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Unicellular but not asocial. Life in community of a bacterium

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Summary. All living organisms have acquired the outstanding ability to overcome the limitations imposed by changeable environments through the gain of genetic traits over years of evolution and the tendency of individuals to associate in communities. The complementation of a singular weakness, the deployment of reinforcement for the good of the community, the better use of resources, or effective defense against external aggression are advantages gained by this communal behavior. Communication has been the cohesive element prompting the global responses that promote efficiency in two features of any community: specialization in differentiated labor and the spatio-temporal organization of the environment. These principles illustrate that what we call human ecology also applies to the cellular world and is exemplified in eukaryotic organisms, where sophisticated cell-to-cell communication networks coordinate cell differentiation and the specialization of multiple tissues consisting of numerous cells embedded in a multifunctional extracellular matrix. This sophisticated molecular machinery appears, however, to be invented by the "simple" but still fascinating bacteria. What I will try to expand in the following sections are notions of how "single prokaryotic cells" organize a multicellular community. [**Int Microbiol** 19(2):81-90 (2016)]

Keywords: evolution · molecular machinery · multicellular community · prokaryotic cells · global responses

From "animalcules" to multicellular bacterial behavior

The first idea of the complexity of the microbial world should likely be attributed to Antonie van Leeuwenhoek (18th century), whose observations established an important principle in microbial ecology: the lives of microbes in multispecies communities embedded in a sort of regenerative shield [66], a description of what is now known as the multispecies biofilm that constitutes dental plaques [39]. It is now believed that any bacterial species is capable of organizing biofilms on any given surface, biotic or abiotic, and research has been devoted to understanding basic aspects of why bacterial cells decide to assemble these multicellular communities and how this process is accomplished.

Prof. Claude ZoBell was one of the first scientists who took an interest in this bacterial behavior, specifically in the bacteria that form "biofouling" on the submerged side of surfaces. In his description of this biofouling, "…our observations show that bacteria […] form a mucilaginous surface to which the fouling organisms adhere. It is entirely possible that bacterial film forms a protecting coating", a fundamental concept of bacterial biofilms emerges: the cells are embedded in a

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multifunctional extracellular matrix [98]. In his study on the relevance of surfaces to the metabolic activity of bacteria, ZoBell concluded that most marine bacterial species must live in association with solid surfaces as a result of the active attachment of bacterial cells [97]. While ZoBell and other authors began suggesting the presence of stalks from some adhered cells to the surfaces, how bacterial cells reach this level of organization and how they respond globally to certain environmental triggers remained to be discovered.

By the mid-20th century, diverse scientists were amazed by the fascinating intrinsic abilities of some organisms to produce light [36,53]. Among these scientists, Prof. Osamu Shimomura was awarded with the 2008 Nobel Prize in Chemistry for the discovery and further development of green fluorescent protein [80]. This phenomenon, called bioluminescence, also occurs in bacteria and was extensively investigated by Prof. J. Woodland Hastings [35]. Prof. Hastings and collaborators elegantly demonstrated that the spent medium of a *Vibrio fischeri* culture accumulated something they intuitively termed autoinducer, a molecule that triggers the production of bioluminescence. This observation established the basis for the further elucidation of the structure of the first bacterial cell-to-cell chemical communication molecule, N-(3-oxohexanoyl)-3-aminodihydro-2(3H)-furanone [25,57]. Further investigation by Prof. Peter Greenberg and Prof. Bonnie Bassler, among others, proved that the accumulation of the autoinducer triggers a global response in the bacterial population, leading to antibiotic production, the expression of virulence factors or the formation of biofilms [58,76]. Continuing research in the field is taking us into a fascinating and unpredictable world of chemical communication networks among cells of the same species, different species and even members of other kingdoms [61,71,89,90].

The concepts introduced above, including bacterial communication, global population response and multicellular behavior, were connected to biofilms by the work of Prof. JW Costerton: "…in all aquatic systems with adequate concentration of nutrients, bacteria form glycocalyx-enclosed biofilms adherent to available surfaces and these sessile populations usually attain numerical and physiological predominance in medical, natural, and industrial aquatic ecosystems." [23,33]. As in human communities, the transition of individual bacterial cells to multicellular communities is attainable due to the combination of the following factors: i) the existence of an inducible communication system ii) the specialization of individuals for different functions, and iii) their spatio-temporal organization through a multifunctional structure called the extracellular matrix.

Lessons in multicellularity from harmless bacteria

The predicted complexity of microbial communities in nature has motivated the use of reductionist approaches to really understand the mechanisms of the developmental program that orchestrates the assembly of single-species biofilms. Pathogenic bacteria have received special attention, since their biofilms are involved in the contamination of medical devices, serving as a reservoir of pathogens that can cause future host infections. They are also involved in the contamination of instruments in the food industry and are difficult to eradicate due to their resilience to many antimicrobials [21,22,34,44,74,95]. Furthermore, studies of the soil-dwelling, non-pathogenic *Bacillus subtilis* have contributed enormously to our understanding of the basis of biofilm formation. At the cellular level, *Bacillus* forms spores that are extremely resistant to environmental offenses [17,54,84]. To form a spore, *B. subtilis* deploys an intricate machinery consisting of receptors, signals and genetic cascades to determine the moment when sporulation is initiated [28,43,88]. The sporulation developmental program and the multifaceted properties of the spores have definitively contributed to making *B. subtilis* a model in studies of gene regulation [3,86].

The complex communication network that coordinates cell differentiation

The joint work of the laboratories of Prof. Roberto Kolter and Prof. Richard Losick, among many other outstanding scientists, has contributed to our understanding of the developmental program that allows *B. subtilis* to form biofilms. In a chemically defined medium, *B. subtilis* assembles either colonies or pellicles with wrinkles as the most visible morphological feature, a simple but extremely effective experimental setup for the screening of genes dedicated to biofilm formation (Fig. 1A-B) [9,10]. Sporulation and biofilm formation are connected by Spo0A, a master regulator that is phosphorylated (Spo0A-P) following a cascade of signals. The intracellular levels of Spo0A determine the fate of the cell, which will become a biofilm producer at an intermediate level of Spo0A or sporulate if the level is high. Readers are highly encouraged to consult other reviews in which this topic is extensively treated [46,93].

The pheromone ComX, which reaches maximum levels during the stationary phase, triggers the development of

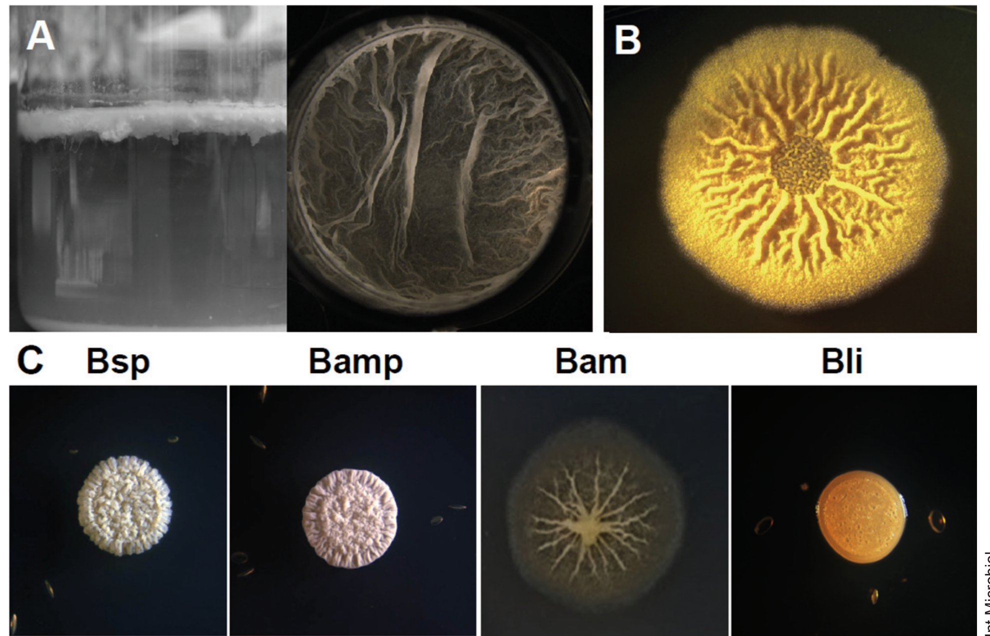

Fig. 1. The experimental set up for the *in vitro* study of *Bacillus* biofilms. (**A**) The strain *Bacillus subtilis* subsp. *subtilis* NCIB 3610 forms pellicles in the biofilm-inducing medium MSgg with no agitation at 30ºC. Left image: side view of the thickness of the pellicle. Right image: Top view of the pellicle with visible wrinkles. (**B**) These wrinkles are also morphological features characterizing *B. subtilis* colonies grown for at least 72 h in MSgg agar at 30ºC. C) *Bacillus* species closely related phylogenetically to *Bacillus subtilis* subsp. *subtilis* NCIB 3610 form morphologically different colonies in the biofilm-inducing medium MSgg agar. Bsp, *Bacillus subtilis* subsp. *spizizenii*; Bamp, *Bacillus amyloliquefaciens* subsp. *plantarum*; Bam, *Bacillus amyloliquefaciens* DSM7; Bli, *Bacillus licheniformis.*

competence, allowing certain cells to take up DNA from their surroundings. ComX also allows the expression of the operon involved in the synthesis of surfactin, a fascinating molecule known for decades but with hidden features still waiting to be discovered. It is the amphiphilic structure of surfactin, a peptide ring fused to a fatty acid tail, that makes it a multivalent molecule in bacterial multicellular behavior: i) the reduction of water surface tension, a physical phenomenon closely linked to the flagella-dependent bacterial social movement called swarming, and ii) insertion in biological membranes, which can lead to cytoplasmic imbalance and cell death, thus providing protection against competitors [79]. This tendency to target membranes appears to be behind the role of surfactin as a self-produced trigger of biofilm formation in *B. subtilis*. Surfactin provokes a leakage of K+ in a certain subpopulation of cells, which is in turn sensed by the histidine kinase KinC, which then, through Spo0A, activates the expression of genes dedicated to the synthesis of the major components of the extracellular matrix: the adhesive protein TasA and the exopolysaccharide EPS [45,47]. In the end, the coordination of these and other signals and their corresponding genetic cascades orchestrates an effective response to a variety of signals and promote divergent cell fates and their coexistence within the same extracellular matrix [12,64,93]. Despite the universality of these genetic factors in the *Bacillus* genus, variations of this developmental program result in variable biofilm architectures. Indeed, bacteria closely related to *B. subtilis* develop visually different biofilms, suggesting the involvement of additional factors (Fig.1C) [49,56].

The integrity and functionality of multicellular organisms are achieved by the processes of cell differentiation and spatial organization of different cell types, and the same is true in bacterial communities [40]. A feature of *Bacillus* biofilms is the spongy appearance of the outermost layers, which, at the microscopic level, reveal the presence of structures reminiscent of the fruiting bodies of *Myxococcus xanthus*, where sporulation preferentially occurs [9,41]. The connection between cell differentiation and spatial localization was further expanded by Hera Vlamakis, Claudio Aguilar and their collaborators in their beautiful anatomical study of biofilms [92]. Using reporter cells for diverse cell fates, they demonstrated that motile cells occupied the bottom of the biofilms, sporulating cells were present in the outer layers, and the matrix producers were embedded in between. This arrangement is characteristic of the wild-type strain; however, a mutant disrupted in the assembly of the extracellular matrix,

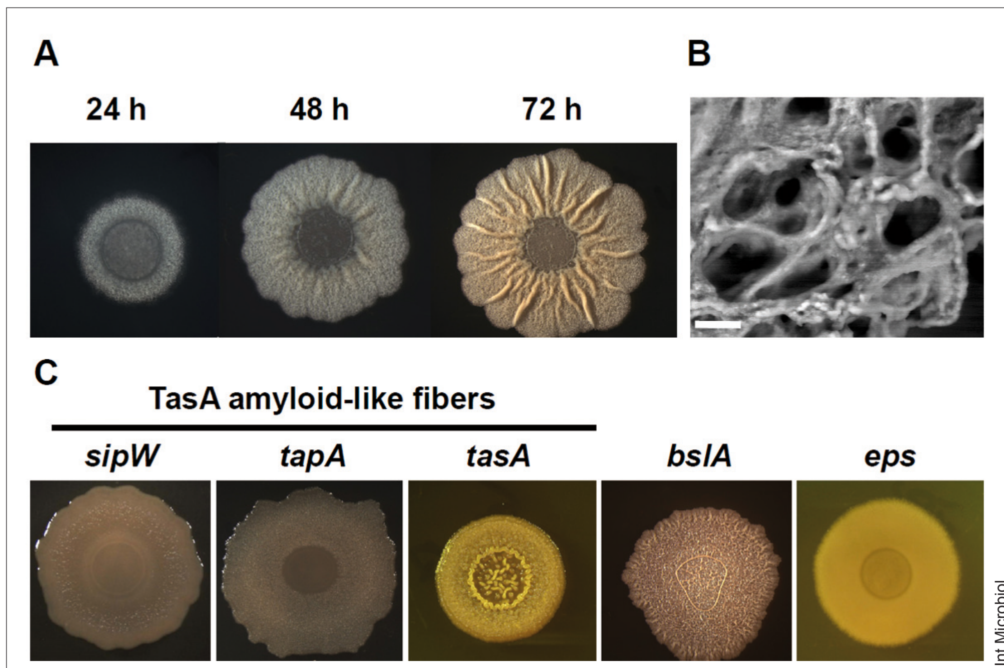

Fig. 2. The extracellular matrix is responsible for the final architecture of *Bacillus subtilis* biofilms. (**A**) The colony morphology of *B. subtilis* changes with time and reaches maturity, characterized by visual wrinkles, after 72 h of growth in MSgg agar at 30ºC. B) The environmental electron micrograph of a 72-h-old *B. subtilis* biofilm reveals the cells and extracellular matrix to be gathered in a tissue-like structure that delimits channels in which nutrients, water, signals and gases flow freely. (**C**) Mutants in any of the main components of the *B. subtilis* extracellular matrix are defective in biofilm formation: (i) the TasA amyloid-like fibers (*sipW* encodes the signal peptidase that processes TapA and TasA; *tapA* encodes the auxiliary protein TapA; and *tasA* encodes the major component of the amyloid-like fibers); (ii) the hydrophobin protein BslA (*bslA*), which forms a hydrophobic coat; and (iii) an exopolysaccharide (*eps* operon). Bar equals 20 µm in B.

and thus lacking the morphological features of the biofilm, failed to achieve cell differentiation and spatial organization. These findings indicate the relevance of the extracellular matrix in maintaining not only the structural integrity of the biofilm but also the regulation of the cell-to-cell communication network. What, then, is the extracellular matrix made of?

The multifunctional extracellular matrix

The biofilm is a dynamic biostructure in which water, nutrients and hazardous compounds flow freely. To reach this goal, cells engineer the assembly of a tissue-like structure, the extracellular matrix [29,94]. The extracellular matrix is responsible for the final architecture of the biofilm: examining *B. subtilis* biofilms by electron microscopy reveals a system of channels delimited by masses of spatially organized cells (Fig. 2A-B), an architecture absent from mutants lacking this tissue-like structure [68,94]. Although not reported directly, this spatial organization of cells prompted by an extracellular matrix should be credited to Ferdinand Cohn. He and another two of the finest scholars in the field, Louis Pasteur and Robert Koch (Koch won the 1905 Nobel Prize in Physiology or Medicine), investigated *B. anthracis,* the etiological agent of a devastating disease called anthrax. In his work, Prof. Cohn described, with great precision, and without the help of potent electron microscopes (not yet available), all the cell types and morphologies of *B. anthracis*. Among them, we note tubular structures consisting of cells within a shield (Fig. 1 reproduces the original color plate in [1]), which, astonishingly, resembles the most recent electron microscopic observations of the channels of cells that characterize the biofilms of *B. subtilis* (Fig. 2B) [68].

The remarkable hydrophobicity of the extracellular matrix promotes the tight adhesion of the colony to surfaces and the enhanced resistance of cells encased in biofilms to antibiotics and other antimicrobials. These two attributes additionally contribute to the difficulty of eradicating biofilms [12,64]. In *B. subtilis,* at least two proteins, TasA and BslA, and an exopolysaccharide, are among the most relevant elements that define the chemical and biological features of the extracellular matrix (Fig. 2C) [8,10,62], although the involvement of

additional molecules should not be excluded [12]. New studies on the extracellular matrices of diverse related bacterial species will contribute exponentially to our understanding of the functionality of each of these components.

The exoskeleton consisting of functional amyloids

Within a biofilm, cells of *B. subtilis* are interconnected by a network of long fibers consisting of TasA (Fig. 3). A deep analysis demonstrated that TasA possesses the intrinsic ability to polymerize to form of fibers that are morphologically and biochemically similar to amyloid proteins [69]. A similar protein called curli was previously reported in *E. coli*, and diverse studies have demonstrated the wide distribution of these proteins in the microbial world [7,16,20,26,30,32,50,51,60,70,75,77,87,91]. Prof. Virchow was the first to use the term amyloid to refer to the *corpora amylacea* of the nervous system, based on the similar appearance of the plaques to starch after staining with iodine. Other authors, however, questioned Virchow's observations and thus the hypothetical starchy nature of the plaques: "…It has been stated by Virchow that, by a dexterous adjustment of sulfuric acid and iodine, a blue tint may be given to the "amyloid" deposit, but, like many other observers, I have never succeeded in obtaining any color but reddish brown, merging into shades of dirty black. This color, due to the precipitation of the iodine by the acid, would probably never have been looked upon as blue except by a person whose impartiality of observation had been warped by a desire to connect the morbid change with the production of starch" [25]. After a long controversy regarding the chemical composition of amyloid plaques, it was demonstrated that amyloid fibers, as observed by electron microscopy, consisted of proteins. However, despite the rejection of the starchy hypothetical composition of the fibers, the pathological term amyloid has prevailed to the present [81,82].

Amyloid proteins do not share similarity at the amino-acid level, but all of them assemble into fibers enriched in β-sheets capable of binding the specific dyes Congo Red and thioflavin T, which resist proteolysis and detergent denaturalization [20,30]. In *B. subtilis*, the TasA amyloid-like fibers form a resistant network that spatially organizes the biofilm, but other amyloids hide cells from the host immune system, protect the cells from the environment or even scavenge toxic monomers, resulting in the term "functional amyloids" in an attempt to distinguish them from pathogenic amyloids [2,5,32,75,85]. Further studies have highlighted interesting differences between functional and pathogenic amyloids, such as the way they polymerize. In general, it can be said that amyloidogenesis in bacteria is the result of an efficient and highly regulated process that defines how and when the fibers are produced [75]. Beyond the intrinsic aggregative nature of amyloid proteins, additional factors assist the monomers in

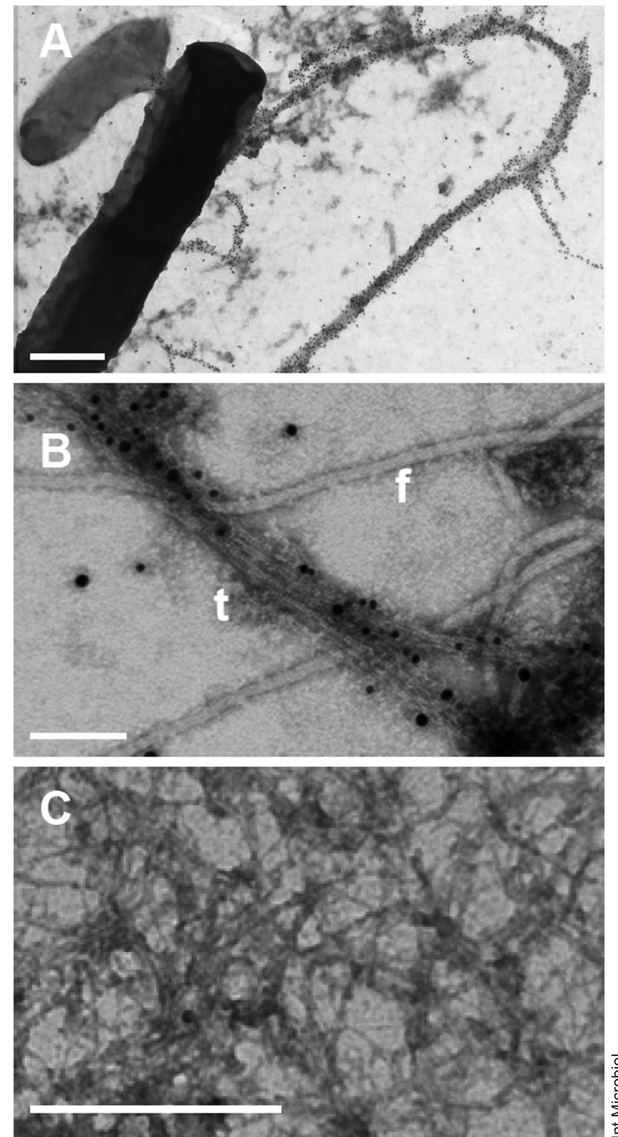

Fig. 3. The extracellular matrix of *Bacillus subtilis* biofilms contains functional amyloids. Transmission electron micrographs of uranyl acetate-contrasted samples show the tendency of TasA to form fibers. (**A**) Anti-TasA immunogold-labeled samples shows TasA fibers emerging from the surfaces of cells. (**B**) Double anti-TasA and anti-TapA immunogold-labeled *B. subtilis* biofilm samples reveal the presence of TasA (10 nm gold particles) and TapA (15 nm gold particles) in the TasA amyloid-like fibers. t, TasA fibers; f, flagella. (**C**) TasA protein purified to homogeneity from *B. subtilis* cells retains the ability to form fibers. Bars equal 500 nm in A and C, 100 nm in B.

the development of fibers. In *B. subtilis*, the accessory protein TapA seems to play two roles: accelerating the polymerization of the TasA fibers and anchoring the growing fibers to the cell envelope [72,73]. These functions, however, are not universal among the *Bacillus* genus. Members of the *B. cereus* group possess three orthologs of TasA but lack orthologs of TapA. Furthermore, TasA in *B. cereus* is still functional and assembles into fibers that resemble the ones in *B. subtilis*. More interestingly, the genomic region dedicated to the synthesis of the fibers in *B. cereus* can be transferred to a *B. subtilis* mutant lacking any known amyloid-related proteins and still promotes the formation of fibers in the cell surfaces [13]. In addition to these proteins, it is thought that the hydrophobicity that characterizes the bacterial cell surfaces and the extracellular matrix also influences the polymerization of the fibers. This idea is based on the fact that aggregates of TasA purified from *B. subtilis* evolve to form fibers when deposited on hydrophobic surfaces but not on hydrophilic ones [15].

The highly hydrophobic members of the extracellular matrix

The exopolysaccharides, and especially the protein BslA, are responsible for the hydrophobicity of the extracellular matrix [37,42]. Exopolysaccharides (EPS) are polymers with hydrophobic or repellent features, two distinctive traits of the extracellular matrix [59]. EPS from different bacteria differ in their chemical composition, which defines their morphological and staining features as well as their biological relevance. *Pseudomonas putida* is known to possess up to four different gene clusters dedicated to the production of EPS [52]. In contrast, *B. subtilis* synthesizes at least one EPS, which is non-cellulosic given the failure of staining with Congo Red [69]. Rheological studies on *Pseudomonas aeruginosa* biofilms, which also contain more than one EPS, have shown the modulatory use of diverse exopolysaccharides as an efficient way for bacterial populations to modify and adapt to the changeable microenvironment[19]. In *B. subtilis*, the osmotic pressure gradients associated with the EPS appear to serve as a driving force to facilitate colony spreading [78]. BslA is a protein that forms a layer covering the entire biofilm of *B. subtilis* and thus contributes greatly, even more than the EPS, to the repellent nature of the extracellular matrix. BslA is similar in size to TasA but polymerizes in the form of regular, markedly hydrophobic aggregates, similarly to fungal hydrophobins [11]. TasA, EPSs, BlsA and other components yet to be found coordinately contribute to the construction of the extracellular matrix, the infrastructure that permits the assembly of this remarkable bacterial community.

From the laboratory to the field

The reductionist approaches have delved into the mechanisms of the sophisticated program by which bacteria form biofilms in the laboratory: external signals, receptors, genetic cascades and structural elements. All this knowledge can now be applied to the study of bacterial biofilms in more complex scenarios and how these factors integrate with many other external/environmental signals that might interfere with the biofilm developmental program. As mentioned above, *B. subtilis* lives in association with plants, a compelling and useful niche for testing all our accumulated knowledge on biofilms.

Plants are truly fascinating living organisms that, due to their static lifestyle, have evolved the ability to handle and respond efficiently to the multitude of changeable abiotic (desiccation, light, UV radiation, drought) and biotic factors (animals, other plants, microbes), thus becoming able to colonize any environment found in the world [65]. Plants live in association with a large variety of microbes, some of them pathogenic and thus responsible for deleterious metabolic imbalances, and others beneficial that may contribute positively to the health of the plants [55]. *B. subtilis* is one of these beneficial microbes: it lacks any virulence factors, such as toxins, and contributes to plant health in a multifaceted way (Fig. 4A-B) [63]. To maintain this mutualistic interaction, *B. subtilis* and plants must use a mutually understandable language (Fig. 4C). Diverse organic acids and polysaccharides secreted by plant roots are sensed by *Bacillus* cells, which activate the formation of biofilms via the histidine kinase KinD. KinD is one of the receptors that triggers the phosphorelay leading to the formation of Spo0A-P, which ultimately activates the expression of the extracellular matrix-related genes [4,18]. Reciprocally, *Bacillus* cells colonizing the roots produce surfactin, which contributes to this beneficial interaction in at least two roles. First, it prompts biofilm formation, probably via KinC, as demonstrated *in vitro*. In this way, *Bacillus* cells efficiently colonize the plant surfaces and secrete antimicrobials, which coordinately and locally repress the spread of pathogens [96]. Second, surfactin activates the immune system of the plants, which thus become better able to locally and systemically defeat pathogens in other parts of the plant. This process of "immunization" is called "priming" and is mediated by the activation of diverse plant hormone

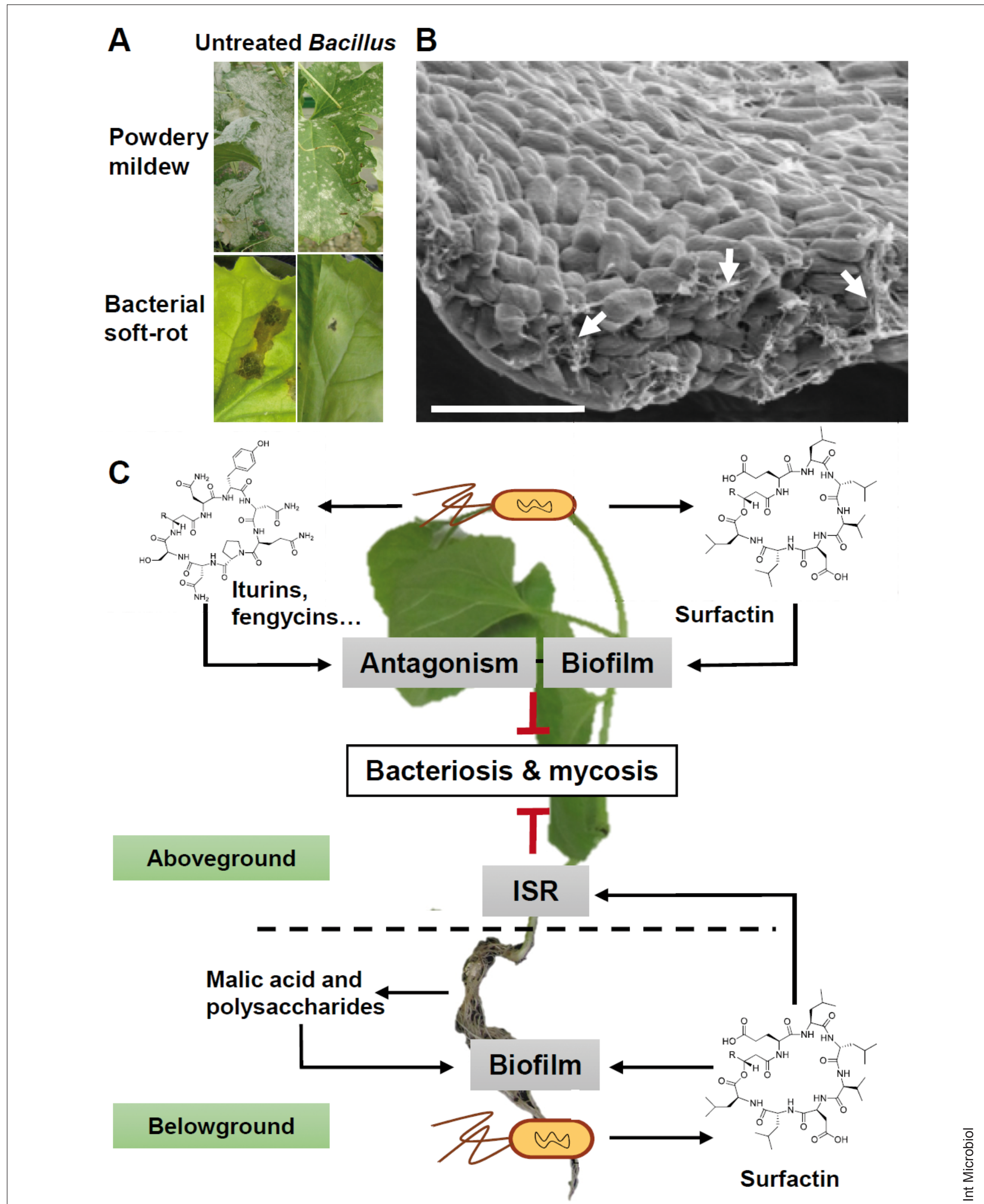

Fig. 4. Biofilms in beneficial *Bacillus*-plant interactions. Diverse *Bacillus* species contribute to plant health in a multifaceted way. (**A**) Cell suspensions of beneficial *Bacillus* strains spread on melon leaves protect the plant against the fungal disease powdery mildew caused by *Podosphaera fusca* and the bacterial soft-rot disease caused by *Pectobacterium carotovorum* subsp. *carotovorum*. (**B**) Scanning electron micrograph of a bacterial biofilm on melon leaves 21 days after the application of the *Bacillus* cell suspension. A section of the biofilm shows multiple layers of cells connected by fibrillar material (arrows). (**C**) The beneficial *Bacillus*-plant interaction is the result of a complex chemical communication network. Aboveground, antimicrobials (e.g., iturins, fengycin) and surfactin, a trigger of biofilm formation self-produced by *Bacillus* cells, contribute to the efficient targeting of pathogens, to protection from other possible competitors and environmental conditions and also to long-term persistence. Belowground, the plants produce diverse organic acids or polysaccharides that trigger the formation of the *Bacillus* biofilm. In parallel, *Bacillus* cells produce surfactin, which reinforces the development of biofilms and also induces systemic resistance of the plant (ISR), providing protection against pathogens in the aerial part of the plant. Bar equals 5 µm in B. Figure 4(B) courtesy of Maria Luisa Antequera.

signaling pathways [14,24,31].

In addition to plants, there are other organisms might affect the fitness of *Bacillus* cells. All the knowledge accumulated over the years on single-species biofilms has prompted the study of multispecies biofilms. Recent research demonstrates that different *Bacillus* species share the same niche, and some molecules of one player can trigger the expression of the biofilm developing program in another, which could benefit the entire community [6]. This interaction, however, does not occur between *Bacillus* and *Pseudomonas* or between *Bacillus* and *Streptomyces*, which prefer to exclude each other, or at least do not cooperate in the formation of a mixed biofilm [38,67]. These examples of interspecies communication validate the sophisticated developmental programs studied in our laboratories and demonstrate the variability of outcomes depending on the repertoire of chemical signals and receptors implicated [48,83].

Bacterial cells have built a sophisticated platform consisting of signals, receptors, and structural components that are finely interconnected to respond efficiently to variations in the environment. One of the most fascinating adaptive responses is the arrangement into perfectly organized communities called biofilms. The chemical communication among bacterial cells promotes a global and therefore more efficient response, and macrostructures made of exopolysaccharides and proteins, among others, constitute the infrastructure that organizes the space. In this way, cells obtain a number of benefits: they are better protected from external aggression and can efficiently manage nutrient limitations or modify the environment. The research on microbial biofilms persuasively argues that bacterial cells may be unicellular but are definitively not asocial.

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References

1. Aguilar C, Vlamakis H, Losick R, Kolter R (2007) Thinking about *Bacillus subtilis* as a multicellular organism. Curr Opin Microbiol 10:638-643
2. Badtke MP, Hammer ND, Chapman MR (2009) Functional amyloids signal their arrival. *Sci Signal* 2:pe43
3. Bate AR, Bonneau R, Eichenberger P (2014) *Bacillus subtilis* systems biology: applications of -omics techniques to the study of endospore formation. Microbiol Spectr 2
4. Beauregard PB, Chai Y, Vlamakis H, Losick R, Kolter R (2013) *Bacillus subtilis* biofilm induction by plant polysaccharides. Proc Natl Acad Sci USA 110:e1621-30
5. Blanco LP, Evans Ml, Smith DR, Badtke MP, Chapman MR (2012) Diversity, biogenesis and function of microbial amyloids. Trends Microbiol 20:66-73
6. Bleich R, Watrous JD, Dorrestein PC, Bowers AA, Shank EA (2015) Thiopeptide antibiotics stimulate biofilm formation in *Bacillus subtilis*. Proc Natl Acad Sci USA 112:3086-3091
7. Bokhove M, Claessen D, De Jong W, Dijkhuizen L, Boekema EJ, Oostergetel GT (2013) Chaplins of *Streptomyces coelicolor* self-assemble into two distinct functional amyloids. J Struct Biol 184:301-309
8. Branda SS, Chu F, Kearns DB, Losick R, Kolter R (2006) A major protein component of the *Bacillus subtilis* biofilm matrix. Mol Microbiol 59:1229-1238
9. Branda SS, Gonzalez-Pastor JE, Ben-Yehuda S, Losick R, Kolter R (2001) Fruiting body formation by *Bacillus subtilis*. Proc Natl Acad Sci USA 98:11621-11626
10. Branda SS, Gonzalez-Pastor JE, Dervyn E, Ehrlich SD, Losick R, Kolter R (2004) Genes involved in formation of structured multicellular communities by *Bacillus subtilis*. J Bacteriol 186:3970-3979
11. Bromley KM, Morris RJ, Hobley L, Brandani G, Gillespie Rm, Mccluskey M, Zachariae U, Marenduzzo D, Stanley-Wall NR, MacPhee CE (2015) Interfacial self-assembly of a bacterial hydrophobin. Proc Natl Acad Sci USA 112:5419-5424
12. Cairns LS, Hobley L, Stanley-Wall NR (2014) Biofilm formation by *Bacillus subtilis*: new insights into regulatory strategies and assembly mechanisms. Mol Microbiol 93:587-598
13. Caro-Astorga J, Perez-Garcia A, De Vicente A, Romero D (2014) A genomic region involved in the formation of adhesin fibers in *Bacillus cereus* biofilms. Front Microbiol 5:745
14. Cawoy H, Mariutto M, Henry G, Fisher C, Vasilyeva N, Thonart P, Dommes J, Ongena M (2014) Plant defense stimulation by natural isolates of *Bacillus* depends on efficient surfactin production. Mol Plant Microbe Interact 27:87-100
15. Chai L, Romero D, Kayatekin C, Akabayov B, Vlamakis H, Losick R, Kolter R (2013) Isolation, characterization, and aggregation of a structured bacterial matrix precursor. J Biol Chem 288:17559-17568
16. Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, Normark S, Hultgren SJ (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. Science 295:851-855
17. Checinska A, Paszczynski A, Burbank M (2015) *Bacillus* and other spore-forming genera: variations in responses and mechanisms for survival. Annu Rev Food Sci Technol 6:351-369
18. Chen Y, Cao S, Chai Y, Clardy J, Kolter R, Guo JH, Losick R (2012) A *Bacillus subtilis* sensor kinase involved in triggering biofilm formation on the roots of tomato plants. Mol Microbiol 85:418-430
19. Chew SC, Kundukad B, Seviour T, van Der Maarel Jr, Yang L, Rice SA, Doyle P, Kjelleberg S (2015) Erratum for "dynamic remodeling of microbial biofilms by functionally distinct exopolysaccharides". Mbio 6:e00688
20. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 75:333-366
21. Chua SL, Yam JK, Hao P, Adav SS, Salido MM, Liu Y, Givskov M, Sze SK, Tolker-Nielsen T, Yang L (2016) Selective labelling and eradication of antibiotic-tolerant bacterial populations in *Pseudomonas aeruginosa* biofilms. Nat Commun 7:10750
22. Clinton A, Carter T (2015) Chronic wound biofilms: pathogenesis and potential therapies. Lab Med 46:277-284

23. Costerton JW, Geesey GG, Cheng KJ (1978) How bacteria stick. Sci Am 238:86-95
24. Debois D, Jourdan E, Smargiasso N, Thonart P, De Pauw E, Ongena M (2014) Spatiotemporal monitoring of the antibiome secreted by *Bacillus* biofilms on plant roots using maldi mass spectrometry imaging. Anal Chem 86:4431-4438
25. Dickinson WH (1867) On the nature of the waxy, lardaceous, or amyloid deposit. Med Chir Trans 50:39-56.3
26. Dueholm MS, Petersen SV, Sonderkaer M, Larsen P, Christiansen G, Hein KL, Enghild JJ, Nielsen JL, Nielsen KL, Nielsen PH, Otzen DE (2010) Functional amyloid in *Pseudomonas*. Mol Microbiol 77:1009-1020
27. Eberhard A, Burlingame Al, Eberhard C, Kenyon GL, Nealson KH, Oppenheimer NJ (1981) Structural identification of autoinducer of *Photobacterium fischeri* luciferase. Biochemistry 20:2444-2449
28. Errington J (2003) Regulation of endospore formation in *Bacillus subtilis*. Nat Rev Microbiol 1:117-126
29. Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623-633
30. Fowler DM, Koulov AV, Balch WE, Kelly JW (2007) Functional amyloid--from bacteria to humans. Trends Biochem Sci 32:217-224
31. Garcia-Gutierrez L, Zeriouh H, Romero D, Cubero J, De Vicente A, Perez-Garcia A (2013) The antagonistic strain *Bacillus subtilis* UMAF6639 also confers protection to melon plants against cucurbit powdery mildew by activation of jasmonate- and salicylic acid-dependent defence responses. Microbial Biotechnology 6:264-274
32. Gebbink MF, Claessen D, Bouma B, Dijkhuizen L, Wosten HA (2005) Amyloids-a functional coat for microorganisms. Nat Rev Microbiol 3:333-341
33. Geesey GG, Richardson WT, Yeomans HG, Irvin RT, Costerton JW (1977) Microscopic examination of natural sessile bacterial populations from an alpine stream. Can J Microbiol 23:1733-1736
34. Gopal N, Hill C, Ross PR, Beresford TP, Fenelon MA, Cotter PD (2015) The prevalence and control of *Bacillus* and related spore-forming bacteria in the dairy industry. Front Microbiol 6:1418
35. Greenberg EP, Nealson KH, Johnson CH (2014) Woody Hastings: 65 years of fun. Proc Natl Acad Sci USA 111:14964-14965
36. Harvey EN (1914) On the chemical nature of the luminous material of the firefly. Science 40:33-34
37. Hobley L, Ostrowski A, Rao FV, Bromley KM, Porter M, Prescott AR, Macphee CE, van Aalten DM, Stanlet-Wall NR (2013) Bsla is a self-assembling bacterial hydrophobin that coats the *Bacillus subtilis* biofilm. Proc Natl Acad Sci USA 110:13600-13605
38. Hoefler BC, Gorzelnik KV, Yang JY, Hendricks N, Dorrestein PC, Straight PD (2012) Enzymatic resistance to the lipopeptide surfactin as identified through imaging mass spectrometry of bacterial competition. Proc Natl Acad Sci USA 109:13082-13087
39. Jakubovics NS (2015) Intermicrobial interactions as a driver for community composition and stratification of oral biofilms. J Mol Biol 427:3662-3675
40. Julien B, Kaiser AD, Garza A (2000) Spatial control of cell differentiation in *Myxococcus xanthus*. Proc Natl Acad Sci USA 97:9098-9103
41. Kaiser D (2004) Signaling in myxobacteria. Annu Rev Microbiol 58:75-98
42. Kobayashi K, Iwano M (2012) Bsla (Yuab) forms a hydrophobic layer on the surface of *Bacillus* subtilis biofilms. Mol Microbiol 85:51-66
43. Kroos L (2007) The *Bacillus* and *Myxococcus* developmental networks and their transcriptional regulators. Annu Rev Genet 41:13-39
44. Lister JL, Horswill AR (2014) *Staphylococcus aureus* biofilms: recent developments in biofilm dispersal. Front Cell Infect Microbiol 4:178
45. Lopez D, Fischbach MA, Chu F, Losick R, Kolter R (2009) Structurally diverse natural products that cause potassium leakage trigger multicellularity in *Bacillus subtilis*. Proc Natl Acad Sci USA 106:280-285
46. Lopez D, Vlamakis H, Kolter R. 2009. Generation of multiple cell types in *Bacillus subtilis*. FEMS Microbiol Rev 33:152-163
47. López D, Vlamakis H, Losick R, Kolter R (2009) Paracrine signaling in a bacterium. Genes Dev 23:1631-1638
48. Lyons NA, Kraigher B, Stefanic P, Mandic-Mulec I, Kolter R (2016) A combinatorial kin discrimination system in *Bacillus subtilis*. Curr Biol 26:733-742
49. Magno-Perez-Bryan MC, Martinez-Garcia PM, Hierrezuelo J, Rodriguez-Palenzuela P, Arrebola E, Ramos C, De Vicente A, Pérez-García A, Romero D (2015) Comparative genomics within the *Bacillus* genus reveal the singularities of two robust *Bacillus amyloliquefaciens* biocontrol strains. Mol Plant Microbe Interact 28:1102-1116
50. Maji SK, Perrin MH, Sawaya MR, Jessberger S, Vadodaria K, Rissman RA, Singru PS, Nilsson KP, Simon R, Schubert D, Eisenberg D, Rivier J, Sawchenko P, Vale W, Riek R (2009) Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. Science 325:328-332
51. Marcoleta A, Marin M, Mercado G, Valpuesta JM, Monasterio O, Lagos R (2013) Microcin e492 amyloid formation is retarded by posttranslational modification. J Bacteriol 195:3995-4004
52. Martinez-Gil M, Quesada JM, Ramos-Gonzalez MI, Soriano MI, de Cristobal RE, Espinosa-Urgel M (2013) Interplay between extracellular matrix components of *Pseudomonas putida* biofilms. Res Microbiol 164:382-389
53. Mcelroy WD, Ballentine R (1944) The mechanism of bioluminescence. Proc Natl Acad Sci USA 30:377-382
54. Mckenney PT, Driks A, Eichenberger P (2013) The *Bacillus subtilis* endospore: assembly and functions of the multilayered coat. Nat Rev Microbiol 11:33-44
55. Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634-663
56. Mielich-Suss B, Lopez D (2015) Molecular mechanisms involved in *Bacillus subtilis* biofilm formation. Environ Microbiol 17:555-565
57. Nealson KH, Platt T, Hastings JW (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. J Bacteriol 104:313-322
58. Ng WL, Bassler Bl (2009) Bacterial quorum-sensing network architectures. Annu Rev Genet 43:197-222
59. Nwodo UU, Green E, Okoh AI (2012) Bacterial exopolysaccharides: functionality and prospects. Int J Mol Sci 13:14002-14015
60. Oli MW, Otoo HN, Crowley PJ, Heim KP, Nascimento MM, Ramsook CB, Lipke PN, Brady LJ (2012) Functional amyloid formation by *Streptococcus mutans*. Microbiology 158:2903-2916
61. Olive AJ, Sassetti CM (2016) Metabolic crosstalk between host and pathogen: sensing, adapting and competing. Nat Rev Microbiol 14:221-234
62. Ostrowski A, Mehert A, Prescott A, Kiley TB, Stanley-Wall NR (2011) Yuab functions synergistically with the exopolysaccharide and TasA amyloid fibers to allow biofilm formation by *Bacillus subtilis*. J Bacteriol 193:4821-4831
63. Perez-Garcia A, Romero D, De Vicente A (2011) Plant protection and growth stimulation by microorganisms: biotechnological applications of *Bacilli* in agriculture. Curr Opin Biotechnol 22:187-193
64. Peterson Bw, He Y, Ren Y, Zerdoum A, Libera MR, Sharma PK, Van Winkelhoff Aj, Neut D, Stoodley P, van Der Mei HC, Busscher HJ (2015) Viscoelasticity of biofilms and their recalcitrance to mechanical and chemical challenges. FEMS Microbiol Rev 39:234-245
65. Pineda A, Zheng SJ, Van Loon JJ, Pieterse CM, Dicke M (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. Trends Plant Sci 15:507-514

66. Porter JR (1976) Antony van Leeuwenhoek: Tercentenary of his discovery of bacteria. Bacteriol Rev 40:260-269
67. Powers MJ, Sanabria-Valentin E, Bowers AA, Shank EA (2015) Inhibition of cell differentiation in *Bacillus subtilis* by *Pseudomonas protegens*. J Bacteriol 197:2129-2138
68. Romero D (2013) Bacterial determinants of the social behavior of *Bacillus subtilis*. Res Microbiol 164:788-798
69. Romero D, Aguilar C, Losick R, Kolter R (2010) Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. Proc Natl Acad Sci USA 107:2230-2234
70. Romero D, Kolter R (2014) Functional amyloids in bacteria. Int Microbiol 17:65-73
71. Romero D, Traxler MF, Lopez D, Kolter R (2011) Antibiotics as signal molecules. Chem Rev 111:5492-5505
72. Romero D, Vlamakis H, Losick R, Kolter R (2011) An accessory protein required for anchoring and assembly of amyloid fibres in *B. subtilis* biofilms. Mol Microbiol 80:1155-1168
73. Romero D, Vlamakis H, Losick R, Kolter R (2014) Functional analysis of the accessory protein tapa in *Bacillus subtilis* amyloid fiber assembly. J Bacteriol 196:1505-1513
74. Sarkar S, Vagenas D, Schembri MA, Totsika M (2016) Biofilm formation by multidrug resistant *Escherichia coli* ST131 is dependent on type 1 fimbriae and assay conditions. Pathog Dis 74(3)
75. Sawyer EB, Claessen D, Gras SL, Perrett S (2012) Exploiting amyloid: how and why bacteria use cross-beta fibrils. Biochem Soc Trans 40:728-734
76. Schuster M, Sexton DJ, Diggle SP, Greenberg EP (2013) Acyl-homoserine lactone quorum sensing: from evolution to application. Annu Rev Microbiol 67:43-63
77. Schwartz K, Syed AK, Stephenson RE, Rickard AH, Boles BR (2012) Functional amyloids composed of phenol soluble modulins stabilize *Staphylococcus aureus* biofilms. Plos Pathog 8:e1002744
78. Seminara A, Angelini TE, Wilking JN, Vlamakis H, Ebrahim S, Kolter R, Weitz DA, Brenner MP (2012) osmotic spreading of *Bacillus subtilis* biofilms driven by an extracellular matrix. Proc Natl Acad Sci USA 109:1116-1121
79. Sen R (2010) Surfactin: biosynthesis, genetics and potential applications. Adv Exp Med Biol 672:316-323
80. Shimomura O (2009) Discovery of green fluorescent protein (GFP) (Nobel Lecture). Angew Chem Int Ed Engl 48:5590-5602
81. Shirahama T, Cohen AS (1967) High-resolution electron microscopic analysis of the amyloid fibril. J Cell Biol 33:679-708
82. Sipe JD, Cohen AS (2000) Review: history of the amyloid fibril. J Struct Biol 130:88-98
83. Stefanic P, Kraigher B, Lyons NA, Kolter R, Mandic-Mulec I (2015) Kin discrimination between sympatric *Bacillus subtilis* isolates. Proc Natl Acad Sci USA 112:14042-14047
84. Stenfors Arnesen LP, Fagerlund A, Granum PE (2008) From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Rev 32:579-606
85. Stevens FJ (2004) Amyloid formation: an emulation of matrix protein assembly? Amyloid 11:232-244
86. Stragier P, Losick R (1996) Molecular genetics of sporulation in *Bacillus subtilis*. Annu Rev Genet 30:297-241
87. Taglialegna A, Navarro S, Ventura S, Garnett JA, Matthews S, Penades JR, Lasa I, Valle J (2016) Staphylococcal bap proteins build amyloid scaffold biofilm matrices in response to environmental signals. PLoS Pathogens 12:e1005711
88. Tan IS, Ramamurthi KS (2014) Spore formation in *Bacillus subtilis*. Environ Microbiol Rep 6:212-225
89. Traxler MF, Seyedsayamdost MR, Clardy J, Kolter R (2012) Interspecies modulation of bacterial development through iron competition and siderophore piracy. Mol Microbiol 86:628-644
90. Vannini C, Carpentieri A, Salvioli A, Novero M, Marsoni M, Testa L, De Pinto Mc, Amoresano A, Ortolani F, Bracale M, Bonfante P (2016) An interdomain network: the endobacterium of a mycorrhizal fungus promotes antioxidative responses in both fungal and plant hosts. New Phytol 211:265-275
91. Villar-Pique A, Espargaro A, Sabate R, De Groot NS, Ventura S (2012) Using bacterial inclusion bodies to screen for amyloid aggregation inhibitors. Microbial Cell Factories 11:55
92. Vlamakis H, Aguilar C, Losick R, Kolter R (2008) Control of cell fate by the formation of an architecturally complex bacterial community. Genes Dev 22:945-53
93. Vlamakis H, Chai Y, Beauregard P, Losick R, Kolter R (2013) Sticking together: building a biofilm the *Bacillus subtilis* way. Nat Rev Microbiol 11:157-68
94. Wilking JN, Zaburdaev V, de Volder M, Losick R, Brenner MP, Weitz DA (2013) Liquid transport facilitated by channels in *Bacillus subtilis* biofilms. Proc Natl Acad Sci USA 110:848-852
95. Winstanley C, O'Brien S, Brockhurst MA (2016) *Pseudomonas aeruginosa* evolutionary adaptation and diversification in cystic fibrosis chronic lung infections. Trends Microbiol 24:327-337
96. Zeriouh H, de Vicente A, Perez-Garcia A, Romero D (2014) Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. Environ Microbiol 16:2196-2211
97. Zobell CE (1943) The effect of solid surfaces upon bacterial activity. J Bacteriol 46:39-56
98. Zobell CE, Allen EC (1935) The significance of marine bacteria in the fouling of submerged surfaces. J Bacteriol 29:239-251

SEM Biennial Prize

The Spanish Society for Microbiology (SEM) Biennial Prize dates back to 1983, when the SEM decided that a lecture should be given by a young researcher at each SEM National Congress. The nominees are selected from among the SEM membership; they must be under 40 years of age, and carrying out research of excellence in a field of microbiology. The following researchers have been awarded the SEM Biennial Prize (the centers indicated are those where the scientists worked when they received the prize).

• First: **Juan Ortín**. Center for Molecular Biology (CBM), CSIC-Autonomous University of Madrid (10th SEM National Congress, Valencia, 1985)

• Second: **Enrique Herrero**. Department of Microbiology, University of Valencia (11th SEM National Congress, Gijón, 1987)

• Third: **Ernesto García López**. Biological Research Center (CIB), CSIC, Madrid (12th SEM National Congress, Pamplona, 1989)

• Fourth: **Antonio Ventosa**. Department of Microbiology, University of Sevilla (13th SEM National Congress, Salamanca, 1991)

• Fifth: **Alicia Estévez Toranzo**. Department of Microbiology, University of Santiago de Compostela (14th SEM National Congress, Zaragoza, 1993)

• Sixth: **Sergio Moreno**, Department of Microbiology, University of Salamanca (15th SEM National Congress, Madrid, 1995)

• Seventh: **Daniel Ramón Vidal**. Department of Biotechnology, Institute for Agrochemistry and Food Technology (IATA), CSIC, Valencia (16th SEM National Congress, Barcelona, 1997). Published in *Microbiología SEM* 1997; 13(4):405-412

• Eighth: **José Antonio Vázquez Boland**. Department of Animal Pathology, Complutense University of Madrid (17th SEM National Congress, Granada, 1999). Published in the special issue on Microbial Patogenesis, Int Microbiol 1999; 2(3):131-198

• Ninth: **Jesús L. Romalde**. Departament of Microbiology and Parasitology, University of Santiago de Compostela (18th SEM National Congress, Alicante, 2001). Published in Int Microbiol 2002; 5(1):3-9

• Tenth: **Eduardo Díaz**. Biological Research Center (CIB), CSIC, Madrid (19th SEM National Congress, Santiago de Compostela, 2003). Published in Int Microbiol 2004; 7(3):171-178

• Eleventh: **Iñigo Lasa**. Institute of Agrobiotechnology and Department of Agrarian Production, Public University of Navarra-CSIC, Pamplona (20th SEM National Congress, Cáceres, 2005). Pubished in Int Microbiol 2006; 9(1):21-28

• Twelveth: **Luis Á. Fernández Herrero**. National Center for Biotechnology, CSIC-Autonomous University of Madrid (21st SEM National Congress, Sevilla, 2007)

• Thirteenth: **Alejandro Mira**. Center for Advanced Research in Public Health (CSISP), Valencia (22nd SEM National Congress, Almería, 2009). Published in Int Microbiol 2010; 13(2):45-57

• Fourteenth: **Bruno González-Zorn**. Department of Aniumal Health, Faculty of Veterinary, Complutense University of Madrid (23rd SEM National Congress, Salamanca, 2011). Published in Int Microbiol 2012; 15(3):101-109

• Fifteenth: **David Rodríguez Lázaro**. Institute of Agricultural Technology of Castilla y León, Valladolid (24th SEM National Congress, L'Hospitalet-Barcelona, 2013).

• Sixteenth: **Diego Romero**. Institute of Subtropical and Mediterranean Horticulture "La Mayora", CSIC-University of Malaga. (25th SEM National Congress, Logroño, 2015). See this issue, pp. 81-90