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Attachment of *Streptomyces coelicolor* is mediated by amyloidal fimbriae that are anchored to the cell surface via cellulose

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Summary

The chaplin proteins ChpA-H enable the filamentous bacterium *Streptomyces coelicolor* to form reproductive aerial structures by assembling into surface-active amyloid-like fibrils. We here demonstrate that chaplins also mediate attachment of *S. coelicolor* to surfaces. Attachment coincides with the formation of fimbriae, which are connected to the cell surface via spike-shaped protrusions. Mass spectrometry, electron microscopy and Congo red treatment showed that these fimbriae are composed of bundled amyloid fibrils of chaplins. Attachment and fimbriae formation were abolished in a strain in which the chaplin genes *chpA–H* were inactivated. Instead, very thin fibrils emerged from the spike-shaped protrusions in this mutant. These fibrils were susceptible to cellulase treatment. This enzymatic treatment also released wild-type fimbriae from the cell surface, thereby abolishing attachment. The reduced attachment of a strain in which the gene of a predicted cellulose synthase was inactivated also indicates a role of cellulose in surface attachment. We propose that the mechanism of attachment via cellulose-anchored amyloidal fimbriae is widespread in bacteria and may function in initiation of infection and in formation of biofilms.

Introduction

Within the bacterial domain streptomycetes are well known for their complex developmental programme.

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Streptomycetes grow by means of hyphae that extend at their apices. This feature makes these soil bacteria particularly successful in colonizing and degrading organic material. After a feeding substrate mycelium has been formed, hyphae grow out of the substrate into the air to form chains of hydrophobic spores. These spores are dispersed by wind or insects enabling this organism to colonize a substrate elsewhere.

Formation of the aerial reproductive structures in *Streptomyces coelicolor* has been studied for several decades (Kelemen and Buttner, 1998; Chater and Horinouchi, 2003; Claessen *et al.*, 2006; Flärdh and Buttner, 2009; de Jong *et al.*, 2009). This has resulted in the identification of a variety of so-called *bld* (bald) genes, the deletion of which leads to an arrest in morphogenesis. Most of the *bld* genes encode proteins with an apparent regulatory role, implying that this developmental switch is subject to extensive regulation (Kelemen and Buttner, 1998; Flärdh and Buttner, 2009). For instance, *bldA* encodes a tRNA required for efficient translation of the rare leucine TTA codon (Leskiw *et al.*, 1991), whereas *bldN* encodes an extracytoplasmic function sigma factor (Bibb *et al.*, 2000).

Several secreted macromolecules play a pivotal role in development of *S. coelicolor.* The *cslA* gene, encoding a cellulose synthase-like protein, was shown to be essential for aerial growth (Xu *et al.*, 2008). $CslA_{SC}$ interacts with the DivIVA protein at hyphal tips, where it synthesizes a β-(1-4) glucan. This secreted polysaccharide is thought to maintain the integrity of the hyphal tip that is subject to constant remodelling due to ongoing cell wall synthesis orchestrated by DivIVA (Flärdh, 2003; Xu *et al.*, 2008). SapB is another macromolecule that is secreted during development (Willey *et al.*, 1991). This lantibiotic-like peptide (Kodani *et al.*, 2004) lowers the surface tension of the aqueous environment to enable hyphae to grow into the air (Tillotson *et al.*, 1998).

Like SapB, chaplins are secreted surface-active molecules. This class of proteins comprises eight members (Claessen *et al.*, 2003; Elliot *et al.*, 2003). The chaplins ChpD–H are about 55 amino acids in length, whereas ChpA–C are approximately fourfold larger. ChpA–C consist of two domains similar to the mature forms of ChpD–H followed by a C-terminal sorting signal that

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anchors them to the peptidoglycan in the cell wall (Elliot *et al*., 2003; Marraffini *et al*., 2006). Two of the small chaplins, ChpE and ChpH, are produced by submerged hyphae and have a function similar to SapB. They enable hyphae to grow into the air by lowering the surface tension of the medium (Claessen *et al*., 2003). These chaplins have another role as well. Together with the other six chaplins they are secreted in the cell walls of aerial hyphae where they provide rigidity and surface hydrophobicity (Claessen *et al*., 2003; 2004). Chaplins carry out these functions by assembling into amyloid fibrils, which, at least on the surface of aerial hyphae, are organized into a pattern of pair-wise aligned rodlets (Claessen *et al*., 2003; 2004). This process seems to be co-ordinated by the rodlins RdlA and RdlB, which are produced and secreted by growing aerial hyphae (Claessen *et al*., 2002). Aerial growth is severely impaired in the absence of chaplins, stressing the important biological function of this class of amyloid proteins during development (Claessen *et al*., 2004; Capstick *et al*., 2007; Di Berardo *et al*., 2008).

Fungi also use amyloid-forming proteins that reduce the surface tension of the aqueous substrate and provide aerial structures with a hydrophobic coating. In many fungi, hydrophobins form these fibrils (Wösten and de Vocht, 2000; Gebbink *et al*., 2005). However in the maize pathogen, *Ustilago maydis*, these proteins have been functionally replaced by repellents (Wösten *et al*., 1996; Teertstra *et al*., 2006; 2009). Repellents and hydrophobins have also been shown to attach hyphae to a hydrophobic solid (Wösten *et al*., 1994; Teertstra *et al*., 2006), which is essential for pathogenicity (Talbot *et al*., 1996) and possibly also for the degradation of organic substrates (Wösten, 2001).

Here, we show that amyloid fibrils of the chaplins are involved in surface attachment of *S. coelicolor.* In contrast to the fungal proteins, the amyloid fibrils of the chaplins are organized into attachment structures known as fimbriae. These fimbriae are anchored to the cell wall via cellulose, which provides an important new insight in the role of this polysaccharide in the bacterial domain.

Results

Chaplins are required for attachment and the formation of Streptomyces *fimbriae*

Previously, we have shown that hyphae of liquid static cultures of streptomycetes attach to polystyrene when grown in gNMMP or mNMMP (Claessen *et al*., 2002; van Keulen *et al*., 2003). Attachment is much stronger in mNMMP compared with gNMMP (D. Claessen and G. van Keulen, unpubl. data). Negative staining showed that *S. coelicolor* formed an extracellular matrix when grown in mNMMP (Fig. 1A), which was absent in gNMMP

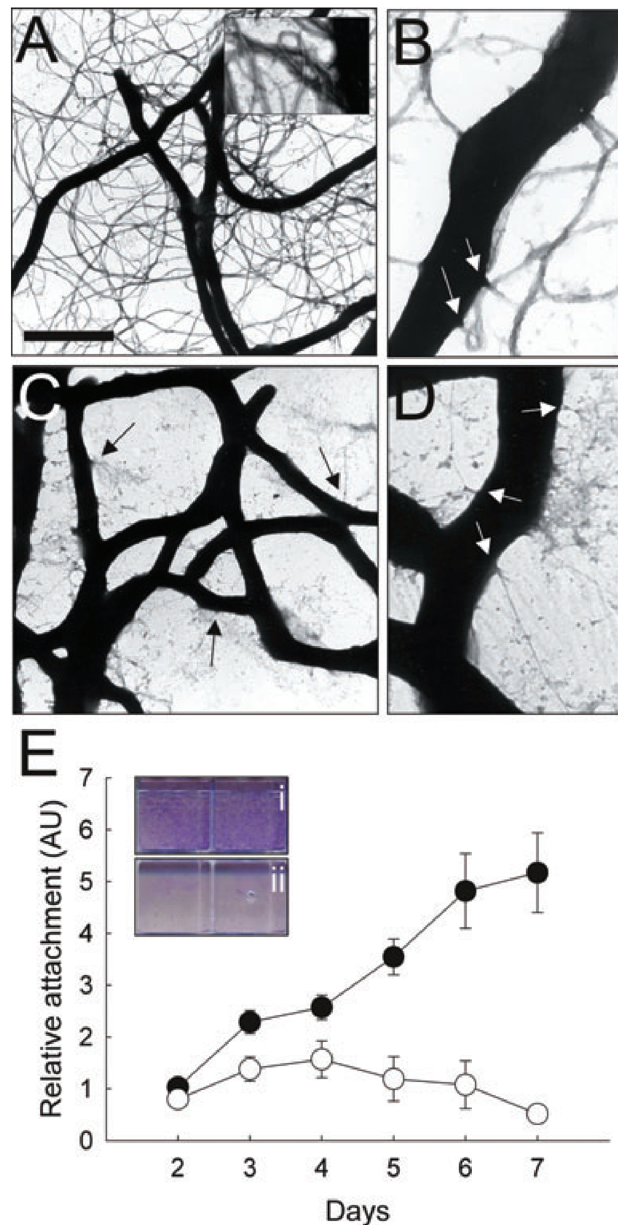

Fig. 1. Formation of fimbriae depends on chaplins.
A. Attachment of *S. coelicolor* in mNMMP medium coincides with the formation of fimbriae in between the adhering hyphae, which are composed of bundled filaments (inlay).
B. Fimbriae emerge from the cell wall of the wild-type strain via spike-shaped protrusions (arrows). In contrast, the Δ*chpABCDEFGH* strain forms thin fibrils (indicated by arrows in C) that protrude from these structures (see arrows in D).
E. Quantification of biomass of the wild-type (filled circles) and the Δ*chpABCDEFGH* mutant (open circles) that has attached to the well. The biomass was stained with crystal violet and related to staining of a fixed amount of mycelium from a liquid-shaken culture. The inlay shows crystal violet stained mycelium of a 7-day-old culture of the wild-type strain (i) and the Δ*chpABCDEFGH* mutant (ii) that resisted washing with water. The scale bar represents 5 μm (A, C), 1.25 μm (B, D) and 100 nm (inlay).

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Table 1. Percentage of the mycelium of *Streptomyces* strains that is attached to the well of the culture plate.

Strain	Attachment (%)
M145	89.3 ± 3.9
ΔchpABCDH	67.8 ± 1.2
ΔchpABCDEH	65.2 ± 3.6
ΔchpABCDEFGH	30.7 ± 13.2
bldN	1.5 ± 0.9
cslA (Tn5062)	56.0 ± 16.4

Cultures were grown for 12 days in liquid static cultures.

(Fig. S1). The matrix consisted of 9- to 100-nm-wide fimbriae, which were present throughout culturing (up to at least 15 days). They were associated with the adhering hyphae via spike-shaped protrusions (see arrows in Fig. 1B). Formation of fimbriae did not require the activity of the rodlin proteins, as the Δ*rdlAB* mutant was shown to make an extracellular matrix indistinguishable from that in the wild-type strain (data not shown). Moreover, attachment of the Δ*rdlAB* mutant was not affected in mNMMP (data not shown). Formation of fimbriae did depend on the chaplins as concluded from the absence of the typical 9- to 100-nm-wide fibres in the Δ*chpABCDEFGH* strain (Fig. 1C). Instead, fibrils with a diameter of 9 ± 2 nm emerged from the spike-shaped protrusions on the cell surface (see arrows in Fig. 1D). These thin fibrils resembled those detected on the cell surface when the wild-type strain was grown in gNMMP liquid standing cultures (Fig. S1). Importantly, attachment was greatly reduced in the *chp*-less mutant (Fig. 1E, Table 1). Compared with the Δ*chpABCDEFGH* strain, attachment was only partially decreased in the Δ*chpABCDH* and Δ*chpABCDEH* strains (Table 1). Both mutants produced fimbriae that were indistinguishable from those produced by the wild-type strain (not shown), although reduced in number (Fig. S2).

Expression of the chaplin genes requires the extracytoplasmic function sigma factor BldN (Elliot *et al*., 2003). In support of a role for chaplins in attachment, we observed that a *bldN* mutant was severely affected in adhesion (Table 1). Moreover, the extracellular matrix produced by the *bldN* mutant resembled that of the chaplin mutant strain (data not shown). Taken together, these results show that formation of the extracellular matrix depends on chaplins and correlates with the capacity of hyphae to attach firmly to the hydrophobic substratum.

Assembly of chaplins into amyloid fibrils on hydrophobic surfaces

The absence of fimbriae in the Δ*chpABCDEFGH* mutant strain prompted us to investigate whether chaplins are part of the extracellular matrix. MALDI-TOF mass spectrometry on intact fimbriae (see *Experimental procedures*) revealed masses corresponding to the mature forms of ChpD, ChpE, ChpF and ChpH (Fig. 2). This shows that fimbriae are, at least in part, composed of chaplins.

Circular dichroism (CD) was used to study structural changes of chaplins in contact with a hydrophobic surface. Previously, it was shown that purified chaplins (ChpD–H) are unstructured when dissolved in water; however, at a water–air interface, these proteins self-assemble into amyloid fibrils, which is accompanied by the formation of β-sheet structure (Claessen *et al*., 2003). The structure of water-soluble chaplins (Fig. 3A, dashed line) also changed rapidly upon adding an excess of colloidal Teflon (Fig. 3A, thin solid line). The CD spectrum indicated formation of α-helix (Chang *et al*., 1978). The conversion towards the α-helical state did not increase

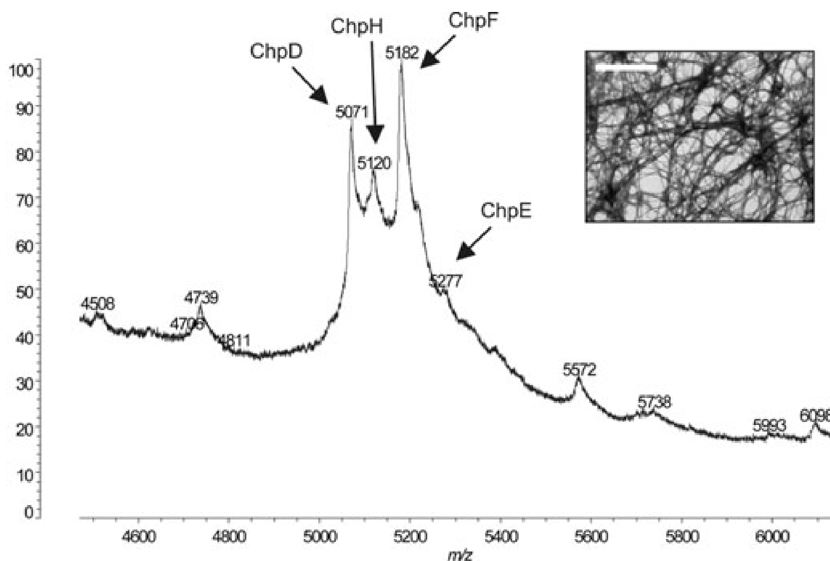

Fig. 2. Identification of chaplins in fimbriae by MALDI-TOF mass spectrometry. Peaks corresponding to the masses of ChpD, ChpE, ChpF and ChpH (Claessen *et al*., 2003) are detected in the fimbrial network (see inlay). The scale bar represents 1 μm.

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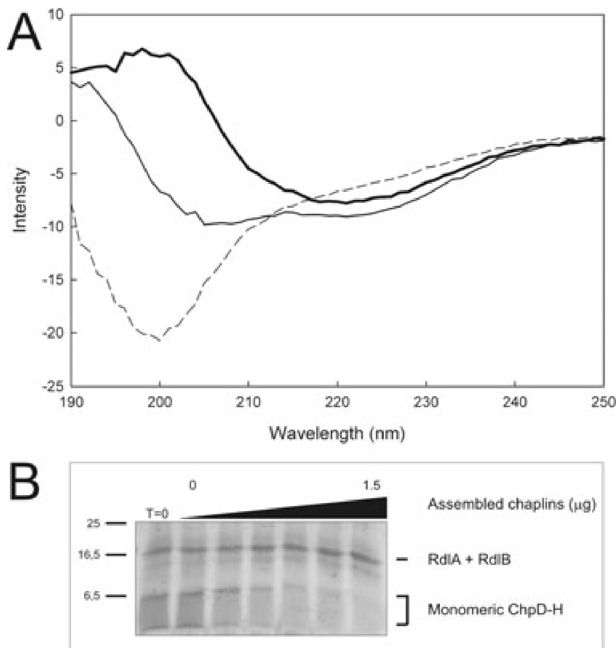

Fig. 3. A. Assembly of chaplins on a hydrophobic surface as determined by CD spectroscopy. Spectrum of ChpD–H before (dashed line) and after (solid line) addition of colloidal Teflon and subsequent treatment with 0.1% Tween-20 at 85°C (thick solid line).
B. Assembly of monomeric chaplins in the presence of the assembled form. Mixtures of chaplins (ChpD–H) and rodlins (RdlA + RdlB) were incubated for 60 min with increasing amounts of seeding chaplin fibrils. Note that the rodlins, serving as a loading control, remain soluble.

the fluorescence of the amyloid specific dye Thioflavin T (ThT; Table 2). The chaplins pelleted together with the Teflon spheres upon centrifugation, indicating that the chaplins were bound to the Teflon (not shown). When the mixture of chaplins associated with the Teflon spheres was heated in the presence of 0.1% Tween-20, thereby promoting lateral interactions between the bound chaplins, the spectrum became indicative for β-sheet structure (Fig. 3A, thick solid line; Sreerama *et al.*, 1999). This treatment increased ThT fluorescence 36-fold (Table 2). This increase was similar to that obtained after vortexing a chaplin solution and showed that all monomers had assembled into amyloid fibrils (Table 2). In the absence of Teflon spheres, a heated solution of chaplin monomers with 0.1% Tween-20 did not increase ThT fluorescence (Table 2). These data show that chaplins can assemble into amyloids on a hydrophobic surface.

Nucleation-driven assembly of chaplins

Amyloid fibrils of ChpD–H (up to 1.5 µg in 25 µl water) were added to 50 µl of a still aqueous solution of their monomers (50 µg ml^{-1}). SDS-PAGE showed that the amount of monomeric chaplins hardly decreased during 1 h incubation when no amyloid fibrils were added (Fig. 3B). In contrast, more and more monomeric chaplin disappeared from solution upon addition of increasing amounts of the assembled form. This coincided with increase in ThT fluorescence showing that the soluble chaplins had assembled into amyloid fibrils (data not shown). Thus, assembly of chaplins becomes independent from a hydrophilic–hydrophobic interface once a nucleus of assembled chaplins is present.

Inhibition of attachment, fimbriae formation and chaplin assembly by Congo red

The capacity of chaplins to assemble into amyloid-like fibrils as well as their involvement in the formation of fimbriae were reason to study the effects of the amyloid inhibitor Congo red (CR) (Findeis, 2000; Kuner *et al.*, 2000). *S. coelicolor* was grown on agar plates in the presence of increasing amounts of CR to assess whether this compound is toxic at high concentrations, as was shown in *Acetobacter xylinum* (Colvin and Witter, 1983). Neither growth nor differentiation was significantly affected on solid MS agar in the presence of up to 200 µg ml^{-1} CR (data not shown). Similarly, no effects were observed in liquid shaken cultures at these concentrations (data not shown). However, CR did affect attachment in liquid static cultures. Attachment was already largely abolished at 5 µg ml^{-1} CR (Fig. 4A and B), which correlated with the absence of fimbriae (Fig. 4C). Instead, thin fibrils with a diameter of about 9 nm were extruded from the spike-shaped protrusions. These fibrils were very similar to those formed in the Δ*chpABCDEFGH* strain (Fig. 1C) and the *bldN* mutant (data not shown). The addition of

Table 2. ThT (3 µM) fluorescence upon interaction with chaplins (14 µg ml^{-1}) in different conformations.

Conformation of chaplin	Relative ThT fluorescence
Water soluble	4.6 (± 0.47)
Water soluble (5 min after addition of 0.1% Tween-20 at 85°C)	5.2 (± 0.58)
α-Helical conformation on Teflon	0.59 (± 0.069)
β-Sheet conformation on Teflon (5 min after addition of 0.1% Tween-20 at 85°C)	166 (± 2.1)
β-Sheet conformation induced by vortexing	159 (± 5.7)

Fluorescence of ThT in the absence of protein was set at 1 and data were corrected for autofluoresence of the Teflon spheres.

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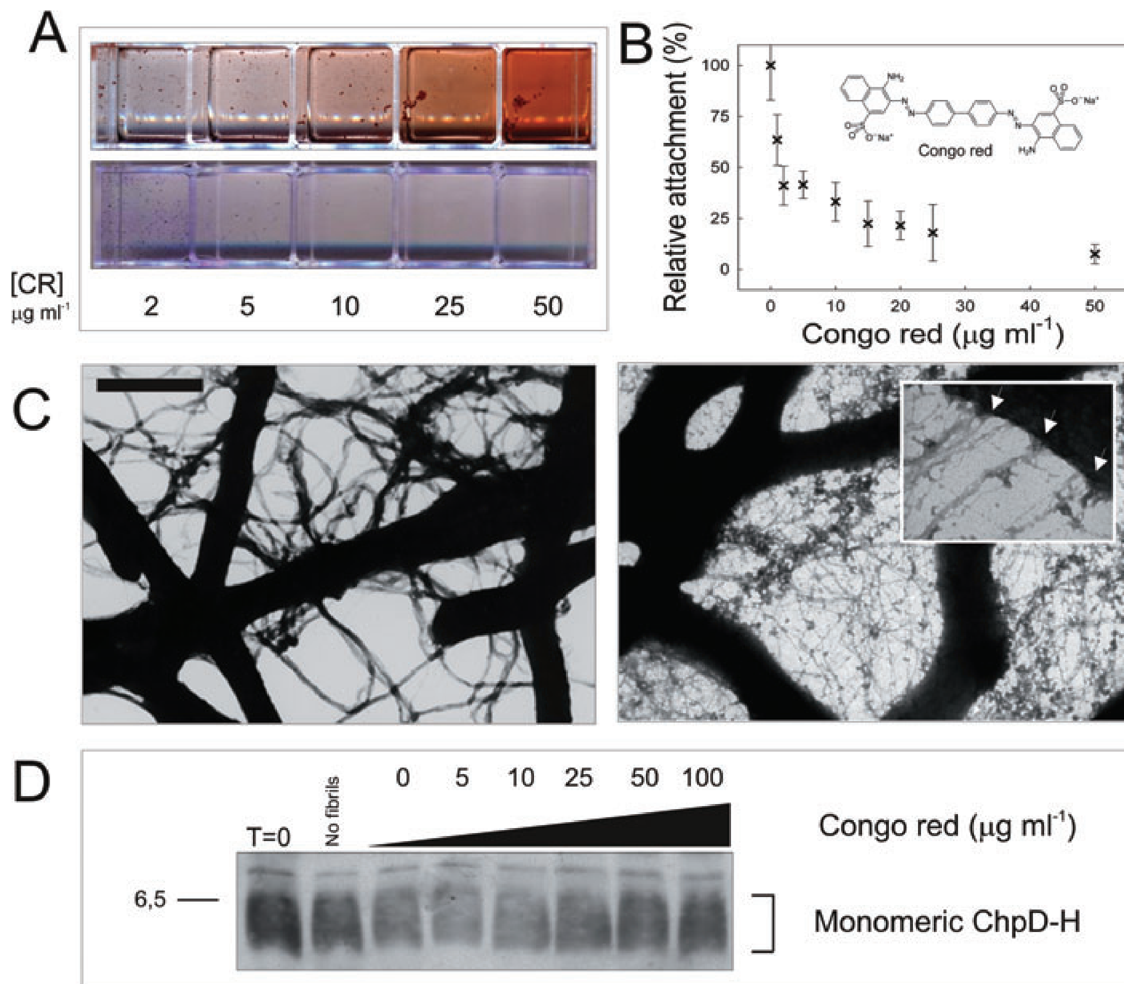

Fig. 4. The effect of Congo red on attachment, fimbriae formation and chaplin assembly.
A. Addition of increasing amounts of Congo red to static liquid cultures decreases attachment (bottom panel) without affecting growth (top panel).
B. Quantitative assessment of the effect of Congo red (inlay) on attachment.
C. Formation of fimbriae (left) is abolished by the addition of 5 μg ml^{-1} Congo red (right panel). Instead, thin fibrils emerge from the spike-shaped protrusions (see arrows in inlay, right panel).
D. The assembly of monomeric chaplin induced by seeding with 1 μg of assembled chaplin could be prevented by the addition of increasing amounts of Congo red. Bar represents 2.5 μm or 125 nm (inlay). Error bars represent the standard error of a biological triplicate.

5 μg ml^{-1} CR had no effect on the formation of the thin fibrils in the *bldN* and *chpABCDEFGH* mutant (data not shown). Notably, the structure of neither the fimbriae nor the thin fibrils was affected when CR was added after their formation (i.e. after 5 days of growth). This indicates that CR does not cause their depolymerization. Accordingly, no decrease in attachment was observed when 5 μg ml^{-1} CR was added to the medium after the formation of fimbriae (Fig. S3). Taken together, these results demonstrate that CR specifically interferes with formation of fimbriae, thereby affecting attachment.

Because chaplins are able to assemble into amyloids when contacting a hydrophobic surface and are part of the fimbriae, we studied the effect of CR on the assembly of chaplins *in vitro*. Importantly, the assembly process was prevented by the addition of increasing amounts of CR to the solution (Fig. 4D). This shows that this compound can directly interfere with chaplin amyloid formation.

The putative cellulose synthase CslA$_{SC}$ is involved in attachment

Previously it was shown that the extracellular matrix produced by several enteric bacteria comprise cellulose, which functions in attachment and biofilm formation (Zogaj *et al.*, 2001; 2003). The operons known to be involved in cellulose biosynthesis in other bacteria are organized in a similar way to a *S. coelicolor* gene cluster

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harbouring a gene encoding a cellulose synthase-like protein, called $CslA_{SC}$ (Zogaj *et al*., 2001; Xu *et al*., 2008). The *cslA* gene was inactivated by a transposon (see *Experimental procedures*) that includes a promoter-less *eGFP* gene. This enables expression analysis of the disrupted gene. Strong expression of *eGFP* was observed in mycelium that had attached (Fig. 5A), while *eGFP* expression was weak in mycelium of a shaken culture (data not shown). These data suggest that $CslA_{SC}$ plays a role in attachment. Indeed, attachment of the transposon mutant strain was approximately 50% reduced (Fig. 5B, Table 1) compared with the wild-type strain. Similar results were obtained with the XE mutant strain (Fig. 5B and data not shown). This strain has an internal fragment of the *cslA* gene replaced with a hygromycin-resistance cassette (Xu *et al*., 2008). Notably, electron microscopy analysis showed that both *cslA* mutants formed fimbriae that were indistinguishable from and similar in number to those produced by the wild-type strain (Fig. 5C; Fig. S2). This implies that $CslA_{SC}$ is not essential for formation of fimbriae.

$CslA_{SC}$-dependent and -independent β-(1-4) glucan formation during attachment

The *S. coelicolor* cellulose synthase-like protein produces a polysaccharide at hyphal tips, which can be visualized with calcofluor white (Xu *et al*., 2008). This fluorescent dye specifically stains β-(1-4)-coupled glucans such as cellulose and chitin. Calcofluor white staining of attached *S. coelicolor* wild-type hyphae revealed the accumulation of β-(1-4) coupled glucans at hyphal tips, consistent with previous results (Xu *et al*., 2008). However, we also detected bright fluorescent spots at considerable distances from hyphal tips and emerging branches, invariably localized at the outer surface of the adhering hyphae (see arrows in Fig. 5D, top panels). These spots were $CslA_{SC}$-dependent, as they were not observed in stained mycelium of the *cslA* mutant (Fig. 5D, bottom panels). The wild-type and the *cslA* mutant strains also showed a weak but detectable staining of the fimbrial network. The stain sometimes extended from the fimbriae to the cell wall of a connecting hypha (Fig. 5D). Taken together, these results demonstrate that during attachment $CslA_{SC}$ is not only active at hyphal tips but also subapically. Here it synthesizes a β-(1-4)-coupled glucan involved in attachment. The results also infer that a related glucan that is part of the fimbriae can be synthesized in a $CslA_{SC}$-independent manner.

Cellulose-mediated anchoring of fimbriae

The homology of $CslA_{SC}$ to known cellulose synthase proteins (Xu *et al*., 2008) and the apparent $CslA_{SC}$-independent formation of a β-(1-4)-coupled glucan suggested potential redundancy in biosynthesis of cellulose-like polysaccharides in *S. coelicolor*. To circumvent redundancy, we decided to study attachment of the wild-type strain in the presence of the cellulose-degrading enzyme cellulase. Strikingly, attachment decreased with increasing amounts of cellulase, and was completely abolished when 1 U of cellulase was added to the well (Fig. 5E). Loss of attachment was also observed when the enzyme was added 9 days after the mycelium had started to attach to the surface (data not shown).

To understand the mechanism by which cellulase inhibited attachment, mycelium grown in the presence of this enzyme was analysed with TEM. Strikingly, cellulase had no discernable effect on formation of the fimbriae. However, the connection of the fimbriae to the mycelium was affected. Fimbriae were abundantly detected in the supernatant dissociated from the surrounding mycelium (Fig. 5F). Notably, hyphae grown in the presence of cellulase still formed the spike-shaped protrusions, indicating that cellulose is not required for their formation. Taken together, these results show that cellulose has a role in anchoring fimbriae to the adhering hyphae.

Extrusion of cellulose at spike-shaped protrusions

The importance of cellulose in attachment, and the identification of thin fibrils emerging from the cell wall-associated protrusions in strains that were no longer able to synthesize or assemble chaplins (see above), were reason to analyse whether these thin fibrils consist of cellulose. We therefore analysed what the effect of the addition of cellulase was on formation of these fibrils in the *bldN* mutant (Fig. 6). Strikingly, the thin fibrils were no longer detected in the presence of cellulase, whereas 5 $\mu g\ ml^{-1}$ CR had no effect. Identical results were obtained with the thin fibrils formed by the Δ*chpABCDEFGH* strain and wild-type strain in the presence of 5 $\mu g\ ml^{-1}$ CR (data not shown). These results show that the spike-shaped protrusions are sites for cellulose biosynthesis, serving as an anchoring platform for the fimbriae.

Discussion

Chaplins of the filamentous bacterium *S. coelicolor* were identified as proteins that function in the formation of spore-forming aerial hyphae (Claessen *et al*., 2003; Elliot *et al*., 2003). Chaplins self-assemble into surface-active amyloid fibrils that enable hyphae to escape the aqueous environment to grow into the air and that provide aerial hyphae and spores with a hydrophobic coating (Claessen *et al*., 2003). The amyloid fibrils of the chaplins are organized by the rodlin proteins into pair-wise aligned rodlets

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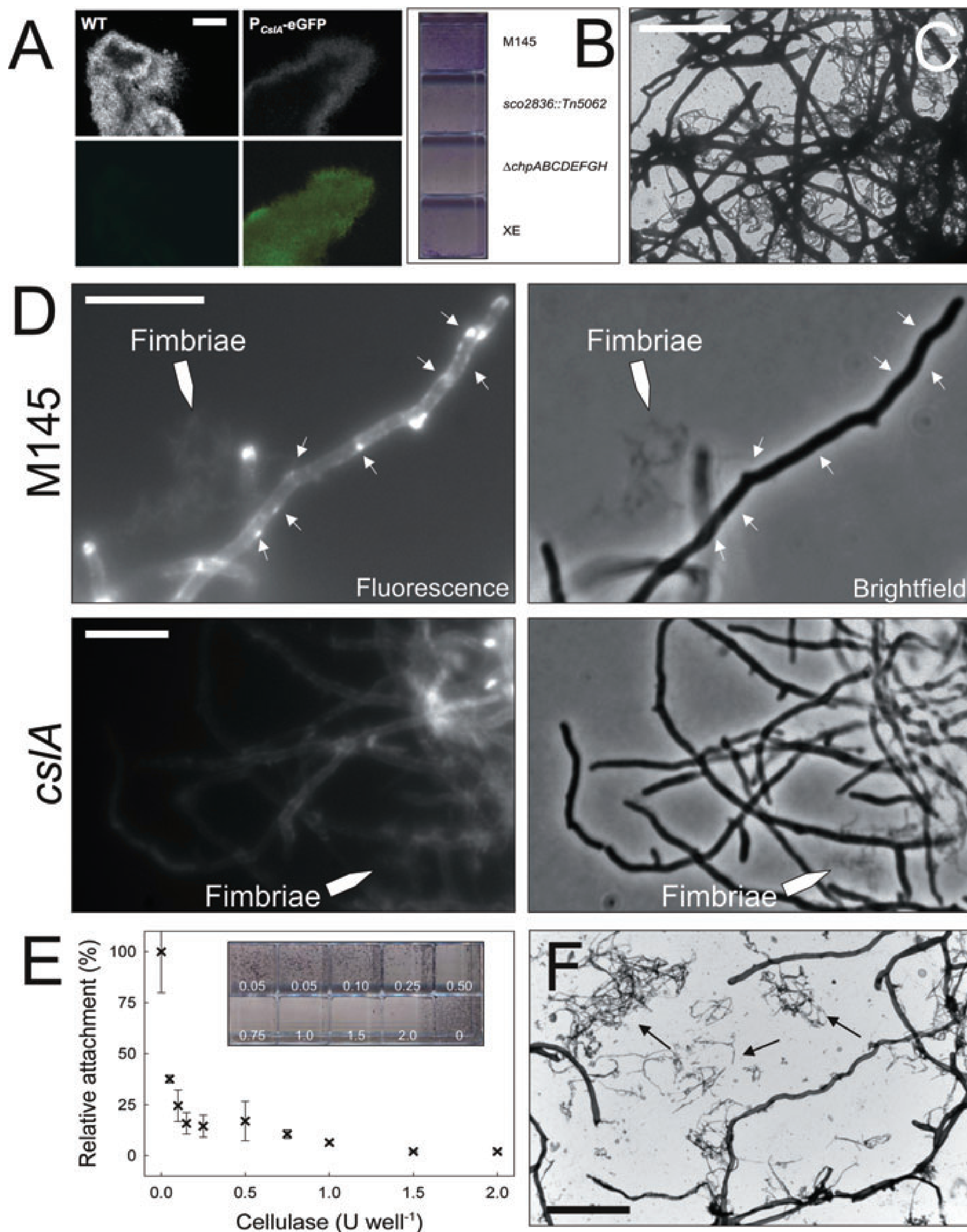

Fig. 5. The role of cellulose in attachment of *S. coelicolor*.
A. Expression of the *cslA* gene in adhering hyphae, the wild-type (left) serving as a control. Light microscopy images are shown at the top, whereas GFP fluorescence is shown at the bottom.
B. Disruption (*cslA::Tn5062*) or replacement (XE) of *cslA* reduces attachment.
C. Formation of fimbriae in the *cslA* (Tn5062) mutant.
D. Calcofluor white stained mycelium (left) of the wild-type (top) and the *cslA* (Tn5062) mutant strain (bottom). Corresponding bright-field images are shown at the right. Note the bright foci at the edge of wild-type hyphae that are absent in the *cslA* mutant (arrows).
E. Quantitative and qualitative (inlay) effect of cellulase on attachment. Increasing amounts of cellulase results in detachment.
F. Cellulase interferes with anchoring of fimbriae to the hyphae. The scale bars represent 100 μm (A), 12.5 μm (C), 5 μm (D), and 15 μm (F). Error bars represent the standard error of a biological triplicate.

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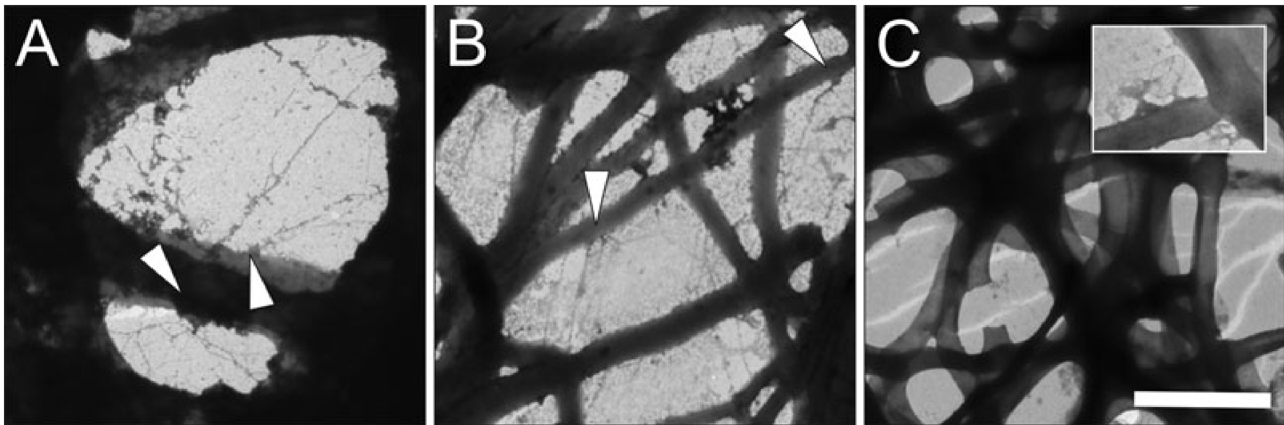

Fig. 6. The thin fibrils formed by the *bldN* mutant consist of cellulose. (A) The *bldN* mutant forms thin fibrils emerging from spike-shaped protrusions (arrow heads) whose formation is not affected by low levels of CR (B). In the presence of CR and cellulase these fibrils are no longer formed (C). Note the material that is expelled from the spike-shaped protrusions (inlay). The scale bar represents 5 μm (A–C) or 2 μm (inlay). Arrowheads point to the spike-shaped protrusions.

at the surface of the aerial structures (Claessen *et al*., 2002; 2003; 2004; Di Berardo *et al*., 2008). Here, we show that the amyloid fibrils of chaplins also function in attachment of hyphae to a hydrophobic surface. To this end, these fibrils are organized into fimbriae that are anchored to the cell wall via cellulose.

Formation of amyloidogenic pili

Several pathogenic and non-pathogenic microorganisms have been shown to produce amyloidogenic fibrils that function in adhesion to abiotic or biotic substrates, such as the fibrils of the SC3 hydrophobin of the filamentous fungus *Schizophillum commune* (Wösten *et al*., 1994), repellents of the phytopathogenic fungus *U. maydis* (Teertstra *et al*., 2006; 2009), curli and tafi of various members of the *Enterobacteriaceae* (Chapman *et al*., 2002; Zogaj *et al*., 2003) and the recently identified *Mycobacterium tuberculosis* pili (Alteri *et al*., 2007). In addition, amyloid adhesins were shown to be abundantly present in various types of natural biofilms (Larsen *et al*., 2007; 2008), indicating that they are important components of the extracellular matrix of these bacterial communities. However, almost nothing is known about the mechanism of amyloidogenic fimbriae formation in Gram-positive bacteria. In fact, details about the formation of Gram-positive pili in general have only recently started to emerge (Telford *et al*., 2006; Mandlik *et al*., 2008; Proft and Baker, 2009). Pili of Gram-negative bacteria are typically formed by non-covalent interactions between pilin subunits. In contrast, pilins of Gram-positives are covalently polymerized by the activity of sortase enzymes. Their genes are typically present near those of the pilin substrate (Mandlik *et al*., 2008). Sortases recognize pilins by their C-terminal cell wall sorting signal, mostly containing a conserved LPXTG motif followed by a hydrophobic domain and a positively charged tail (Marraffini *et al*., 2006). Strikingly, the three large chaplin variants, ChpA–C, also have a sortase recognition sequence (although with a variation of the LPXTG motif, namely LAXTG), but are not located close to any of the seven sortase homologues on the *S. coelicolor* chromosome (Pallen *et al*., 2001). Our work demonstrates that these three chaplins are not essential for formation of the fimbriae as these structures were still produced in the Δ*chpABCDH* mutant strain. This shows that the *S. coelicolor* fimbriae are atypical Gram-positive pili that resemble the *M. tuberculosis* pili, which are also polymerized in a sortase-independent manner (Alteri *et al*., 2007). However, co-assembly of the small and large chaplins and the covalent coupling of ChpA–C to the cell wall could contribute, in addition to cellulose (see below), to anchoring of the fimbriae to the cell surface (see Fig. 7).

The best-studied amyloidogenic pili are curli. *Escherichia coli* has a specific nucleation-precipitation machinery for the assembly of curli, which might help to prevent self-assembly of monomers inside the cell, and accelerate polymerization of curli on the cell surface *in vivo*. The major constituent of curli fibrils is the CsgA protein, which *in vitro* is capable of self-assembling into fibrils indistinguishable from those observed on surfaces of wild-type *E. coli* cells. However, CsgB and CsgF are involved in nucleation of CsgA *in vivo* (Hammar *et al*., 1996; Chapman *et al*., 2002; Hammer *et al*., 2007; Nenninger *et al*., 2009). How is the assembly process of chaplins initiated? Although we cannot exclude the involvement of a nucleator (Fig. 7), as observed for curli, chaplins can assemble without one. When mixtures of small chaplins were dried down on a surface, a regular pattern of fibrils was formed (Claessen *et al*., 2003). Under these condi-

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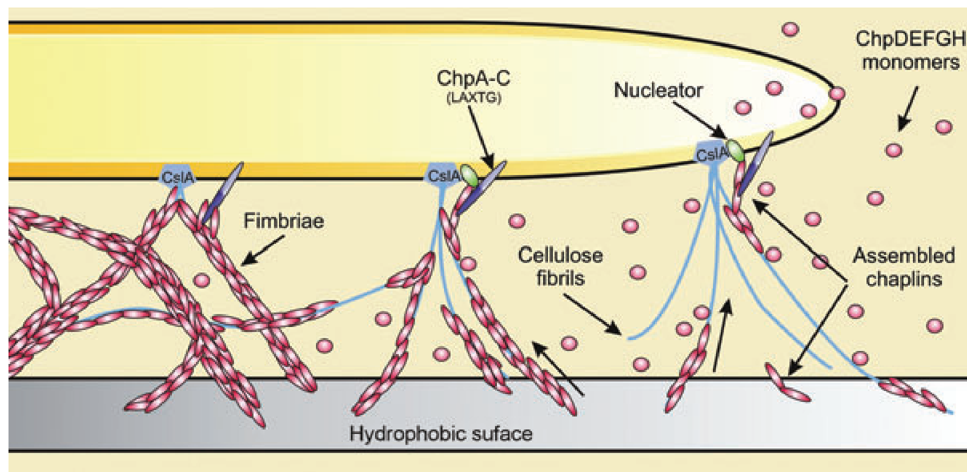

Fig. 7. The role of chaplins and cellulose in fimbriae formation in *S. coelicolor*. CslA is involved in the extrusion of cellulose fibrils from the spike-shaped protrusions along the cell wall of adhering hyphae. Chaplin monomers are secreted and assemble into amyloids when contacting either assembled chaplin fibrils or a hydrophobic surface. In addition, assembly may be triggered by the activity of a (unknown) nucleator. The assembled chaplin amyloids interact with the cellulose fibrils and each other, leading to fimbriae formation. Possibly, the large chaplins (ChpA–C) may contribute to the covalent coupling of fimbriae to the cell wall.

tions, the protein concentration will increase over time due to evaporation of the solvent, leading to self-assembly, as observed for other amyloid-forming proteins (Wösten *et al.*, 1993). A similar process can happen when chaplins bind to a hydrophobic surface. A certain amount of bound monomers could result in the formation of an amyloid nucleus, which then induces other chaplins to adopt the amyloidal end-state.

When chaplins were incubated with Teflon spheres, they initially did not form amyloid fibrils. Instead, the protein adopted a conformation rich in α-helix. This so called α-helical state probably represents an intermediate of the assembly process analogous to that observed for other amyloid-forming proteins (de Vocht *et al.*, 1998; 2002; Giacomelli and Norde, 2003). This intermediate seems to proceed to the amyloid state when the local concentration of protein is increased as was recently shown for the SC3 hydrophobin (K. Scholtmeijer and H.A.B. Wösten, unpublished). Alternatively, the conversion to the amyloid state is obtained by promoting lateral interactions between the bound protein (e.g. chaplins and hydrophobin) by treating the coated surface with diluted detergent at high temperature. It may also be that cellulose induces the conversion to the amyloid conformation (see below).

A novel role for cellulose in fimbrial anchoring and attachment

Various bacteria produce cellulose during formation of biofilms (Zogaj *et al.*, 2001; Römling, 2002), and adherence to plant tissues (Matthysse *et al.*, 1981; Matthysse and McMahan, 1998). For instance, the extracellular matrix produced by *Salmonella enterica* comprises, in addition to curli fimbriae, cellulose and one or more other polysaccharides (White *et al.*, 2003). Cellulose was shown to be tightly associated with the curliated structures on the cell surface, but was not required for their formation. These data are consistent with our results in *S. coelicolor*. In addition, we observed detachment of the fimbriae from the cell surface by enzymatic treatment with cellulase, revealing an important novel role for cellulose in fimbrial anchoring (Fig. 7). Cellulose fibrils were shown to emerge from spike-shaped protrusions along the hyphal cell wall in the absence of chaplins, indicating that their formation and anchoring occur at these sites. A role for $CslA_{SC}$ in this process is envisaged as this protein was shown to polymerise a β-(1-4) glucan along the periphery of adhering hyphae. However, other, yet unknown proteins also contribute to this process as fimbriae of the *cslA* mutant still stained with calcofluor white.

The observed spike-shaped protrusions are morphologically reminiscent of cellulosomes that are present on the cell surface of anaerobic cellulolytic bacteria such as *Clostridium thermocellum* and *Ruminococcus albus* (Felix and Ljungdahl, 1993). Cellulosomes are large, multicomponent complexes consisting of tens of polypeptides, which are responsible for the binding to, and hydrolysis of cellulose (Felix and Ljungdahl, 1993). It is tempting to speculate that the *Streptomyces* protrusions are cellulosome variants designated to synthesize, anchor and expel cellulose. Anchoring of cellulose could be mediated by one of the many cellulose-binding proteins encoded in the *S. coelicolor* genome, such as CbpC (Walter and Schrempf, 2008), or homologues of AbpS of *Streptomyces reticuli* (Walter *et al.*, 1998). In this respect, it is

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Table 3. Strains used in this study.

Strain or plasmid	Description	Reference or source
S. coelicolor strains		
M145	Wild-type SCP1- SCP2-	Kieser *et al.* (2000)
Δ*rdlAB*	*rdlAB::aac(3)IV*	Claessen *et al.* (2004)
Δ*chpABCDH*	*chpAD::aac(3)IV chpB::vph chpCH::aadA*	Claessen *et al.* (2003)
Δ*chpABCDEH*	*chpAD::scar chpB::vph chpCH::aadA chpE::aac(3)IV*	Claessen *et al.* (2003)
Δ*chpABCDEFGH*	*chpAD::scar chpB::vph chpCH::aadA chpE::scar chpF::scar chpG::aac(3)IV*	Claessen *et al.* (2004)
J2177	*bldN::hyg*	Bibb *et al.* (2000)
cslA (Tn5062)	M145 *sco2836::Tn5062*	This work
XE	M145 *cslA$_{SC}$::hyg*	Xu *et al.* (2008)
E. coli strains		
DH5α	F^- Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *recA1 endA1 hsdR17*(r_K^-, m_K^-) *phoA supE44 thi-1 gyrA96 relA1* λ^-	Hanahan (1983)
ET12567	F^-*dam-13::*Tn9 *dcm-6 hsdM hsdR recF143 zjj-202::*Tn10 *galK2 galT22 ara14 lacY1 xyl-5 leuB6 thi-1 tonA31 rpsL136 hisG4 tsx78 mtl-1 glnV44*	MacNeil *et al.* (1992)
BW25113	Δ*araBAD$_{AH33}$* Δ*rhaBAD$_{LD78}$* *lacI^q* *rrnB3* Δ*lacZ4787 hsdR514 rph-1*	Datsenko and Wanner (2000)

S. coelicolor-derived rodlin and chaplin mutant strains were created in the M145 genetic background.

interesting to note that CbpC is a designated sortase substrate.

The identification of cellulose fibrils in the absence of chaplins indicates that cellulose acts as a scaffold for the bundling of chaplin amyloid fibrils into fimbriae (Fig. 7). This implies that cellulose has a high affinity for assembled amyloids. However, cellulose could also directly contribute to the formation of chaplin amyloid fibrils, not only during attachment, but also during formation of aerial hyphae. A role for glycans in inducing amyloid formation has been observed with disease-associated amyloids, where proteoglycans and heparin-like polymers promote amyloid fibrillogenesis (van Horssen *et al.*, 2003; Bellotti and Chiti, 2008). The formation of cellulose at the periphery of adhering hyphae and at the hyphal tip during aerial growth could thus contribute to the co-ordination in space and time of the chaplin assembly process.

Experimental procedures

Strains, plasmids and culture conditions

The *E. coli* and *Streptomyces* strains used in this study are shown in Table 3. *E. coli* was grown at 37°C in LB medium with or without antibiotics. *Streptomyces* strains were grown at 30°C on R5 or MS agar medium (Kieser *et al.*, 2000) or in liquid YEME (Kieser *et al.*, 2000), mNMMP or gNMMP medium (van Keulen *et al.*, 2003). Briefly, mNMMP and gNMMP are minimal media containing either mannitol (25 mM) or glucose (25 mM) and casamino acids (0.25%) as the carbon source respectively.

For growth in static liquid cultures, mycelium was harvested from 1 ml of a 2-day-old YEME liquid shaken culture in early stationary phase. The dispersed mycelium was washed twice with 0.1 M NaH_2PO_4/K_2HPO_4 buffer (pH 6.8) and taken up in 1 ml of the same buffer. This suspension was diluted (1:1000) in fresh gNMMP or mNMMP medium and subsequently used to fill 25-well flat-bottomed polystyrene plates (Greiner Bio-One) with 4 ml per well.

Attachment assay

Attachment of hyphae in static liquid cultures in 25-well plates was quantified as follows: 100 μl crystal violet solution (0.5%; Acros Organics) was added to each well after 2–10 days of growth and left at room temperature for 10 min. For gNMMP cultures, non-adherent cells were removed by washing three times with 5 ml water using a 25 ml glass pipette attached to a motorized pipettor. For mNMMP cultures, plates were vigorously washed with running tap water (tap diameter 1.7 cm; distance to the tap 40 cm; water flow 9 l min^{-1}). After drying at 50°C, crystal violet associated with the attached biomass was solubilized in 5 ml 10% SDS on a platform rocking shaker for 30 min. The OD_{570} of 200 μl aliquots was determined in a microtiter plate reader. If necessary, dilutions were made in 10% SDS. For total biomass quantification, mycelium from single wells was collected and 100 μl crystal violet solution was added. After 30 min, the mycelium was centrifuged for 10 min at 4300 *g* and washed three times with 15 ml water. After drying the mycelium, crystal violet was solubilized with 5 ml 10% SDS and processed as described above. The relative attachment values were calculated by dividing the value of the OD_{570} of the attached mycelium by the value of the OD_{570} of the total biomass.

Construction of the cslA (Tn5062) mutant strain

Cosmid StE20 carrying the Tn5062 transposon in the *SCO2836* gene (Bishop *et al.*, 2004) was transferred by conjugation to *S. coelicolor* M145 to disrupt the *cslA* gene. Aparamycin-resistant ex-conjugants were screened for kanamycin sensitivity. Inactivation of the *cslA* gene was verified by PCR and Southern analyses (data not shown).

Isolation of fimbriae

The *S. coelicolor* wild-type strain was grown in 4 ml mNMMP medium supplemented with 0.25 U ml^{-1} cellulase from

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Aspergillus niger (Sigma-Aldrich). After 10 days of growth, the culture was mixed by pipetting and allowed to stand for 10 min. Two millilitres of the supernatant, containing the fimbriae, was centrifuged at 10 700 *g* for 10 min. The pellet was washed twice with water and taken up in 50 μl water.

Purification of chaplins from S. coelicolor

Chaplins were extracted with trifluoroacetic acid (TFA) from SDS-treated cell walls of sporulating cultures of the *S. coelicolor* Δ*rdlAB* strain (Claessen *et al.*, 2003), as described (Wösten *et al.*, 1993; Claessen *et al.*, 2002). TFA extracts were taken up in water (50–200 μg ml^{-1}) and, if necessary, adjusted to pH 7 with diluted ammonia.

Gel electrophoresis

SDS-PAGE was done in 16% gels as described (Laemmli, 1970). Pre-stained broad range molecular weight markers of Fermentas were used. After separation, proteins were stained with the Bio-Rad Silver Stain Plus kit, according to instructions of the manufacturer.

Maldi-TOF mass spectrometry of fimbriae

Purified fimbriae were analysed with an Axima Performance Maldi-TOF mass spectrometer (Shimadzu Biotech) using a sinapinic acid matrix that had been dissolved in a mixture of 40% acetonitril/0.1% TFA.

Fluorescence microscopy

Fluorescence of GFP and calcofluor white was monitored with a Zeiss Axioskop 50 wide-field fluorescence microscope. All images in a given figure were taken on the same day with the same excitation and camera gain. In case of GFP, samples were analysed using a 470/40 nm bandpass filter, with a 495 nm beamsplitter and a 525/50 nm emission bandpass filter, while for calcofluor white stained samples, a 365/12 nm excitation filter, with a 395 nm beamsplitter and a 397 nm long-pass filter was used. For calcofluor white staining, adhering colonies were carefully removed from the polystyrene microtiter plate with a pipet, mounted on an agarose-covered glass slide, and stained for 5 min with a 0.1% (w/w) solution of the dye (Sigma-Aldrich).

Electron microscopy

For negative staining, mycelium was transferred to Formvar-coated nickel grids. After extensive washing with water, staining was done for 2 s with 2% uranyl acetate. Samples were analysed with a Philips CM12 transmission electron microscope, connected to a MegaView III CCD camera (Soft Imaging System).

Quantification of fimbriae

To quantify fimbriae, random EM images were taken (see below). Positions of the fimbriae were indicated on transparencies, which were subsequently digitized using a Canon 8800F scanner. Scanned images were loaded into ImageJ (1.42). Average pixel densities (from 6 to 10 scanned images) were determined and used as a measure for the number of fimbriae produced by each strain.

Circular dichroism

The CD spectra were recorded over the wavelength region 190–250 nm on an Aviv 62A DS Circular dichroism spectrometer, using a 5 mm quartz cuvette. The temperature was kept constant at 25°C and the sample compartment was flushed with a continuous stream of N_2. Spectra represent the average of three scans using a bandwidth of 1 nm, a step width of 1 nm and a 5 s averaging per point. The spectra were corrected by using a reference solution without the protein. Typically, a protein concentration of 50–200 μg ml^{-1} was used.

To determine the secondary structure of chaplins interacting with a hydrophobic support, an amount of colloidal Teflon was added such that the protein in solution could cover 10% of the surface of the solid (de Vocht *et al.*, 1998). Spectra were taken before and after heating at 85°C in the presence of 0.1% Tween.

Fluorescence spectroscopy

Amyloid fibrils of chaplins were stained with 3 μM of the fluorescent dye ThT. Fluorescence was followed at 482 nm (excitation = 450 nm) on an Aminco-Bowman series 2 luminescence spectrometer (SLM-Aminco·).

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References

Alteri, C.J., Xicohténcatl-Cortes, J., Hess, S., Caballero-Olin, G., Girón, J.A., and Friedman, R.L. (2007) *Mycobacterium tuberculosis* produces pili during human infection. *Proc Natl Acad Sci USA* **104:** 5145–5150.

Bellotti, V., and Chiti, F. (2008) Amyloidogenesis in its biological environment: challenging a fundamental issue in protein misfolding diseases. *Curr Opin Struct Biol* **18:** 771–779.

Bibb, M.J., Molle, V., and Buttner, M.J. (2000) ó (BldN), an extracytoplasmic function RNA polymerase sigma factor required for aerial mycelium formation in *Streptomyces coelicolor* A3(2). *J Bacteriol* **182:** 4606–4616.

Bishop, A., Fielding, S., Dyson, P., and Herron, P. (2004) Systematic insertional mutagenesis of a streptomycete genome: a link between osmoadaptation and antibiotic production. *Genome Res* **14:** 893–900.

Capstick, D.S., Willey, J.M., Buttner, M.J., and Elliot, M.A.

13652958, 2009, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2958.2009.06838.x by Utrecht University Library, Wiley Online Library on [07/12/2022]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(2007) SapB and the chaplins: connections between morphogenetic proteins in *Streptomyces coelicolor*. *Mol Microbiol* **64:** 602–613.

Chang, C.T., Wu, C.S., and Yang, J.T. (1978) Circular dichroic analysis of protein conformation: inclusion of the β-turns. *Anal Biochem* **91:** 13–31.

Chapman, M.R., Robinson, L.S., Pinkner, J.S., Roth, R., Heuser, J., Hammar, M., *et al.* (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* **295:** 851–855.

Chater, K.F., and Horinouchi, S. (2003) Signalling early developmental events in two highly diverged *Streptomyces* species. *Mol Microbiol* **48:** 9–15.

Claessen, D., Wösten, H.A.B., van Keulen, G., Faber, O.G., Alves, A.M., Meijer, W.G., and Dijkhuizen, L. (2002) Two novel homologous proteins of *Streptomyces coelicolor* and *Streptomyces lividans* are involved in the formation of the rodlet layer and mediate attachment to a hydrophobic surface. *Mol Microbiol* **44:** 1483–1492.

Claessen, D., Rink, R., de Jong, W., Siebring, J., de Vreugd, P., Boersma, F.G., *et al.* (2003) A novel class of secreted hydrophobic proteins is involved in aerial hyphae formation in *Streptomyces coelicolor* by forming amyloid-like fibrils. *Genes Dev* **17:** 1714–1726.

Claessen, D., Stokroos, I., Deelstra, H.J., Penninga, N.A., Bormann, C., Salas, J.A., *et al.* (2004) The formation of the rodlet layer of streptomycetes is the result of the interplay between rodlins and chaplins. *Mol Microbiol* **53:** 433–443.

Claessen, D., de Jong, W., Dijkhuizen, L., and Wösten, H.A.B. (2006) Regulation of *Streptomyces* development: reach for the sky!. *Trends Microbiol* **14:** 313–319.

Colvin, J.R., and Witter, D.E. (1983) Congo red and calcofluor white inhibition of *Acetobacter xylinum* cell growth and of bacterial cellulose microfibril formation: isolation and properties of a transient, extracellular glucan related to cellulose. *Protoplasma* **116:** 34–40.

Datsenko, K.A., and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* **97:** 6640–6645.

Di Berardo, C., Capstick, D.S., Bibb, M.J., Findlay, K.C., Buttner, M.J., and Elliot, M.A. (2008) Function and redundancy of the chaplin cell surface proteins in aerial hypha formation, rodlet assembly, and viability in *Streptomyces coelicolor*. *J Bacteriol* **190:** 5879–5889.

Elliot, M.A., Karoonuthaisiri, N., Huang, J., Bibb, M.J., Cohen, S.N., Kao, C.M., and Buttner, M.J. (2003) The chaplins: a family of hydrophobic cell-surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor*. *Genes Dev* **17:** 1727–1740.

Felix, C.R., and Ljungdahl, L.G. (1993) The cellulosome: the exocellular organelle of *Clostridium*. *Annu Rev Microbiol* **47:** 791–819.

Findeis, M.A. (2000) Approaches to discovery and characterization of inhibitors of amyloid beta-peptide polymerization. *Biochim Biophys Acta* **1502:** 76–84.

Flärdh, K. (2003) Essential role of DivIVA in polar growth and morphogenesis in *Streptomyces coelicolor* A3(2). *Mol Microbiol* **49:** 1523–1536.

Flärdh, K., and Buttner, M.J. (2009) *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium. *Nat Rev Microbiol* **7:** 36–49.

Gebbink, M.F.B.G., Claessen, D., Bouma, B., Dijkhuizen, L., and Wösten, H.A.B. (2005) Amyloids – a functional coat for microorganisms. *Nat Rev Microbiol* **3:** 333–341.

Giacomelli, C.E., and Norde, W. (2003) Influence of hydrophobic Teflon particles on the structure of amyloid beta-peptide. *Biomacromolecules* **4:** 1719–1726.

Hammar, M., Bian, Z., and Normark, S. (1996) Nucleator-dependent intercellular assembly of adhesive curli organelles in *Escherichia coli*. *Proc Natl Acad Sci USA* **93:** 6562–6566.

Hammer, N.D., Schmidt, J.C., and Chapman, M.R. (2007) The curli nucleator protein, CsgB, contains an amyloidogenic domain that directs CsgA polymerization. *Proc Natl Acad Sci USA* **104:** 12494–12499.

Hanahan, D. (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* **166:** 557–580.

van Horssen, J., Wesseling, P., van den Heuvel, L.P.W.J., de Waal, R.M.W., and Verbeek, M.M. (2003) Heparan sulphate proteoglycans in Alzheimer's disease and amyloid-related disorders. *Lancet Neurol* **2:** 482–492.

de Jong, W., Manteca, A., Sanchez, J., Bucca, G., Smith, C.P., Dijkhuizen, L., *et al.* (2009) NepA is a structural cell wall protein involved in maintenance of spore dormancy in *Streptomyces coelicolor*. *Mol Microbiol* **71:** 1591–1603.

Kelemen, G.H., and Buttner, M.J. (1998) Initiation of aerial mycelium formation in *Streptomyces*. *Curr Opin Microbiol* **1:** 656–662.

van Keulen, G., Jonkers, H.M., Claessen, D., Dijkhuizen, L., and Wösten, H.A.B. (2003) Differentiation and anaerobiosis in standing liquid cultures of *Streptomyces coelicolor*. *J Bacteriol* **185:** 1455–1458.

Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F., and Hopwood, D.A. (2000) *Practical Streptomyces Genetics*. Norwich: The John Innes Foundation.

Kodani, S., Hudson, M.E., Durrant, M.C., Buttner, M.J., Nodwell, J.R., and Willey, J.M. (2004) The SapB morphogen is a lantibiotic-like peptide derived from the product of the developmental gene *ramS* in *Streptomyces coelicolor*. *Proc Natl Acad Sci USA* **101:** 11448–11453.

Kuner, P., Bohrmann, B., Tjernberg, L.O., Naslund, J., Huber, G., Celenk, S., *et al.* (2000) Controlling polymerization of beta-amyloid and prion-derived peptides with synthetic small molecule ligands. *J Biol Chem* **275:** 1673–1678.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Larsen, P., Nielsen, J.L., Dueholm, M.S., Wetzel, R., Otzen, D., and Nielsen, P.H. (2007) Amyloid adhesins are abundant in natural biofilms. *Environ Microbiol* **9:** 3077–3090.

Larsen, P., Nielsen, J.L., Otzen, D., and Nielsen, P.H. (2008) Amyloid-like adhesins produced by floc-forming and filamentous bacteria in activated sludge. *Appl Environ Microbiol* **74:** 1517–1526.

Leskiw, B.K., Lawlor, E.J., Fernandez-Abalos, J.M., and Chater, K.F. (1991) TTA codons in some genes prevent their expression in a class of developmental, antibiotic-negative, *Streptomyces* mutants. *Proc Natl Acad Sci USA* **88:** 2461–2465.

MacNeil, D.J., Gewain, K.M., Ruby, C.L., Dezeny, G., Gibbons, P.H., and MacNeil, T. (1992) Analysis of *Streptomyces avermitilis* genes required for avermectin biosyn-

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thesis utilizing a novel integration vector. *Gene* **111:** 61–68.

Mandlik, A., Swierczynski, A., Das, A., and Ton-That, H. (2008) Pili in Gram-positive bacteria: assembly, involvement in colonization and biofilm development. *Trends Microbiol* **16:** 33–40.

Marraffini, L.A., Dedent, A.C., and Schneewind, O. (2006) Sortases and the art of anchoring proteins to the envelopes of Gram-positive bacteria. *Microbiol Mol Biol Rev* **70:** 192–221.

Matthysse, A.G., and McMahan, S. (1998) Root colonization by *Agrobacterium tumefaciens* is reduced in *cel*, *attB*, *attD*, and *attR* mutants. *Appl Environ Microbiol* **64:** 2341–2345.

Matthysse, A.G., Holmes, K.V., and Gurlitz, R.H. (1981) Elaboration of cellulose fibrils by *Agrobacterium tumefaciens* during attachment to carrot cells. *J Bacteriol* **145:** 583–595.

Nenninger, A.A., Robinson, L.S., and Hultgren, S.J. (2009) Localized and efficient curli nucleation requires the chaperone-like amyloid assembly protein CsgF. *Proc Natl Acad Sci USA* **106:** 900–905.

Pallen, M.J., Lam, A.C., Antonio, M., and Dunbar, K. (2001) An embarrassment of sortases – a richness of substrates? *Trends Microbiol* **9:** 97–102.

Proft, T., and Baker, E.N. (2009) Pili in Gram-negative and Gram-positive bacteria – structure, assembly and their role in disease. *Cell Mol Life Sci* **66:** 613–635.

Römling, U. (2002) Molecular biology of cellulose production in bacteria. *Res Microbiol* **153:** 205–212.

Sreerama, N., Venyaminov, S.Y., and Woody, R.W. (1999) Estimation of the number of α-helical and β-strand segments in proteins using circular dichroism spectroscopy. *Protein Sci* **8:** 370–380.

Talbot, N.J., Kershaw, M.J., Wakley, G.E., De Vries, O.M.H., Wessels, J.G.H., and Hamer, J.E. (1996) MPG1 encodes a fungal hydrophobin Involved in surface interactions during infection-related development of *Magnaporthe grisea*. *Plant Cell* **8:** 985–999.

Teertstra, W.R., Deelstra, H.J., Vranes, M., Bohlmann, R., Kahmann, R., Kamper, J., and Wösten, H.A.B. (2006) Repellents have functionally replaced hydrophobins in mediating attachment to a hydrophobic surface and in formation of hydrophobic aerial hyphae in *Ustilago maydis*. *Microbiology* **152:** 3607–3612.

Teertstra, W.R., van der Velden, G.J., de Jong, J.F., Kruijtzer, J.A., Liskamp, R.M., Kroon-Batenburg, L.M., *et al.* (2009) The filament-specific Rep1-1 repellent of the phytopathogen *Ustilago maydis* forms functional surface-active amyloid-like fibrils. *J Biol Chem* **284:** 9153–9159.

Telford, J.L., Barocchi, M.A., Margarit, I., Rappuoli, R., and Grandi, G. (2006) Pili in Gram-positive pathogens. *Nat Rev Microbiol* **4:** 509–519.

Tillotson, R.D., Wösten, H.A.B., Richter, M., and Willey, J.M. (1998) A surface active protein involved in aerial hyphae formation in the filamentous fungus *Schizophillum commune* restores the capacity of a bald mutant of the filamentous bacterium *Streptomyces coelicolor* to erect aerial structures. *Mol Microbiol* **30:** 595–602.

de Vocht, M.L., Scholtmeijer, K., van der Vegte, E.W., de Vries, O.M.H., Sonveaux, N., Wösten, H.A.B., *et al.* (1998) Structural characterization of the hydrophobin SC3, as a monomer and after self-assembly at hydrophobic/hydrophilic interfaces. *Biophys J* **74:** 2059–2068.

de Vocht, M.L., Reviakine, I., Ulrich, W.-P., Bergsma-Schutter, W., Wösten, H.A.B., Vogel, H., *et al.* (2002) Self-assembly of the hydrophobin SC3 proceeds via two structural intermediates. *Protein Sci* **11:** 1199–1205.

Walter, S., and Schrempf, H. (2008) Characteristics of the surface-located carbohydrate-binding protein CbpC from *Streptomyces coelicolor* A3(2). *Arch Microbiol* **190:** 119–127.

Walter, S., Wellmann, E., and Schrempf, H. (1998) The cell wall-anchored *Streptomyces reticuli* avicel-binding protein (AbpS) and its gene. *J Bacteriol* **180:** 1647–1654.

White, A.P., Gibson, D.L., Collinson, S.K., Banser, P.A., and Kay, W.W. (2003) Extracellular polysaccharides associated with thin aggregative fimbriae of *Salmonella enterica* serovar enteritidis. *J Bacteriol* **185:** 5398–5407.

Willey, J., Santamaria, R., Guijarro, J., Geistlich, M., and Losick, R. (1991) Extracellular complementation of a developmental mutation implicates a small sporulation protein in aerial mycelium formation by *S. coelicolor*. *Cell* **65:** 641–650.

Wösten, H.A.B. (2001) Hydrophobins: multipurpose proteins. *Annu Rev Microbiol* **55:** 625–646.

Wösten, H.A.B., and de Vocht, M.L. (2000) Hydrophobins, the fungal coat unravelled. *Biochim Biophys Acta* **1469:** 79–86.

Wösten, H.A.B., De Vries, O.M.H., and Wessels, J.G.H. (1993) Interfacial self-assembly of a fungal hydrophobin into a hydrophobic rodlet layer. *Plant Cell* **5:** 1567–1574.

Wösten, H.A.B., Schuren, F.H.J., and Wessels, J.G.H. (1994) Interfacial self-assembly of a hydrophobin into an amphipathic protein membrane mediates fungal attachment to hydrophobic surfaces. *EMBO J* **13:** 5848–5854.

Wösten, H.A.B., Bohlmann, R., Eckerskorn, C., Lottspeich, F., Bölker, M., and Kahmann, R. (1996) A novel class of small amphipathic peptides affect aerial hyphal growth and surface hydrophobicity in *Ustilago maydis*. *EMBO J* **15:** 4274–4281.

Xu, H., Chater, K.F., Deng, Z., and Tao, M. (2008) A cellulose synthase-like protein involved in hyphal tip growth and morphological differentiation in *Streptomyces*. *J Bacteriol* **190:** 4971–4978.

Zogaj, X., Nimtz, M., Rohde, M., Bokranz, W., and Römling, U. (2001) The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. *Mol Microbiol* **39:** 1452–1463.

Zogaj, X., Bokranz, W., Nimtz, M., and Römling, U. (2003) Production of cellulose and curli fimbriae by members of the family *Enterobacteriaceae* isolated from the human gastrointestinal tract. *Infect Immun* **71:** 4151–4158.

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