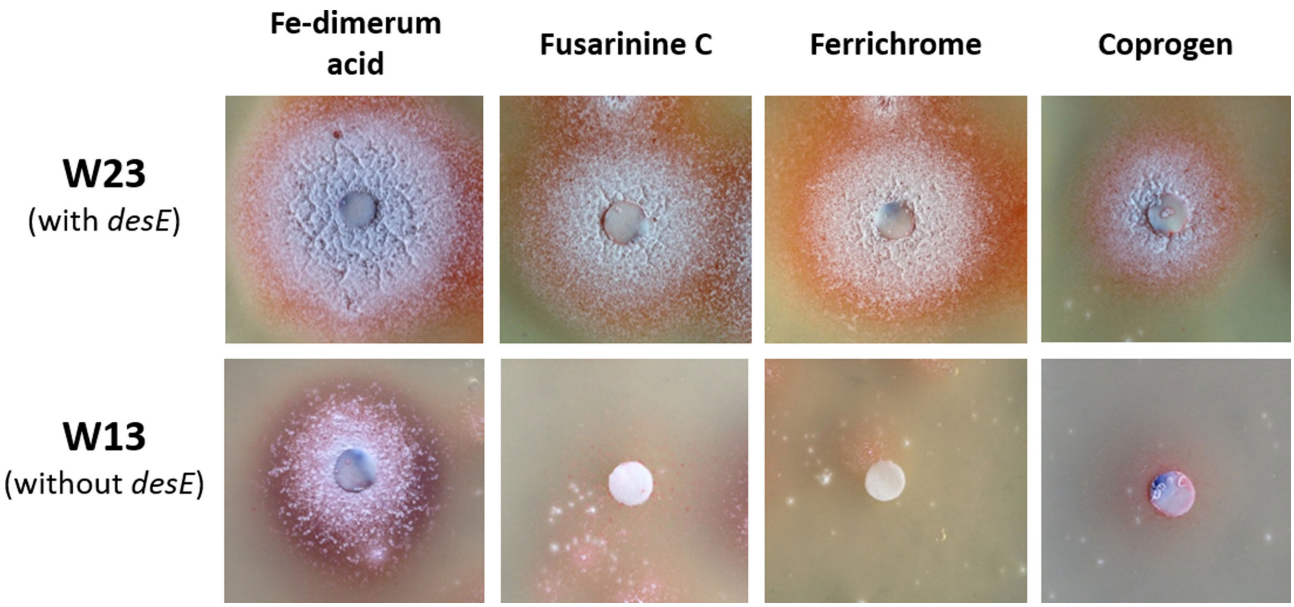

Figure 5. The restored growth of *S. coelicolor* DFO-non-producers requires DesE-dependent siderophore uptake. Effect of siderophore-containing HPLC fractions on the growth of the *S. coelicolor* W13 and W23 mutants. Note that fractions with fusarinine C, coprogen and ferrichrome are not able to restore growth of the *S. coelicolor* DFO-non-producer W13 mutant which lacks the *desE* gene.

siderophore production in streptomycetes by the cell wall component N-acetylglucosamine (GlcNAc) (Craig *et al.* 2012). The transport of GlcNAc by streptomycetes would involve lysis of the chitin-containing cell wall of fungal competitors and would result in repression of endogenous siderophore biosynthesis in order to force the utilization of siderophores produced by the antagonistic microorganism. This strategy would spare streptomycetes the energy required to produce their own siderophores and would lower the amount of siderophores available for the neighbouring fungus.

Is xenosiderophore piracy in nature just a kind of cheating behaviour only beneficial to one of the two interacting partners? Remarkably, our initial observation itself runs contrary to Fleming's well-known observation of an accidental antagonistic fungus–bacterium interaction which led to the discovery of penicillin (Fleming 2001; Houbraken, Frisvad and Samson 2011). Rather, we observed a sort of stimulatory interaction as the survival of the bacterium is fungus dependent. Overall, our work confirms that xenosiderophore utilization plays crucial roles in both commensal and antagonistic interactions between taxonomically unrelated microorganisms (D'Onofrio *et al.* 2010).

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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