PLOS GENETICS

RESEARCH ARTICLE

A natural single nucleotide mutation in the small regulatory RNA ArcZ of *Dickeya solani* switches off the antimicrobial activities against yeast and bacteria

Typhaine Brual[1☯], Géraldine Effantin[1☯], Julie Baltenneck[1], Laetitia Attaiech[2], Cloé Grosbois[1], Monique Royer[3], Jérémy Cigna[4], Denis Faure[5], Nicole Hugouvieux-Cotte-Pattat[1], Erwan Gueguen[1]*

1 Univ Lyon, Université Claude Bernard Lyon1, CNRS, INSA Lyon, UMR5240 MAP LYON, France, **2** CIRI, Centre International de Recherche en Infectiologie, Team "Horizontal gene transfer in bacterial pathogens" (Horigene), Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, Lyon, France, **3** CIRAD, UMR PHIM, Montpellier, France, **4** French Federation of Seed Potato Growers (FN3PT-inov3PT), Paris, France, **5** Paris-Saclay University, CNRS, CEA, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

☯ These authors contributed equally to this work.
* erwan.gueguen@univ-lyon1.fr

Citation: Brual T, Effantin G, Baltenneck J, Attaiech L, Grosbois C, Royer M, et al. (2023) A natural single nucleotide mutation in the small regulatory RNA ArcZ of *Dickeya solani* switches off the antimicrobial activities against yeast and bacteria. PLoS Genet 19(4): e1010725. https://doi.org/10.1371/journal.pgen.1010725

Editor: Pascale Romby, UPR 9002 CNRS-ARN, FRANCE

Received: November 25, 2022

Accepted: March 30, 2023

Published: April 27, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by grants from Agence Nationale de la Recherche to L.A. (Project RNAchap, ANR-17-CE11-0009-01) and to E.G. (Project Tn-Phyto, ANR-19-CE35-0016). E.G was also supported by the FR BioEEnVis, and by annual credits from the University Lyon I and the CNRS at regular basis. T.B. was supported by a PhD grant

Abstract

The necrotrophic plant pathogenic bacterium *Dickeya solani* emerged in the potato agrosystem in Europe. All isolated strains of *D. solani* contain several large polyketide synthase/non-ribosomal peptide synthetase (PKS/NRPS) gene clusters. Analogy with genes described in other bacteria suggests that the clusters *ooc* and *zms* are involved in the production of secondary metabolites of the oocydin and zeamine families, respectively. A third cluster named *sol* was recently shown to produce an antifungal molecule. In this study, we constructed mutants impaired in each of the three secondary metabolite clusters *sol*, *ooc*, and *zms* to compare first the phenotype of the *D. solani* wild-type strain D s0432-1 with its associated mutants. We demonstrated the antimicrobial functions of these three PKS/NRPS clusters against bacteria, yeasts or fungi. The cluster *sol*, conserved in several other *Dickeya* species, produces a secondary metabolite inhibiting yeasts. Phenotyping and comparative genomics of different *D. solani* wild-type isolates revealed that the small regulatory RNA ArcZ plays a major role in the control of the clusters *sol* and *zms*. A single-point mutation, conserved in some *Dickeya* wild-type strains, including the *D. solani* type strain IPO 2222, impairs the ArcZ function by affecting its processing into an active form.

Author summary

The development of new antibacterial molecules is critical in tackling the emergence of new pathogens or bacterial strains resistant to already available antibiotics. Bacterial phytopathogens can potentially synthesize novel compounds capable of targeting a specific type of microorganism. *Dickeya solani* is the only of the twelve described *Dickeya* species

from the Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

that has the three secondary metabolic pathways *sol*, *ooc* and *zms*. An investigation of the functions of these three clusters allowed us to identify the anti-yeast activity of the *sol* cluster, a potential new molecule of clinical importance. By comparing the antimicrobial activity of several *Dickeya solani* strains, we identified the small RNA regulator ArcZ as a critical regulator in the activation of the *sol* and *zms* clusters. Our study showed that single-nucleotide polymorphisms of sRNA encoding genes can have huge impacts on bacterial phenotypes. It is thus critical to pay attention to the allele diversity of sRNA genes.

Introduction

Bacterial phytopathogens of the genus *Dickeya* and *Pectobacterium* are pectinolytic necrotrophic bacteria with a broad host plant spectrum [1,2]. These members of the family *Pectobacteriaceae* [3] cause substantial agricultural losses worldwide by affecting many vegetables, ornamentals and crops, of which the potato is the most important economically. These bacteria are able to invade and degrade the plant tissues through the coordinated expression of genes encoding virulence factors, with a major role of pectate lyases that dissociate the plant cell wall constituents [4].

The species *D. solani* was officially established in 2014 [5] but *D. solani* isolates have attracted attention since their emergence on the potato agrosystem in Europe in the early 2000s. It causes symptoms in both subtropical and temperate climates. Many scientific efforts have been made to provide information on this phytopathogen, resulting in 76 *D. solani* genomes available in May 2021 [6]. Comparative genomics analysis was performed to identify the genetic basis for the different levels of virulence between *D. solani* strains [6–11]. Most *D. solani* strains isolated from different regions show a low level of genetic variation, suggesting a clonal origin [11]. The *D. solani* genomes share a high similarity and synteny with those of the model strain *D. dadantii* 3937, prompting a comparison between the two species. Only a few hundred genes were specific to each species, including a few dozen distinctive genomic regions [7,8]. Three of these regions encode polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS) and amino acid adenylation domain proteins, which are typically involved in the production of secondary metabolites [7].

PKSs and NRPSs are able to synthesize molecules by sequential condensation of acyl-CoAs and amino acids, respectively. PKS and NRPS modules can combine together to form hybrid PKS/NRPS systems capable of producing compounds of great structural diversity [12]. The molecules synthesized may have siderophore, antibiotic or phytotoxic properties that promote the virulence of a plant pathogen. Three PKS/NRPS clusters are present in all sequenced *D. solani* strains [13] and found in a few other *Dickeya* species and related genera: *ooc*, *zms*, and a cluster of unknown function at the beginning of our study, which was recently named *sol* [14]. In *Serratia plymuthica*, the cluster *ooc* is involved in the synthesis of oocydin A, a halogenated macrolide with antioomycete and antiascomycete activities [15,16]. The cluster *zms*, previously found in the genomes of *S. plymuthica* and *Dickeya oryzae*, leads to the biosynthesis of a polyamino-amide antibiotic, zeamine [17,18]. The cluster *sol* (for solanimycin) is found in a few *Dickeya* species. To elucidate the contribution of the *sol*, *ooc* and *zms* clusters of *D. solani* in competition with other living organisms, we constructed mutants of each of the three loci involved in the biosynthesis of secondary metabolites in the highly virulent *D. solani* strain D s0432-1. We showed that the *D. solani* D s0432-1 clusters *zms* and *ooc*, encoding zeamine and oocydin biosynthesis, are involved in growth inhibition of bacteria and fungi, respectively. Recently, we, and others identified the third cluster *sol* as implicated in *Dickeya solani*'s ability

to inhibit the growth of different yeast species. We had previously named this cluster *ssm* (for *solani* secondary metabolite) [19], and it was later renamed *sol* [14]. Finally, the comparative analysis of the genomes and phenotypes of several wild-type *D. solani* strains revealed the fundamental role of the small RNA ArcZ. This sRNA plays a major role in the regulation of the *sol* and *zms* clusters, which can be disrupted by a mutation in the *arcZ* sequence, conserved in some natural isolates.

Results

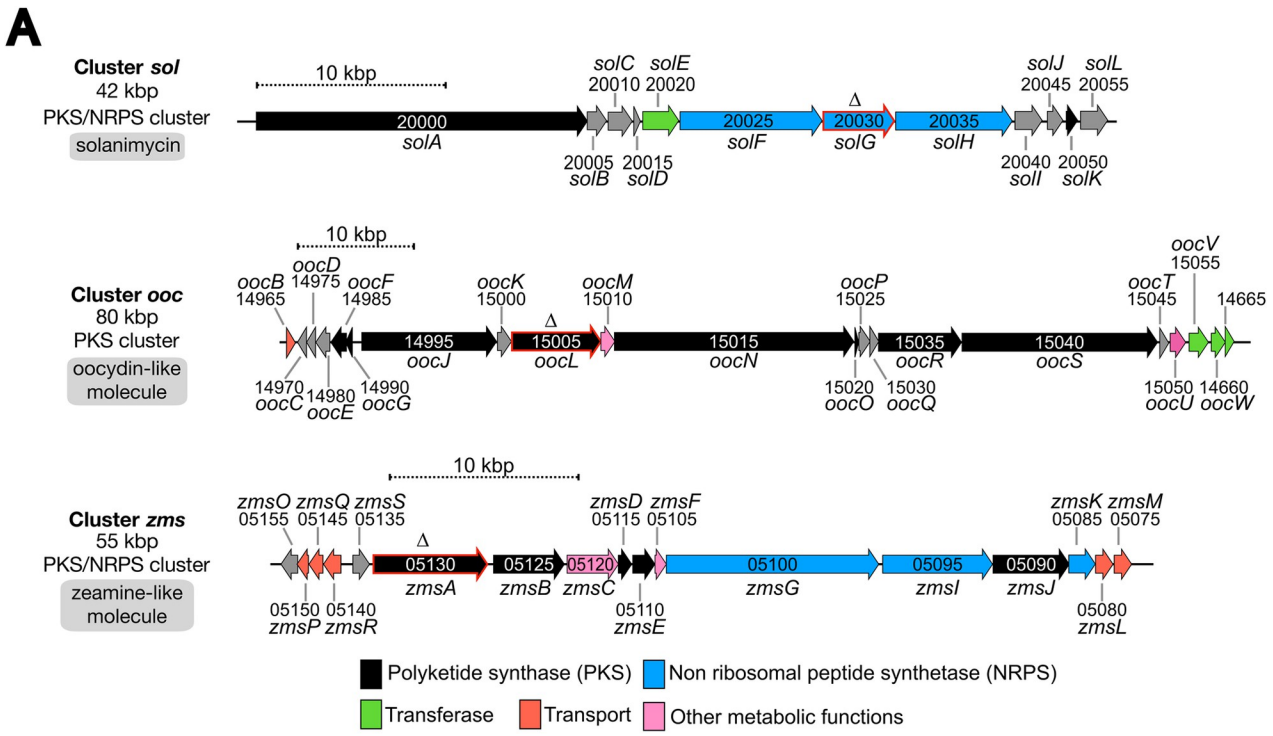
Description and distribution of the three selected PKS/NRPS secondary metabolite clusters of *D. solani*

The gene clusters encoding complex NRPS and PKS involved in the production of secondary metabolites were named *sol*, *ooc* and *zms*, respectively (Fig 1A). Their distribution in *Dickeya* species is summarized in Fig 1B (see also S1 Table for a complete list of the *Dickeya* strains with the indication of the presence or absence of each cluster).

The ∼42-kbp cluster *sol* contains the 12 genes *solABCDEFGHIJKL* (Fig 1A). It is widely conserved in the genus *Dickeya*, *i.e*., in all sequenced *D. solani*, *D. aquatica*, *D. fangzhongdai*, *D. poaceiphila* and *D. zeae* genomes, in some *D. dadantii* strains (such as NCPPB 898, NCPPB 3537, DSM18020, but not the model strain 3937), in some *D. undicola* strains (FVG1, FVG10), and in some *D. oryzae* strains (such as S20 and FVG03). The structure of the metabolite produced from this cluster has not been elucidated yet but the function of this cluster has been described by us in our 2021 preprint article [19] and others in 2022 [14]. The *sol* cluster produces solanimycin that targets fungi and yeast of the *Ascomycota* phylum.

The ∼80-kbp cluster *ooc* (Fig 1A) is highly similar to the *oocBCDEFGJKLMNOPQRSTUVW* cluster of *S. plymuthica* A153. It is present in all the sequenced genomes of *D. solani* and *D. dianthicola*, in some *D. oryzae* strains (such as ZYY5, EC1, DZ2Q and ZJU1202) and in a few other species. In *S. plymuthica* A153, disruption of this gene cluster abolished bioactivity against the ascomycete *Verticillium dahliae* and the oomycetes *Pythium ultimum*. This cluster produces oocydin A [15], a chlorinated macrolide, powerfully active against plant pathogenic oomycetes [20]. Since various *D. solani* strains inhibit *V. dahliae* and *P. ultimum* growth [16], it was suggested, on the basis of gene sequence homologies and similar cluster organization, that *D. solani* also produces oocydin A.

The 55-kbp cluster *zms* encodes mixed fatty acid synthase FAS/PKS and hybrid NRPS/PKS enzymes (Fig 1A). Its genomic organization is identical to the *D. oryzae* EC1 zeamine cluster and related to the *S. plymuthica* AS12 zeamine cluster [17,21]. While the *zmsABCDEFGIJKLMNPQRS* cluster directing zeamine biosynthesis is present only in a few *D. oryzae* strains, it is conserved in all the sequenced *D. solani* and *D. fangzhongdai* genomes, which suggest secondary acquisition by horizontal gene transfer in *D. oryzae* [13,21]. After reclassification of several *D. zeae* strains in the novel species *D. oryzae* [22], the *zms* cluster appeared to be absent in the genomes of true *D. zeae* strains. It is present in some, but not all, *D. oryzae* rice strains (ZYY5, EC1, DZ2Q and ZJU1202) and in only one *D. dadantii* strain (A622-S1-A17). The zeamine biosynthetic clusters from *D. oryzae* EC1 and *D. solani* Ds0432-1 share from 59 to 94% identity at individual protein level [21]. Zeamine-related antibiotics are polyaminoamide molecules toxic to a wide range of pro- and eukaryotic organisms such as bacteria, fungi, oomycetes, plants, and nematodes [23]. Mutation of the zeamine synthase gene *zmsA* in *D. oryzae* EC1 attenuates the inhibition of rice seed germination [17] and suppresses antibacterial activity against *E. coli* [17]. Zeamine produced by *S. plymuthica* kills nematodes and ascomycete yeast [24]. *D. solani* IPO 2222 can also kill the nematode *Caenorhabditis elegans* but not as quickly as *S. plymuthica* [24].


A
Cluster sol
42 kbp
PKS/NRPS cluster
solanimycin
10 kbp
20000 solA
solC 20010
solE 20020
20005 solB
20015 solD
20025 solF
Δ
20030 solG
20035 solH
solJ 20045
solL 20055
20040 solI
20050 solK
Cluster ooc
80 kbp
PKS cluster
oocydin-like molecule
10 kbp
oocB 14965
oocD 14975
oocF 14985
14970 oocC
14980 oocE
14990 oocG
oocK 15000
14995 oocJ
oocM 15010
Δ
15005 oocL
15015 oocN
oocP 15025
15020 oocO
15030 oocQ
15035 oocR
15040 oocS
oocT 15045
oocV 15055
14665
15050 oocU
14660 oocW
Cluster zms
55 kbp
PKS/NRPS cluster
zeamine-like molecule
10 kbp
zmsO 05155
zmsQ 05145
zmsS 05135
05150 zmsP
05140 zmsR
Δ
05130 zmsA
05125 zmsB
05120 zmsC
zmsD 05115
zmsF 05105
05110 zmsE
05100 zmsG
05095 zmsI
05090 zmsJ
zmsK 05085
zmsM 05075
05080 zmsL
Polyketide synthase (PKS)
Non ribosomal peptide synthetase (NRPS)
Transferase
Transport
Other metabolic functions

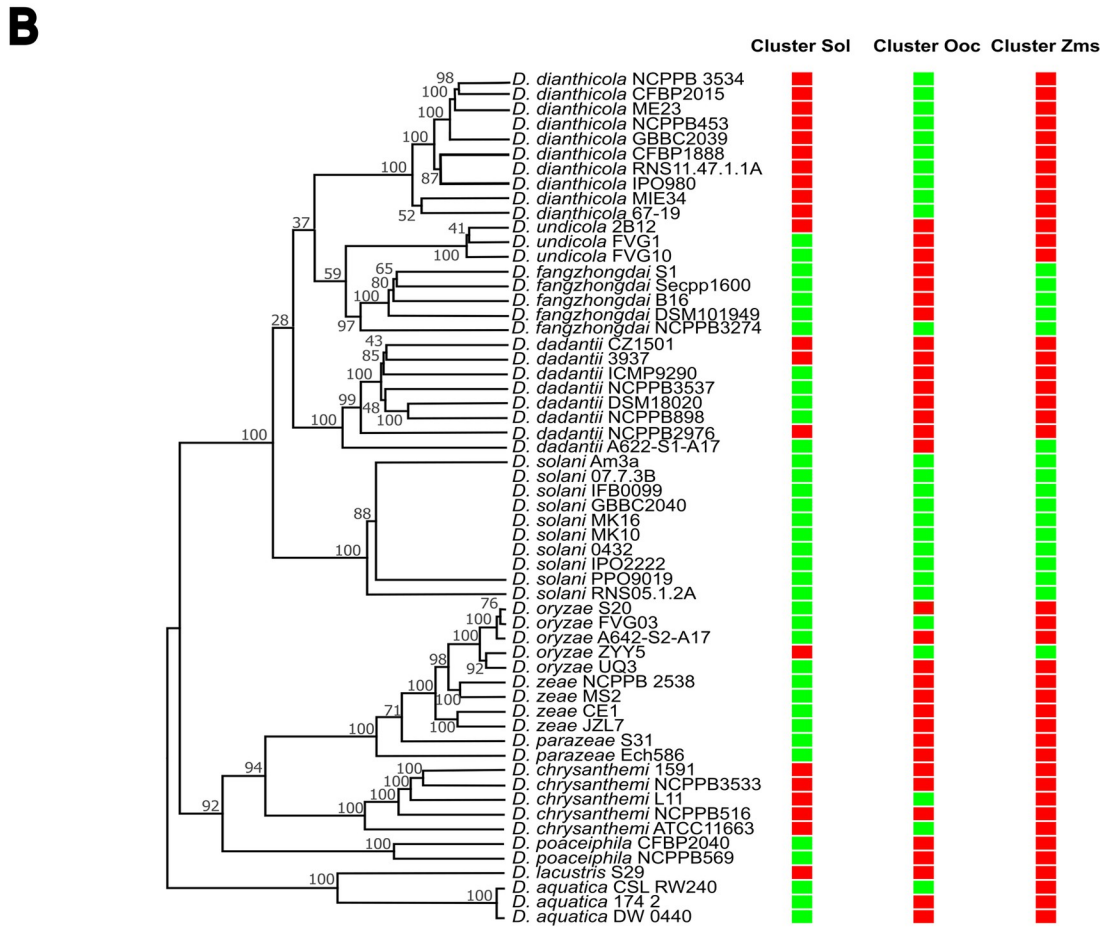

B
Cluster Sol
Cluster Ooc
Cluster Zms
D. dianthicola NCPPB 3534
D. dianthicola CFBP2015
D. dianthicola ME23
D. dianthicola NCPPB453
D. dianthicola GBBC2039
D. dianthicola CFBP1888
D. dianthicola RNS11.47.1.1A
D. dianthicola IPO980
D. dianthicola MIE34
D. dianthicola 67-19
D. undicola 2B12
D. undicola FVG1
D. undicola FVG10
D. fangzhongdai S1
D. fangzhongdai Secpp1600
D. fangzhongdai B16
D. fangzhongdai DSM101949
D. fangzhongdai NCPPB3274
D. dadantii CZ1501
D. dadantii 3937
D. dadantii ICMP9290
D. dadantii NCPPB3537
D. dadantii DSM18020
D. dadantii NCPPB898
D. dadantii NCPPB2976
D. dadantii A622-S1-A17
D. solani Am3a
D. solani 07.7.3B
D. solani IFB0099
D. solani GBBC2040
D. solani MK16
D. solani MK10
D. solani 0432
D. solani IPO2222
D. solani PPO9019
D. solani RNS05.1.2A
D. oryzae S20
D. oryzae FVG03
D. oryzae A642-S2-A17
D. oryzae ZYY5
D. oryzae UQ3
D. zeae NCPPB 2538
D. zeae MS2
D. zeae CE1
D. zeae JZL7
D. parazeae S31
D. parazeae Ech586
D. chrysanthemi 1591
D. chrysanthemi NCPPB3533
D. chrysanthemi L11
D. chrysanthemi NCPPB516
D. chrysanthemi ATCC11663
D. poaceiphila CFBP2040
D. poaceiphila NCPPB569
D. lacustris S29
D. aquatica CSL RW240
D. aquatica 174 2
D. aquatica DW 0440

Fig 1. Organization and distribution of the secondary metabolite clusters *sol*, *ooc*, and *zms* of *D. solani* D s0432-1. (A) Organization of the clusters *sol*, *ooc*, and *zms*. Genes are indicated using the NCBI nomenclature of the NCBI reference genome sequence NZ_CP017453.1 of *D. solani* D s0432-1. XXXXX is the digital number in BJD21_RSXXXXX, which corresponds to the locus tag. Arrowheads show gene orientations. Color code indicates gene function. The red-framed arrows indicate genes targeted for in-frame deletion performed in this study. (B) MLSA tree positioning strains within the *Dickeya* genus. The evolutionary history was inferred by using the Maximum Likelihood method. The presence (green frame) or absence (red frame) of the cluster *sol*, *ooc*, and *zms* are indicated in front of each strain. The distribution of these clusters in 155 *Dickeya* strains whose genome has been sequenced is given in S1 Table.

https://doi.org/10.1371/journal.pgen.1010725.g001

To interrupt the synthesis of the secondary molecules produced by these three clusters, we constructed from a Nal^R Gm^R derivative of WT *D. solani* D s0432-1 in-frame deletion mutants inactivating a PKS or NRPS gene of each cluster, which is predicted to interrupt metabolite synthesis. The inhibitory effects of the mutants Δ*solG*, Δ*oocL* and Δ*zmsA* against fungi, bacteria and yeasts were compared with that of the parental strain. To further analyze possible synergistic effects, double mutants Δ*solG*Δ*oocL*, Δ*solG*Δ*zmsA* and Δ*oocL*Δ*zmsA* as well as the triple mutant (hereafter called Δ3) were also constructed.

The *D. solani* oocydin cluster ooc inhibits growth of phytopathogenic ascomycete fungi, and the *sol* cluster is also involved in the inhibition of some fungi

We compared the ability of the WT *D. solani* strain D s0432-1 and its derived mutants to inhibit the growth of *Botrytis cinerea*, *Magnaporthe oryzae* and *Sclerotinia sclerotiorum*, three fungi eukaryotes of the phylum *Ascomycota*. The center of a potato dextrose agar (PDA) plate was inoculated with fungal mycelium, and 5 μl of overnight bacterial culture of each *D. solani* strain grown in M63 minimal medium with sucrose was deposited at the periphery of the plate (Fig 2). The different *D. solani* strains showed similar overall growth in liquid culture (S1 Fig). After incubation at 25˚C for several days, we observed a growth inhibition of the three fungi by the *D. solani* WT and the Δ*solG* and Δ*zmsA* mutants. In contrast, the Δ*oocL* mutant seemed less effective: *B. cinerea* and *M. oryzae* mycelium growth was completely unaffected, while a small zone of inhibition was observed for *S. sclerotiorum* (Fig 2). This suggests that the Δ*oocL* mutant may also produce another molecule that inhibits this fungus. The phenotypes were fully restored in the reverted strain Δ*oocL*/*oocL*$^+$. Double and triple mutants of the secondary metabolite clusters containing the *oocL* deletion showed no differences from the Δ*oocL* mutant with *B. cinerea* and *M. oryzae*. However, the Δ*oocL*Δ*solG* double mutant and the Δ3 triple mutant were completely unable to inhibit *S. sclerotiorum* growth (Fig 2). These data suggest that, in our assay on PDA plate, the *ooc* cluster is mainly responsible for the majority of ascomycete inhibition and that the *sol* cluster is secondary involved in the inhibition of some fungi. In contrast, the *zms* cluster does not seem to be involved in fungal inhibition.

The *D. solani* zeamine cluster inhibits bacterial growth

The zeamine cluster of *D. oryzae* EC1 is responsible for a bactericidal activity against *Escherichia coli* DH5α [17]. *D. oryzae* EC1 and *D. solani* zeamine biosynthetic genes share a high degree of similarity [21]. We thus evaluated the capacity of the *D. solani* strain D s0432-1 and its derived mutants to inhibit the growth of Gram-positive and Gram-negative bacteria (Fig 3). Only the Δ*zmsA* mutant was unable to inhibit *Bacillus subtilis* growth, indicating that WT *D. solani* produces an active zeamine antibiotic (Fig 3). The WT phenotype was restored in the reverted Δ*zmsA*/*zmsA*+ strain. We also tested the ability of *D. solani* to inhibit growth of the other Gram-positive bacteria *Streptomyces scabiei*, a plant pathogen causing the potato disease common scab [25]. An inhibition of *S. scabiei* growth was observed, except with the Δ*zmsA* mutant. No phenotype difference was observed between the single Δ*zmsA* mutant and the double and

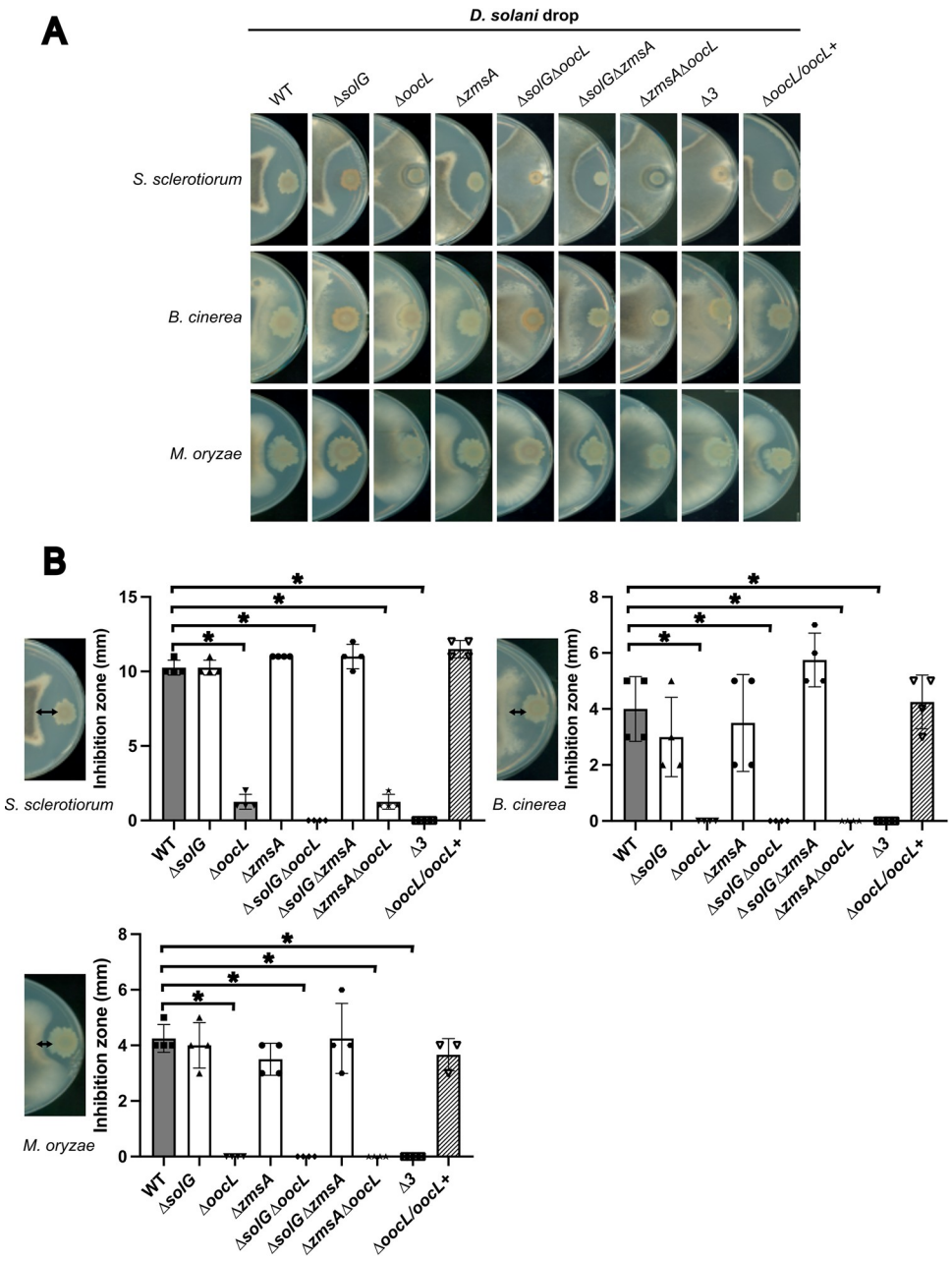


Fig 2. Inhibition of fungal growth by *D. solani* Ds0432-1 and mutant derivatives. 5 μL of bacterial culture at OD_{600nm} = 2 from *D. solani* Ds0432-1 (WT) or derivatives were spotted onto PDA plates inoculated with plugs of *S. sclerotiorum*, *B. cinerea or M. oryzae mycelium*. Plates were incubated at 25˚C until the mycelium covers the plate. Lengths of fungi inhibition zone were measured in 4 independent experiments. A statistical difference was significant only between the WT and Δ*oocL* mutants (Mann-Whitney test; * p-value < 0.05).

https://doi.org/10.1371/journal.pgen.1010725.g002

triple mutants containing the *zmsA* deletion, suggesting that, out of the three clusters, only *zms* is required for Gram-positive bacterial growth inhibition. We also tested the ability of *D. solani* to inhibit growth of the Gram-negative bacteria *E. coli*, *D. dadantii* and *Pectobacterium atrosepticum* (Fig 3). *E. coli* DH5α was, like *B. subtilis* and *S. scabiei*, inhibited by the WT strain through *zms* activity, albeit much less potently (Figs 3 and S2). No inhibition of the two other pectinolytic bacteria *D. dadantii* and *P. atrosepticum* growth was observed. In conclusion, out of the

three clusters analyzed, only the zeamine-like molecule potentially produced by the *zms* cluster has an antibacterial activity against some Gram-positive and Gram-negative bacteria.

The *D. solani* clusters *sol* and *zms* inhibits ascomycete yeast growth

Since zeamine produced by *S. plymuthica* A153 has previously been shown to be toxic to the ascomycete yeast *Saccharomyces cerevisiae* [24], we tested the capacity of *D. solani* D s0432-1 and its derivative mutant to inhibit the growth of the yeasts *S. cerevisiae*, *Kluyveromyces lactis*, a predominant eukaryote during cheese productions [26], and *Candida albicans*, an opportunistic human pathogen. In a growth inhibition assay performed in Yeast Peptone Dextrose (YPD) solid agar medium, we observed a strong inhibition of *K. lactis* by WT *D. solani*, a lower inhibition of *S. cerevisiae*, and a slight inhibition of *C. albicans* (Fig 4). The Δ*solG* mutant was almost completely deficient in its ability to inhibit yeast growth, while the Δ*solG*/*solG*$^{+}$ revertant strain showed a restored WT phenotype. The cluster *sol* is thus primarily responsible for the antieukaryotic action against these yeasts on YPD (Fig 4). Nevertheless, a weak halo of inhibition of the yeasts *K. lactis* and *S. cerevisiae* was observable around the mutant Δ*solG*. This halo disappeared around the double mutant Δ*solG*Δ*zmsA* and the triple mutant *Δ3*, suggesting that the *zms* cluster is also implicated in yeast inhibition, but at a lower level than that observed for the *sol* cluster. In conclusion, although the zeamine cluster of *D. solani* D s0432-1 is involved in the inhibition of yeast growth, as shown for *S. plymuthica* A153 [24], *D. solani* D s0432-1 also possesses the cluster *sol* that produces another antiyeast molecule.

The WT *D. solani* strains IPO 2222 and IFB0223 are unable to inhibit yeast and bacterial growth

We showed that *D. solani* D s0432-1 has the capacity to inhibit the growth of a wide range of microorganisms, including filamentous fungi, bacteria, and yeasts. We therefore tested

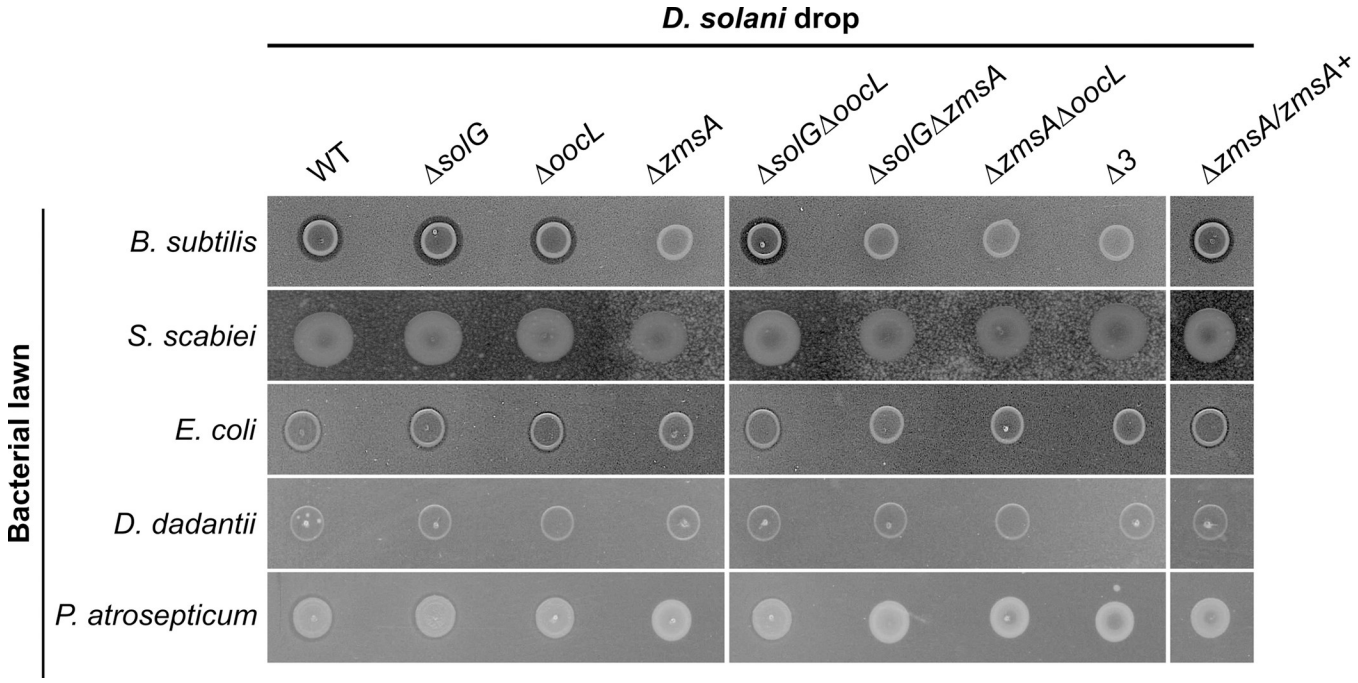


Fig 3. Inhibition of bacteria by *D. solani* Ds0432-1 and mutant derivatives. Bioassay plates were prepared by mixing bacterial culture (*B. subtilis*, *S. scabiei*, *E. coli*, *D. dadantii and P. atrosepticum*) with warm LB agar. 5 μL of bacterial cultures at OD_{600nm} = 2 from *D. solani* Ds0432-1 (WT) or derivatives were spotted onto the plate and incubation was performed at 30˚C during 48 h. A slight inhibition zone was observed with *B. subtilis* and *E. coli*, except with the *Δzms* mutants. All experiments were performed in 4 replicates.

https://doi.org/10.1371/journal.pgen.1010725.g003

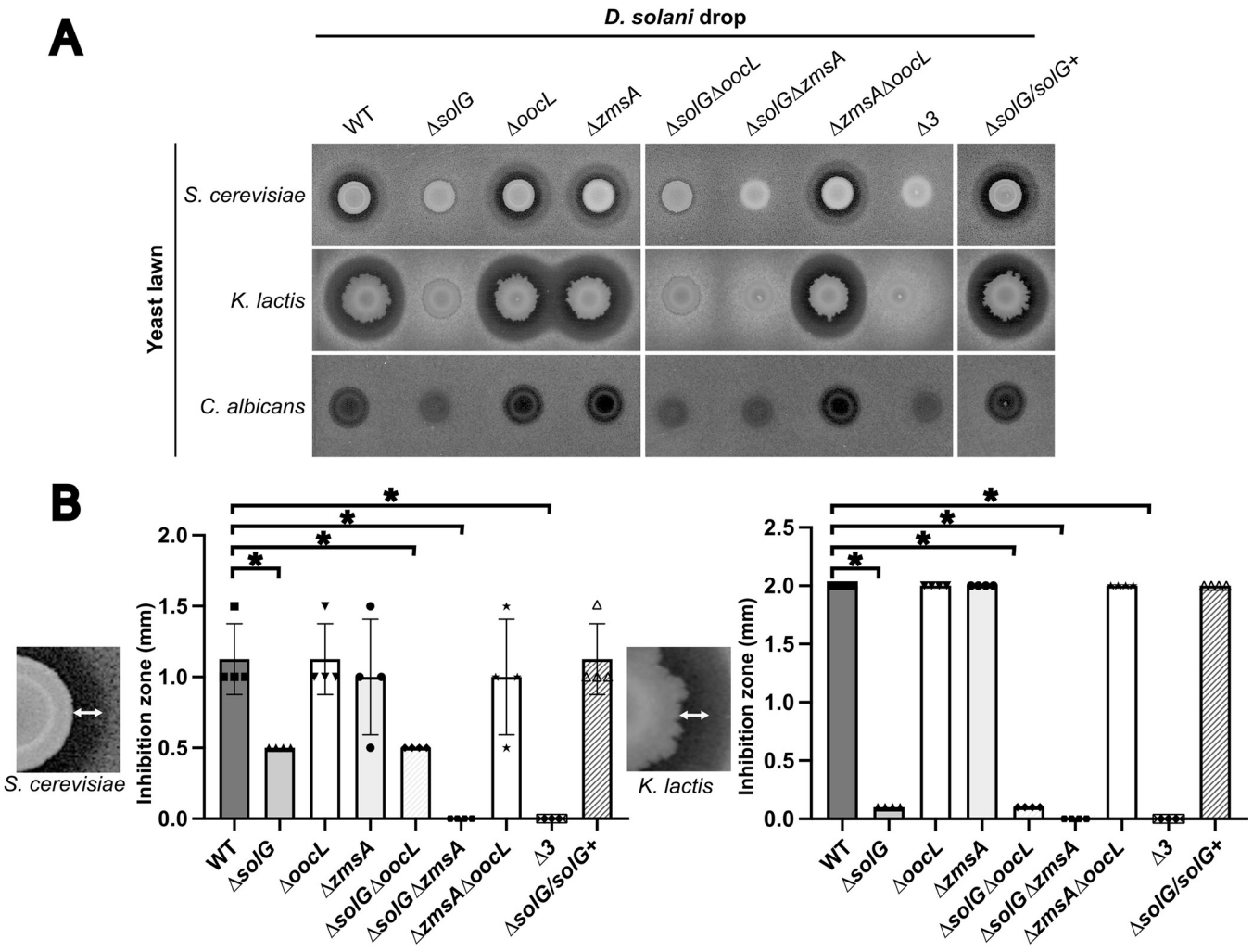


Fig 4. Inhibition of yeasts by *D. solani* Ds0432-1 and mutant derivatives. Bioassay plates were prepared by mixing yeast culture (*S. cerevisiae*, *K. lactis*, *C. albicans*) with warm YPD agar. (A) 5 μL of bacterial culture at OD_{600nm} = 2 from *D. solani* Ds0432-1 (WT) or derivatives were spotted onto the plates and incubation was performed at 30˚C during 48 h. (B) The radius of each inhibition zone was measured. A statistical difference was significant only between the WT and the *Δsol* mutants (Mann-Whitney test; * p-value < 0.05). For *C. albicans*, the inhibition zone was too small to be quantified. All experiments were performed in 4 replicates.

https://doi.org/10.1371/journal.pgen.1010725.g004

whether other WT *D. solani* strains isolated from the environment in Europe, either from lesions of potato tubers or plants, or from potato rhizosphere, possess this ability. We focused our work on *D. solani* strains available in national strain collections and whose genome sequence is available. These strains are D s0432-1, IFB0099, IFB0158, IFB0223, IFB0484, IPO 3337, IPO 3494, IPO 3793 and the *D. solani* type strain IPO 2222. We tested their capacity to inhibit the bacterium *B. subtilis*, the yeast *K. lactis* and the fungus *S. sclerotiorum* (Fig 5A). All strains were able to inhibit the growth of *S. sclerotiorum*. The inhibition of *B. subtilis* and *K. lactis* was clearly observed for all the strains except IPO 2222 and IFB0223. These strong phenotypic differences between IPO 2222 and IFB0223 versus the other WT *D. solani* strains could not be explained by any nucleotide variabilities in the *sol*, *zms*, and *ooc* clusters. All the selected strains carry the *sol*, *zms*, and *ooc* clusters, with 100% conservation at the nucleotide level (including in the promoter regions). Nonetheless, there are certain changes at the genome level, most notably some nucleotide variability with the presence of SNPs (single-nucleotide

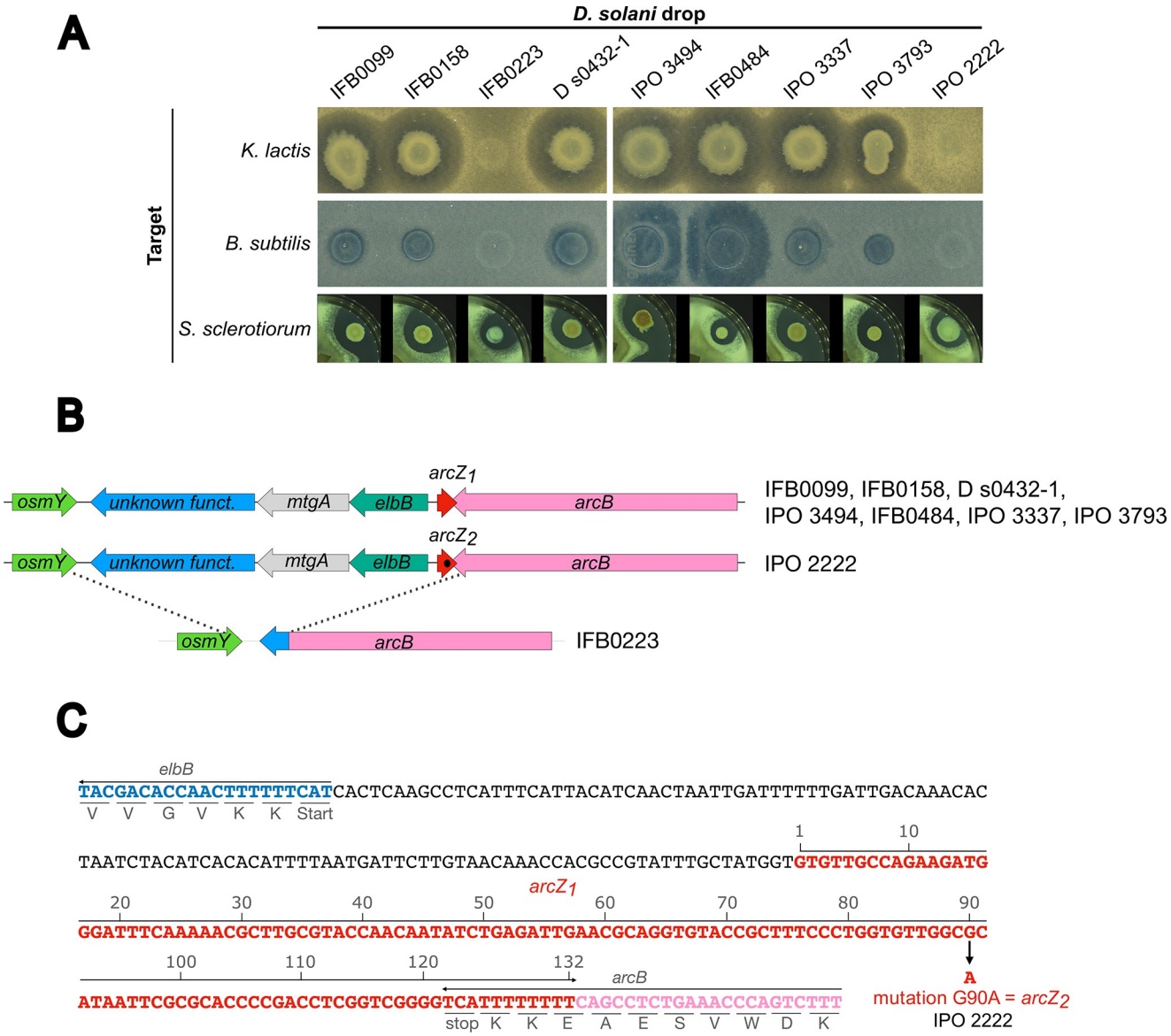


Fig 5. *D. solani* WT strains deficient for antimicrobial activity are mutated for the sRNA *arcZ*. (A) Inhibition of *B. subtilis*, *K. lactis* and S. *sclerotiorum* by diverse WT *D. solani* strains. The tests were performed as previously described using the nine strains IFB 0099 (NZ_CP024711), IFB 0158 (NZ_PENA00000000), IFB 0223 (NZ_CP024710), D s0432-1 (NZ_CP017453), IPO 3494 (NZ_CM001842), IFB 0484 (NZ_CM001860), IPO 3337 (NZ_CP016928), IPO 3793 (NZ_CP017454) or the type strain IPO 2222 (NZ_CP015137). Only IFB 0223 and IPO 2222 were unable to inhibit *B. subtilis* and *K. lactis*. (B) Organization and conservation of the *arcZ* locus in the nine *D. solani* strains. The single mutation G90A in IPO 2222 *arcZ* is symbolized by a dark spot in the red *arcZ* arrow. (C) Zoom on the nucleotide sequence around *arcZ* of *D. solani* D s0432-1. This allele is named $arcZ_1$. Positions and orientations of the genes *elbB*, *arcZ* and *arcB* are shown. The location of the G90A mutation present in IPO 2222 is indicated. This allele is named $arcZ_2$.

https://doi.org/10.1371/journal.pgen.1010725.g005

polymorphism) and InDel (insertion or deletion), outside the clusters *sol*, *zms*, and *ooc*. Hence, we searched for variations in the whole genomes by comparing two groups: strains IFB0223 and IPO 2222 that are defective for the growth inhibition of yeast and bacteria, versus strains D s0432-1, IPO 3337 and IFB0099 that are efficient for these inhibitions (S2 Table).

A SNP was observed with an identical nucleotide in the strains D s0432-1, IPO3337 and IFB0099 (a G at position 90 of *arcZ*) but different nucleotide in the strain IPO2222 (a A at position 90 of *arcZ*) (position 2530087 according to IPO 2222 genome). This variation is positioned in the 3' region of the small RNA *arcZ*, known to be a key area interacting with targeted

mRNA for post-translational regulation in other bacterial species (Fig 5C). The ArcZ variants of D s0432-1 and IPO 2222 will be referred to as $ArcZ_1$ and $ArcZ_2$ respectively in the remainder of this article. In the strain IFB0223, we observed a 3-kbp chromosomal deletion covering the gene *arcZ*, and extending between the genes *arcB* and *osmY* (Fig 5B). In other *D. solani* strains, this region includes *arcZ*, *elbB*, *mtgA*, and a gene encoding an alginate lyase domain (pfam05426). These discoveries led us to hypothesize that the inability of strains IFB0223 and IPO 2222 to inhibit yeast and bacterial growth could be due to an ArcZ deficiency.

Before going further, we ordered the *D. solani* IPO 2222 type strain stored in the BCCM collection (LMG 25993) to analyze its genotype and phenotype. Using PCR amplification and Sanger sequencing of *arcZ*, we confirmed that the *arcZ* allele of LMG 25993 is $arcZ_2$, similarly to the IPO 2222 strain used in our experiments. Our laboratory strain IPO 2222 and LMG 25993 have the same inhibition phenotype, i.e., an inability to inhibit yeast and bacterial growth (S3 Fig).

The $arcZ_1$ allele is required for *zms* and *sol* antimicrobial activities

sRNAs are posttranscriptional regulators that most commonly influence gene translation by base-pairing to target mRNAs. In *E. coli*, this regulation often requires the RNA chaperones Hfq or ProQ [27–29]. ArcZ belongs to what is called the "core sRNAs" because it is highly conserved among enterobacterial species [30]. In order to determine if ArcZ is required for secondary metabolite production, the Δ*arcZ* mutants of *D. solani* strains D s0432-1 and IPO 2222 were constructed, and their phenotypes compared to those of the WT strains (Fig 6A). In M63 sucrose, the mutants grew as well as the parental WT strains (S4 Fig). In our inhibition assay against *B. subtilis* and *K. lactis*, no inhibition zones were observed around the $\Delta arcZ_1$ mutant of D s0432-1 (Fig 6A), demonstrating that $ArcZ_1$ is required for the synthesis of the metabolites produced from the *sol* and *zms* clusters in D s0432-1. However, the $\Delta arcZ_1$ mutant of *D. solani* D s0432-1 was still able to inhibit the growth of the fungus *S. sclerotiorum*, although a slight decrease in the antifungal effect was observed. Thus, ArcZ does not appear to be fully required for *ooc* cluster expression, which is consistent with the results obtained with *D. solani* IFB0223, the naturally *arcZ* deletion mutant capable of inhibiting *S. sclerotiorum* growth (Figs 5A and 6A). *D. solani* IPO 2222 WT inhibited neither bacteria nor yeast but inhibited the growth of fungi, and the phenotype of the IPO 2222 $\Delta arcZ_2$ mutant was similar to that of the IPO 2222 WT or the D s0432-1 $\Delta arcZ_1$ mutant. This suggests that $ArcZ_2$ might be inactive.

The *sol* and *zms* clusters' expression is promoted by the $arcZ_1$ allele, but not $arcZ_2$

To further demonstrate that $ArcZ_1$ controls the *sol* and *zms* clusters, we constructed transcriptional fusions between the promoter region of the *sol*, *zms*, and *ooc* clusters, and the *luxCDABE* operon. We introduced *sol*::*luxCDABE*, *zms*::*luxCDABE and ooc*::*luxCDABE* into WT D s0431-1, WT IPO 2222, WT IFB0223, and the mutants $\Delta arcZ_1$ D s0432-1 and $\Delta arcZ_2$ IPO 2222. Luminescence of the strains were measured (Fig 6B). We detected a strong transcriptional activity of the *sol* cluster in WT D s0432-1, while its activity was 229-fold lower in the $\Delta arcZ_1$ background. WT IPO 2222, its $\Delta arcZ_2$ mutant, and IFB0223 all exhibited low *sol* transcriptional activity similar to the $\Delta arcZ_1$ D s0432-1 mutant. Regarding the transcriptional activity of the cluster *zms*, WT D s0432-1 had a significant ∼3-fold stronger transcriptional activity than the $\Delta arcZ_1$ D s0432-1 mutant, WT IPO 2222, and its $\Delta arcZ_2$ mutant. Surprisingly, the $\Delta arcZ_1$ D s0432-1 mutant, WT IPO 2222, and its $\Delta arcZ_2$ mutant had a stronger *zms* transcriptional activity compared to IFB0223, suggesting the existence of other genomic differences between IPO 2222 and IFB0223 that could modulate *zms* transcriptional activity.

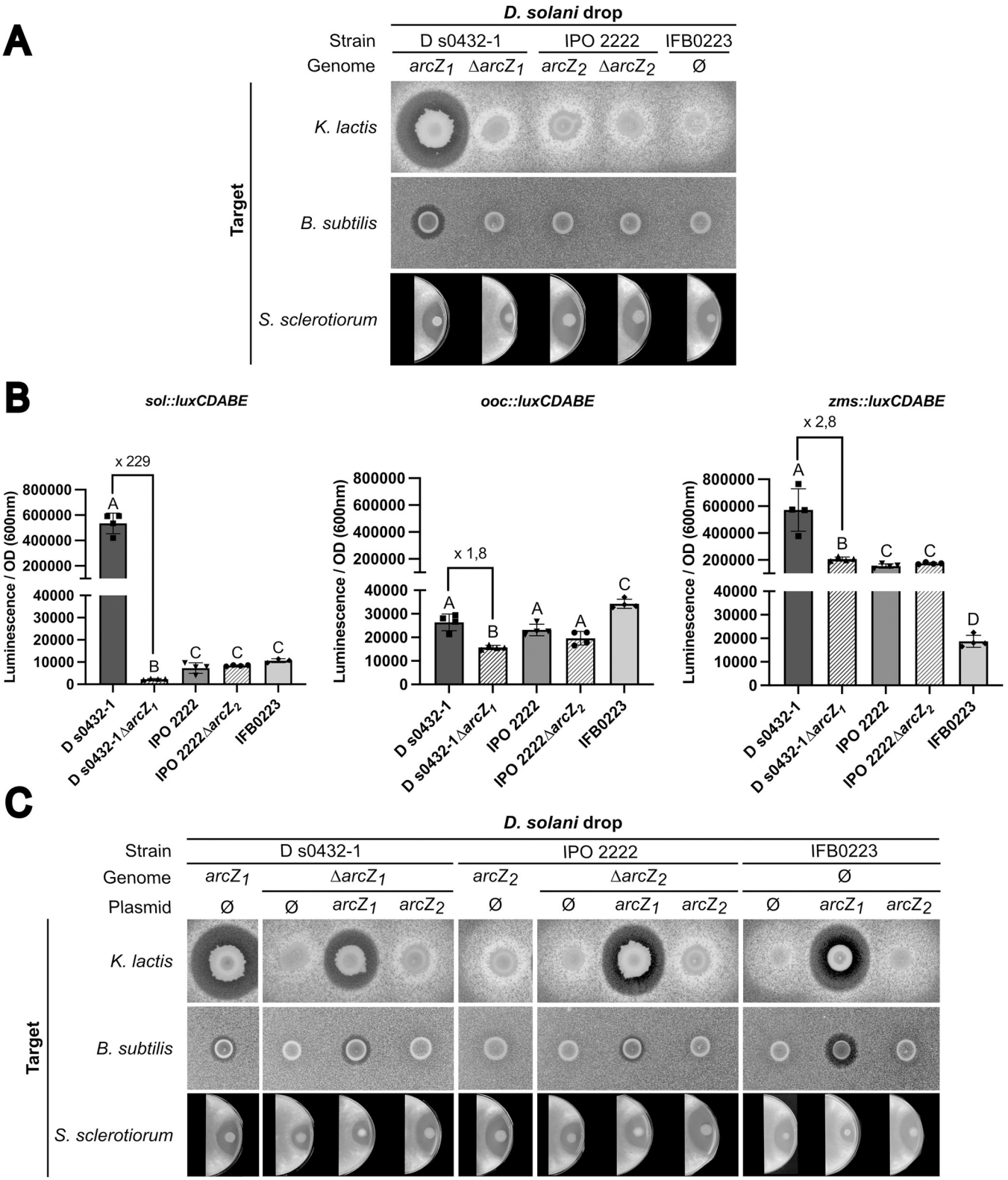


Fig 6. Inhibition of *K. lactis* and *B. subtilis* by *D. solani* is regulated by ArcZ. (A) Yeast bioassay plates were prepared by mixing culture of *K. lactis* with warm YPD agar. Bacteria bioassay plates were prepared by mixing culture of *B. subtilis* with warm LB agar. PDA plates were inoculated with plugs of *S. sclerotiorum*. For each experiment, 5 μL of bacterial culture at OD_{600nm} = 2 from the different *D. solani* strains were spotted onto the plate. Inhibition zones observed with D s0432-1, IPO 2222, their respective *ΔarcZ* mutants and IFB0223. ArcZ1 is required for the synthesis of metabolites produced by the clusters *sol*

and *zms* clusters (inhibition of *K. lactis* and *B. subtilis*, respectively) but not for the metabolite produced by the cluster *ooc* (inhibition of *S. sclerotiorum*). (B) Expression of *sol*::*luxCDABE*, *ooc*::*luxCDABE* and *zms*::*luxCDABE* fusions in the different *D. solani* strains. Cultures of the different strains with the plasmid pSEVA421 carrying a transcriptional fusion of each promoter of the 3 clusters into a 96-well plate. The luminescence values shown here were measured when all cultures reached an OD_{600nm} of 0.3. A, B, C, D represent groups with significantly different means (MannWhitney test, p-value < 0.05). The expression of the 3 clusters is higher in D s0432-1, especially for the *sol* and *zms* clusters. (C) Complementation assays and heterologous expression of the alleles $arcZ_1$ or $arcZ_2$ in the three *D. solani* strains. Yeast bioassay plates were prepared as in (A). ArcZ1 activates the anti-microbial activities of *sol* and *zms* clusters in IPO 2222 and IFB0223. All experiments were performed in 4 replicates.

https://doi.org/10.1371/journal.pgen.1010725.g006

Finally, a slight 1.8-fold decrease of *ooc* transcriptional activity was measured between WT D s0432-1 and its Δ$arcZ_1$ D s0432-1 mutant. These findings indicate that ArcZ has a modest effect on *ooc* transcriptional activity while promoting the expression of *zms* and *sol* clusters.

We next looked for predictions of $ArcZ_1$ interactions with mRNA targets in the -200/+100 region around the start codon (+1) of each *D. solani* D s0432-1 gene using the CopraRNA algorithm [31] (S3 Table). No putative interactions were predicted around the start codons of the first genes of each cluster (*solA*, *zmsA* and *oocJ)*. This result, together with transcriptional fusion analyses, suggests that ArcZ may not interact directly with the 5' ends of the *sol* and *zms* mRNAs but rather indirectly by regulating the expression of a transcriptional factor that regulates these clusters at the transcriptional level.

The G90A mutation in $arcZ_2$ of *D. solani* IPO 2222 causes a loss of function of *zms* and *sol* clusters

To verify the effect of the G90A mutation in *arcZ*, we performed a heterologous complementation experiment by transferring the allele $arcZ_1$ from D s0432-1 or $arcZ_2$ from IPO 2222 in *D. solani* WT strains D s0432-1 or IPO 2222 or their Δ*arcZ* derivatives. The alleles $arcZ_1$ and $arcZ_2$ were also transferred into *D. solani* IFB0223 naturally deleted for *arcZ*.

The two allelic forms $arcZ_1$ and $arcZ_2$ were cloned with their own promoter in the plasmid pWSK29-oriT, a mobilizable low copy plasmid with pSC101 origin and ampicillin resistance gene. DNA sequencing confirmed that the only difference between the two plasmids is the G90A mutation in pWSK29-oriT-$arcZ_2$. The recombinant plasmids and the empty vector were transferred by conjugation into WT or Δ*arcZ D. solani* D s0432-1 or IPO 2222 strains, as well as WT *D. solani* IFB0223 (naturally deleted for *arcZ*). The phenotypes of the conjugants were determined by testing their antimicrobial activity against *B. subtilis*, *K. lactis* and *S. sclerotiorum* (Fig 6C).

The transfer of $arcZ_1$ in Δ$arcZ_1$ Ds0432-1 restored the WT phenotypes, proving that the allele $arcZ_1$ activates the antimicrobial activities of the *sol* and *zms* clusters, while the antifungal activity was unaffected. In contrast, the transfer of $arcZ_2$ in the Δ$arcZ_1$ DS0432-1 strain did not restore the WT phenotypes. Thus, the difference in complementation between $arcZ_1$ and $arcZ_2$ is due to the presence of the G90A mutation that impairs $ArcZ_2$ function.

An identical result was obtained with the Δ$arcZ_2$ mutant of IPO 2222 or the IFB0223 strain carrying the pWSK29-oriT, pWSK29-oriT-$arcZ_1$ and pWSK29-oriT-$arcZ_2$. Indeed, the transfer of $arcZ_1$ in strains WT IPO 2222, Δ$arcZ_2$ IPO 2222 or IFB0223 activates the antimicrobial activities of the *sol* and *zms* clusters. In contrast, the transfer of $arcZ_2$ in these strains did not. These data demonstrate that the presence of *arcZ* and the nature of the *arcZ* allele are the main factors governing the difference in phenotypes for antimicrobial activities between the *D. solani* strains D s0432-1 and IPO 2222 or IFB0223.

Finally, we questioned if an *arcZ* mutation could be found in additional *D. solani* natural isolates. We extended this genomic analysis to 57 *D. solani* genomes, including IPO 2222. It revealed that only four strains have an *arcZ* single-nucleotide mutation, all at different positions in the 3' region of *arcZ*. It is not known whether these other mutations affect the

antimicrobial activity of these strains. The *arcZ* DNA sequence of *D*. *solani* IPO 2222 was also used as a query in a BlastN search against the NCBI nucleotide database. Three *D*. *fangzhongdai strains* DSM 101947 (CP025003.1), QZH3 (CP031507.1), and LN1 (CP031505.1) had the G90A mutation in *arcZ*. A G90T mutation was also discovered in *D*. *parazeae* Ech586 (CP001836.1) (S5 Fig). Therefore, the G90 mutation to A or T of *arcZ* can be found in other *Dickeya* species.

The G90A mutation in $arcZ_2$ of *D. solani* IPO 2222 alters $ArcZ_2$ processing

To gain further insight into the consequence of the G90A mutation in *arcZ*, Northern-blot experiments were performed to detect the sRNAs $ArcZ_1$ in WT D s0432-1 and $ArcZ_2$ in WT IPO 2222 (Fig 7). A probe specific to the 3' part of $ArcZ_1$ and $ArcZ_2$ was used (annealing from nucleotide 91 to 132). An abundant smaller transcript (~60nt) representing the processed 3′ fragment of $ArcZ_1$ was detected in WT D s0432-1 but not in the mutant D s0432-1 Δ$arcZ_1$ (Fig 7, compared lanes 1 and 2). The full-length form corresponding to the $ArcZ_1$ precursor was barely detected. This confirms that the processed form of $ArcZ_1$ is predominant, like observed in *E*. *coli*, *Salmonella*, and *Photorhabdus* [30,32,33]. In IPO 2222, only the full-length $ArcZ_2$ was detected in the WT strain whereas no $ArcZ_2$ transcript was detected in the IPO 2222 Δ$arcZ_2$ mutant (Fig 7, compared lanes 5 and 6). This result suggests that the G90A mutation present in the $arcZ_2$ allele of IPO 2222 prevents $ArcZ_2$ processing in a shorter functional transcript.

Next, we realized Northern-blot with the *D*. *solani ΔarcZ* strains expressing either the $arcZ_1$ or $arcZ_2$ allele from the plasmid pWSK29-oriT-*arcZ*. On one hand, Northern blot showed that D s0432-1Δ$arcZ_1$, IPO 2222 Δ$arcZ_2$ and IFB0223 (natural *ΔarcZ*) expressing $arcZ_1$ from D s0432-1 accumulate the processed short transcript of $ArcZ_1$ (Fig 7, lanes 3, 7 and 10) and have the ability to inhibit yeast and bacteria (Fig 6). On the other hand, D s0432-1Δ$arcZ_1$, IPO 2222 Δ$arcZ_2$ and IFB0223 expressing $arcZ_2$ from IPO 2222 showed the accumulation of the non-processed transcript of $ArcZ_2$ (Fig 7, lanes 3, 7 and 10) and an inability to inhibit yeast and bacteria (Fig 6). These results support the hypothesis that the G90A mutation in ArcZ alters the processing of the ArcZ precursor in a shorter form, leading to its inability to play a correct regulatory function on the *sol* and *zms* clusters.

Discussion

PKS/NRPS secondary metabolite pathways are a great source of molecules with antieukaryotic or antibacterial activity, giving the bacteria that synthesize them a competitive advantage over other organisms. Targeted mutagenesis of the *D*. *solani* D s0432-1 chromosome has allowed us to specifically study the involvement of three secondary metabolite biosynthesis clusters encoded by all the *D*. *solani* strains for which genome sequence is available. We focused our work on strain D s0432-1, one of the most virulent *D*. *solani* strains [9]. We have demonstrated that *D*. *solani* D s0432-1 is able to inhibit the growth of a variety of living microorganisms. We tested bio-activities against Gram-negative bacteria (*E*. *coli*, *P*. *atrosepticum*, *D*. *dadantii*), Gram-positive bacteria (*B*. *subtilis*, *S*. *scabiei*), ascomycete yeasts (*S*. *cerevisiae*, *K*. *lactis*, *C*. *albicans*), and ascomycete fungi (*B*. *cinerea*, *M*. *oryzae* and *S*. *sclerotiorum*). The cluster *ooc* encodes a biosynthesis pathway that produces an oocydin-like molecule, a chlorinated macrocyclic lactone molecule having antifungal, antioomycete and antitumor activities in *S*. *plymuthica* [15,16]. It was previously shown that the *D*. *solani* strains MK10, MK16 and IPO 2222, all encoding the oocydin cluster, inhibit the growth of the ascomycete fungus *V*. *dahliae* and the oomycete *P*. *ultimum* [16]. Our study showed that *D*. *solani* D s0432-1 prevents the growth of the ascomycete fungi *B*. *cinerea*, *M*. *oryzae* and *S*. *sclerotiorum*, and that inactivation

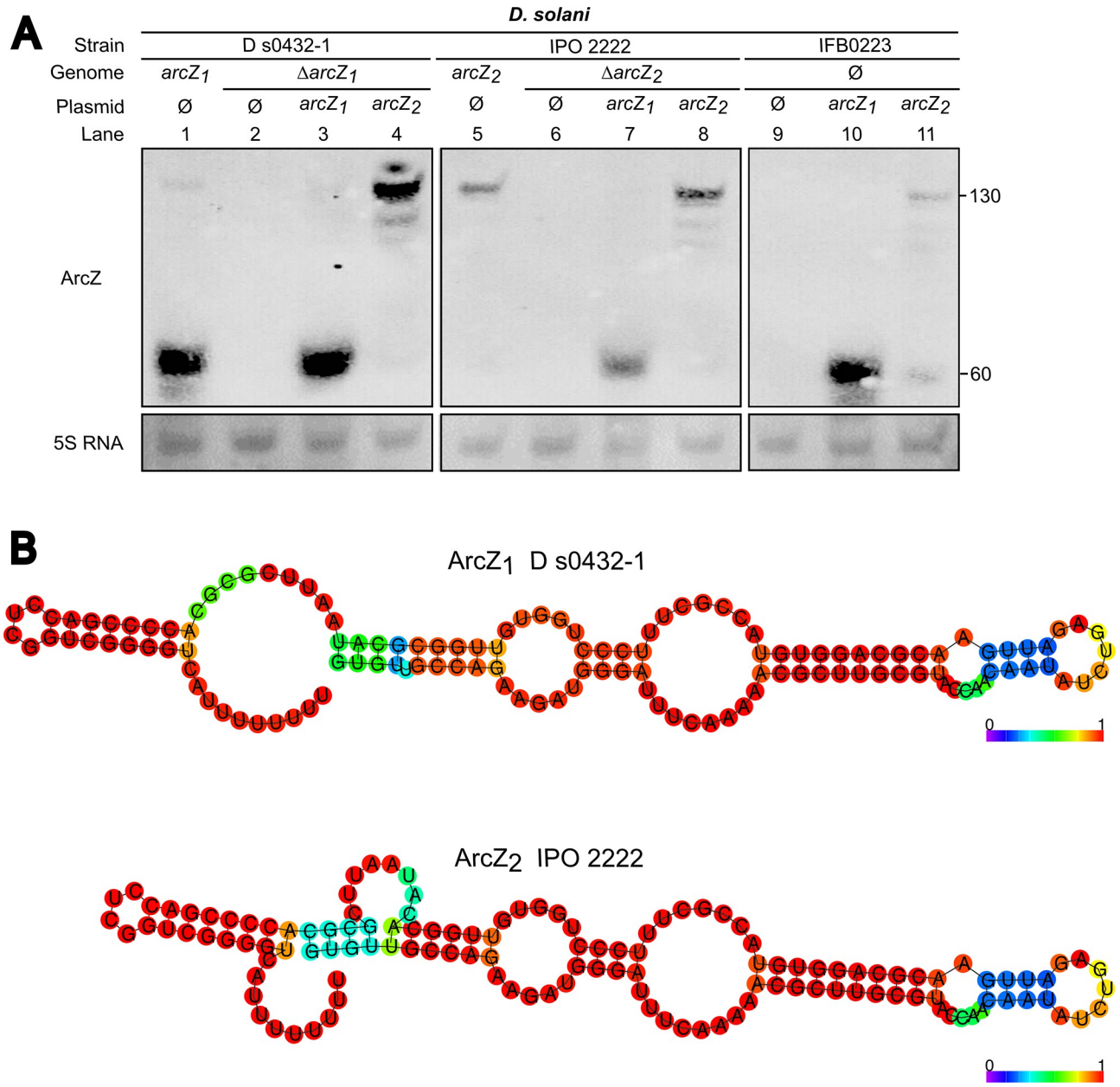


Fig 7. Expression of different ArcZ alleles: the G90A mutation in $ArcZ_2$ does not prevent its production but its processing. (A) Detection of the sRNA $ArcZ_1$ and $ArcZ_2$ by Northern Blot in *D. solani* strains. A small transcript (~60 nt) corresponding to the processed 3′ fragment of $ArcZ_1$ was detected in D s0432-1 and in all strains expressing *arcZ*$_1$ on plasmid. In IPO 2222, only the full-length form (~132nt) $ArcZ_2$ was detected, as well as in strains expressing the allele *arcZ*$_2$ on plasmid. The 5S RNA was used as a loading control. This experiment was performed twice with biological replicates and gave identical results. (B) Prediction of the secondary structure of the precursor forms of $ArcZ_1$ and $ArcZ_2$ by RNA fold. Bases are colored according to base-pairing probabilities (see color scale). The G90A mutation present in $ArcZ_2$ seems to modify the structure of the ArcZ sRNA precursor.

https://doi.org/10.1371/journal.pgen.1010725.g007

of the *oocL* gene suppressed the inhibition of the three fungi without any visible effect on the bacteria or yeasts tested. We can thus conclude that the *ooc* cluster of *D. solani* produces an antiascomycete molecule.

By testing other mutants of the other secondary metabolite clusters, we showed that the mutant Δ*solG* was nearly completely unable to prevent the growth of the ascomycete yeast *S. cerevisiae* and *K. lactis*. Therefore, the *sol* cluster appears to be responsible for yeast inhibition.

D. solani D s0432-1 also inhibits the human pathogen yeast *C. albicans*, but its inhibitory activity appears to be weaker than that observed with *S. cerevisiae* and *K. lactis* in our inhibition assay. This observation on the *sol* cluster paves the way for the identification of a new molecule with highly specific inhibitory activity against yeasts. The *sol* cluster is conserved in several *Dickeya* genomes but rarely in other bacterial genera, except in some *Rouxiella* species. The structure of the molecule and its target remains to be elucidated.

Our study also showed that *D. solani* D s0432-1 has an antibacterial activity linked to the cluster *zms*, which encodes a zeamine biosynthetic pathway. Zeamine produced by *S. plymuthica* kills *B. subtilis*, the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe*, and the nematode *Caenorhabditis elegans* [24]. We showed that *D. solani* D s0432-1, but not the Δ*zmsA* mutant, can clearly inhibit the growth of two Gram-positive bacteria, *B. subtilis* and *S. scabiei*. Since *D. solani* and *S. scabiei* are two potato pathogens, they might be in competition for the same ecological niche. We also observed a slight inhibition exerted by *D. solani* D s0432-1 against *E. coli*, but no inhibition towards *D. dadantii* and *P. atrosepticum*. Zeamine resistance of *D. dadantii* could be explained by the presence in its genome of the *desAB* genes encoding an RND pump identified in *D. oryzae* EC1 and involved in zeamine efflux [34]. In-frame deletion of *desA* or *desB* in *D. oryzae* EC1 leads to a zeamine sensitive phenotype [34]. In *in planta* Tn-seq experiments with *D. dadantii* 3937 [35], mutants of the genes *desA* and *desB* (Dda3937_00787 and Dda3937_00786 respectively) did not display a significant negative or positive variation (Log2 fold-changes -0.09 and +0.44, respectively), suggesting that this RND efflux pump does not play a significant role during *D. dadantii* chicory infection, contrary to the *D. dadantii* RND efflux pump AcrAB that appeared to be essential for virulence [35]. While *P. atrosepticum* is not inhibited by *D. solani* D s0432-1, it does not have the *desAB* genes. Zeamine resistance could be provided by another efflux pump or by a different mechanism in *P. atrosepticum*. It is worth remembering that the *D. solani* D s0432-1 Δ*zmsA* mutant grows slightly faster than the WT strain after reaching OD_{600nm} 0.4 (S1 Fig). This small difference in growth might be explained by the lack of need of the mutant to adapt to the presence of zeamine in the medium. We also observed that *D. solani* D s0432-1 Δ*solG* produced a very slight halo of inhibition against *S. cerevisiae* and *K. lactis*, barely visible in our tests. This weak halo disappears in the double mutant Δ*solG*Δ*zmsA* and the triple mutant *ΔsolGΔoocLΔzmsA*, showing that the zeamine produced by the *D. solani zms* cluster has also an anti-yeast action. It is important to note that zeamine produced by *S. plymuthica* A153 is bioactive against *S. cerevisiae* and *S. pombe* [24]. Thus, the presence of three clusters encoding secondary metabolites allows *D. solani* D s0432-1 to produce an arsenal of bioactive secondary metabolites against a variety of living microorganisms. The cluster *sol* produces an unknown molecule active against ascomycete yeasts, the cluster *ooc* produces an oocydin-like molecule active against ascomycete fungi and the cluster *zms* produces a zeamine-like molecule active against bacteria and ascomycete yeast. It is now clear that *D. solani* synthesizes several molecules to inhibit the growth of ascomycetes. Through a variety of regulatory mechanisms, the bacterium may always be able to express at least one of the *sol*, *zms*, and *ooc* clusters. This can make it particularly effective in the struggle against rival microorganisms. In future studies, it would be instructive to investigate *D. solani* behavior toward other organisms found in the environment, such as amoebas, nematodes, or even insects.

We then expanded our studies to a larger set of *D. solani* strains isolated in different European countries and years. Through the combination of phenotypic analysis and comparative genomics, confirmed by mutant construction, we demonstrated that the ArcZ sRNA plays a major role in activating the antimicrobial activities of the clusters *sol* and *zms*. We first observed that some *D. solani* WT strains are defective for yeast inhibition and possess different *arcZ* alleles, a deletion in the case of strain IFB0223, or the presence of the SNP G90A in the 3'

part of *arcZ* in the type strain IPO 2222. We demonstrated that these arcZ alterations significantly impair the *D. solani* ability to inhibit other bacteria and yeasts. These *arcZ*-related genomic changes represent a minority of *D. solani* isolates, only two of the nine strains phenotypically tested. Hence, our results show that despite a probable clonal origin, environmental strains of *D. solani* can present very different characteristics due to a single mutation into a regulatory sRNA. The *arcZ* polymorphism may be one of the explanations for the variability of phenotypes and virulence observed between genetically close *D. solani* strains. A global investigation of the polymorphism of all the small RNAs within the genomes of the same species could give surprising results. This study shows that it is critical to pay attention to the allelic diversity due to SNPs present not only in protein coding genes but also in sRNA genes.

ArcZ is a well-known regulatory sRNA that is associated with the chaperone Hfq. The ArcZ primary transcript (121 to 134nt-long) is converted by the essential endoribonuclease RNAse E [36] to a shorter stable of about 60 nt RNA retaining the seed region. The precise length of this short processed RNAs depends on the bacterial species [30,33,37,38]. In *E. coli*, the processed short form of ArcZ promotes *rpoS* translation while suppressing the expression of several other genes. In anaerobic conditions, *arcZ* is repressed and translation of RpoS is low [37]. RpoS is the RNA polymerase alternative sigma factor that governs the general stress response, which is activated by a wide range of conditions. It controls 10% of the *E. coli* genome [39]. RpoS can control positively or negatively the production of metabolites such as antibiotics [16,40]. The *rpoS* mRNA is not the only ArcZ direct target. In *Photorhabdus* and *Xenorhabdus*, ArcZ pairs with the mRNA encoding HexA, a transcriptional repressor of the expression of specific metabolite gene clusters [33]. In *Dickeya* species, the regulator gene *pecT* is the orthologous gene to *hexA*. In *D. dadantii* 3937, the *pecT* mRNA is also a direct target of ArcZ. Short processed ArcZ binds to Hfq and basepairs with the 5'UTR region of *pecT* mRNA, repressing its translation [38]. PecT is, directly and indirectly, involved in the expression of genes encoding plant cell wall degrading enzymes and virulence factors [38]. In the phytopathogen *Erwinia amylovora*, ArcZ also participates in the positive control of T3SS, exopolysaccharide production, biofilm formation, and motility [41]. Finally, another known direct target of ArcZ is the *flhD* mRNA [42,43]. FlhD forms with FlhC the master regulator FlhDC that controls motility in bacteria [44]. Although we demonstrated the critical positive role of ArcZ in the regulation of *sol* and *zms* expression, and secondary metabolite production in *D. solani*, it remains to understand the precise mechanism of ArcZ action on the *sol and zms* clusters. Our CopraRNA analysis indicates no putative binding between the processed form of $ArcZ_1$ and the 5'UTR region of *solA* and *zmsA*. However, the *pecT* 5'UTR, but not *flhD* and *rpoS* 5'UTRs, is detected as a putative target of $ArcZ_1$ (S3 Table). It should be kept in mind that bioinformatics analyses remain predictions. For example, CopraRNA does not detect the interaction between *E. coli* ArcZ and the 5'UTR of the *rpoS* mRNA. Much work remains to be done to elucidate the mode of action of ArcZ in *Dickeya solani*.

Another point to consider is a more global role of *D. solani* ArcZ exerted on other cellular functions such as motility and virulence. ArcZ targets in *D. solani* need to be investigated in the future. It is worth pointing out that the G90 nucleotide is in the 3' region of ArcZ that interacts with the target mRNAs *rpoS*, *flhD*, or *pecT* in other bacteria (S5 Fig). Northern-blot analyses revealed that $ArcZ_2$ of *D. solani* IPO 2222 is not processed in the stable shorter sRNA as $ArcZ_1$ of *D. solani* D s0432-1. The G90A mutation in IPO 2222 $ArcZ_2$ is predicted to modify the secondary structure of the ArcZ precursor form (Fig 7B). Since maturation by RNase E is essential for target regulation by the ArcZ sRNA in *Salmonella* [36], we conclude that the absence of a short form of ArcZ in IPO 2222 is probably the main cause of the loss of regulatory function of the IPO 2222 $ArcZ_2$.

The type strain *D. solani* IPO 2222 is largely used in academic laboratories as a model to study the *D. solani* species. In light of our findings, it could be important to compare data obtained with the strain IPO 2222 with those obtained with other *D. solani* strains. The *arcZ* mutations may be responsible, at least partially, for the lower virulence of IPO 2222 and IFB0223 observed previously in comparison to D s0432-1 [9]. ArcZ is expressed in aerobic condition in stationary phase. Thus, in IFB0223 and the type strain IPO 2222, one can assume that each gene controlled by ArcZ in this condition will not be properly regulated at the post-transcriptional level. IPO 2222 exhibited a low number of genes with oxygen-dependent transcription [45]. It might be possible that the missing functional ArcZ in this strain prevents the oxygen-dependent response of some genes. Besides, IPO 2222 was isolated from infected seed potatoes in the Netherlands in 2007 [46]. It is less virulent than D s0432-1 [9] but can still successfully colonize potato and bittersweet nightshade (*Solanum dulcamara*) plants [47,48]. These observations imply that ArcZ might not be necessary for a successful plant infection. However, when *D. solani* is present on the phylosphere, where oxygen is available, ArcZ might be crucial for outcompeting other microorganisms. It will be necessary to conduct more research to validate these hypotheses.

Even strains with the same *arcZ*, *sol*, *ooc*, and *zms* genomic sequences as D s0432-1, showed heterogeneity in their ability to inhibit other microorganisms. For example, IPO 3494 and IFB0484 inhibited *B. subtilis* better than other *D. solani* strains (Fig 5A). Other specific mutations in the genomes of these strains, which could also modify the activity of secondary metabolite clusters, could explain these phenotypic variations.

We first described the inhibitory function of the *sol* cluster of the *D. solani* strain D s0432-1 against the yeasts *S. cerevisiae* and *K. lactis* in a 2021 bioRxiv preprint [19]. Very recently, in November 2022, Matilla *et al.* published results on the *sol* cluster of the *D. solani* strain MK10 [14]. The authors described the antifungal activity of the uncharacterized molecule that they called solanimycin [14]. Matilla *et al.* also reported the inhibition observed with the *D. solani* strain IPO 2222 against *Verticillium dahliae* and *S. pombe* ([14], S1 Fig of the article). In our conditions, strain IPO 2222 was able to inhibit the fungus *S. sclerotiorum* but not the yeast *K. lactis* (Fig 5). In comparison to our study, Matilla *et al* used different fungal species but also different growth conditions (medium, temperature, exposure time, etc). By random mutagenesis of strain MK10, they identified RsmA and the two *Dickeya* quorum-sensing systems ExpI/R and Vfm as regulators of the *sol* cluster [14]. Interestingly, the Rsm regulatory pathway of *D. dadantii* 3937 is regulated by PecT, which is under the ArcZ control [38]. Our CopraRNA analysis identified *pecT*, and *vfmX*, one of the Vfm quorum sensing genes, as putative targets of ArcZ, but not *rsmA*, *expI* and *expR*, (S3 Table).

Further studies are now necessary to specify the biological targets of solanimycin and to clarify the role in *sol* expression of the sRNA ArcZ, quorum-sensing Vfm and Exp, and transcriptional regulators PecT or RsmA, in order to better understand the regulatory network controlling the production of secondary metabolites in *D. solani*. We are currently investigating the regulatory pathway of secondary metabolite clusters involving ArcZ.

In conclusion, our results provide the basis for further investigation of a novel metabolite capable of limiting yeast growth and, at the same time, open doors to understand how the ArcZ sRNA acts as a global regulator to regulate two different clusters of secondary metabolites in *D. solani*. It also shows how a single mutation in a small RNA can greatly modify the phenotype of bacterial wild-type strains that are very close phylogenetically.

Material and methods

Bacterial and fungal strains, plasmids and growth conditions

The *E. coli* and *Dickeya* bacterial strains, plasmids and oligonucleotides used in this study are described in S4 and S5 Tables. The genome accession number of *D. solani* D s0432-1 is

NZ_CP017453. The following strains have been used in the study: *Sclerotinia sclerotiorum* S5, *Botrytis cinerea* B05.10, *Magnaporthe oryzae* Guy11, *Saccharomyces cerevisiae* BY4743 (*MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0)*, *Kluyveromyces lactis* MWL9S1 [49], *Candida albicans* SC5314, *Dickeya dadantii* 3937, *Pectobacterium atrosepticum* SCRI1043, *Streptomyces scabiei* CFBP4517, *Bacillus subtilis* PY79. *E. coli* was grown routinely at 37˚C in LB. Fungus strains were grown at 25˚C onto Potato Dextrose Agar (PDA). *S. scabiei* was grown in tryptic soy broth (TSB) medium at 28˚C. *B. subtilis*, *P. atrosepticum* and the *Dickeya* strains were cultivated in LB unless specified. Yeast cells were grown at 30˚C in rich medium consisting of complete yeast extract-peptone (YP) medium containing 1% Bacto yeast extract, 1% Bacto peptone (Difco) supplemented with 2% glucose (yeast extract-peptone-dextrose [YPD] medium). For the bacteria, yeast and fungi inhibition assay, M63 medium supplemented with sucrose (2 g $(NH_4)_2SO_4$, 13.6 g KH_2PO_4, 2.5 mg $FeSO_47H_2O$, 0.2 g $MgSO_47H_2O$, 10 g sucrose, per liter) were employed to grow overnight the *D. solani* strains before performing the assay.

When required, antibiotics were added at the following concentrations: ampicillin (Amp), 100 μg/L; nalidixic acid (Nal), 10 μg/mL. streptomycin (Sm), 50 μg/mL; Diaminopimelic acid (DAP) (57 μg/mL) was added for the growth of the *E. coli* MFDpir strain. Media were solidified with 12 g/L agar.

Growth inhibition assay of bacteria and yeast

D. solani strains were grown for 24 h at 30˚C with shaking in M63 medium supplemented with 1% sucrose. *B. subtilis*, *E. coli*, *D. dadantii* and *P. atrosepticum* were grown in LB at 30˚C with shaking overnight. The next day, the OD_{600} of the cultures of *D. solani* were adjusted to 2. The temperature of melted LB agar was lowered to around 40˚C (just before the agar resolidified). 100 mL of the LB agar in surfusion were mixed with 100 μl of the OD_{600} 1 culture of *B. subtilis*, *E. coli*, *D. dadantii* or *P. atrosepticum*. 30 mL of inoculated LB agar were poured in 12- by 12-cm square plates. Then 5 μl of the OD_{600} 2 cultures of *D. solani* were spotted onto the inoculated square plates which were incubated at 30˚C for 24–48 h before visualization of the inhibition zone.

The same experiment was conducted with *S. scabiei*, except that it was grown for 3 days in TSB at 28˚C and TSB agar was poured in the square plates. With the yeasts *S. cerevisiae*, *K. lactis*, and *C. albicans*, a similar protocol was used with YPD medium and with OD_{600} 2 culture of yeast per 30mL of medium.

Growth inhibition of fungal strains

S. sclerotiorum, *B. cinerea* and *M. oryzae* were grown onto PDA plates at 25˚C for 5, 7 and 10 days respectively. *D. solani* strains were grown for 24 h at 30˚C with shaking in M63 medium supplemented with 1% sucrose. The OD_{600} of these overnight cultures were adjusted to 2. Then, 5 μl of the bacterial suspensions were spotted onto PDA plates with 5 mm agar plugs of fungus at the center of the Petri dish. The radius of fungus inhibition zone was measured.

Construction of the MLSA tree positioning strains within the *Dickeya* genus

Details on the methods used for the construction of MLSA tree positioning strains are given in S1 Supplementary Methods.

Constructions of the mutant strains and plasmids

All the material and methods for the construction of the strains and plasmids used in this study are given in S1 Supplementary Methods.

RNA isolation and Northern detection

All the material and methods for RNA isolation and Northern detection performed in this study are given in S1 Supplementary Methods.

Supporting information

S1 Fig. Growth curves of *D. solani* strains and mutant derivatives. A 96-well plate containing M63 medium with 1% sucrose was inoculated with the strains used in this study at an OD of 0.06. The growth of each strain was determined by measuring OD_{600} every 20 minutes during 2 days in a TECAN device. The different strains showed similar overall growth, except for the light grey points where a low but significant fitness gain was observed for the mutants *Δzms*, *Δsol Δzms*, *Δooc Δzms* and Δ*sol Δooc Δzms* (Mann-Whitney test, p-value<0.05).
(TIF)

S2 Fig. Inhibition of *E. coli* growth by *D. solani* D s0432-1 and mutant derivatives (zoom on drop borders). Bioassay plates were prepared by mixing *E. coli* culture with melted LB agar as described in the experimental procedures. 5 μL of bacterial culture at OD_{600nm} = 2 of *D. solani* D s0432-1 (WT) or derivatives were spotted onto the plate and incubated at 30˚C during 48 h. A slight inhibition zone was observed except with the *Δzms* mutant. All experiments were carried in 4 replicates.
(TIF)

S3 Fig. Phenotypes and *arcZ* sequence of WT *D. solani* IPO 2222 from the BCCM collection (LMG 25993). (A) Inhibition assay of *K. lactis*, *B. subtilis* and *S. sclerotiorum* by WT *D. solani* IPO 2222 LMG 25993 from the BCCM collection, compared to the strains used in this study. (B) Sanger sequencing results of *arcZ of D. solani* IPO 2222 (LMG 25993). The mutation G90A is highlighted by a red frame.
(TIF)

S4 Fig. Growth curves of WT and Δ*arcZ D. solani* strains used in this study. (A) Growth curves of D s0432-1, IPO 2222, their respective *ΔarcZ* mutants and IFB0223. The latter (in grey) has a slight growth delay (Mann-Whitney test, p-value<0.05). (B) Growth curves of strains used in complementation and heterologous expression tests. The plasmids do not cause any growth defect in D s0432-1, IPO 2222 and *ΔarcZ* mutants. IFB0223 derivatives containing a plasmid (in blue) have a growth delay already observed in the strain without plasmid (Mann-Whitney test, p-value<0.05). All experiments were performed in 4 biological replicates.
(TIF)

S5 Fig. Alignment of *arcZ* sequences. The *arcZ* DNA sequences were retrieved by running a BlastN on the NCBI database using the *arcZ* sequence of *D. solani* D s0432-1 as query. Search was limited to the *Dickeya* genomes. Then, the *Dickeya arcZ* sequences were aligned with *E. coli* MG1655 *arcZ* by using Jalview [50] and Muscle [51]. Known regions of interactions with the *pecT*, *flhD* and *rpoS* 5'UTR mRNA in *D. dadantii* and *E. coli* are indicated.
(TIF)

S1 Supplementary Methods. DOCX file with supplementary material and methods.
(DOCX)

S1 Table. Distribution of the clusters *sol*, *ooc*, and *zms* in 155 *Dickeya* strains whose genome is sequenced. For each *Dickeya* species, the strains were classified on the basis of the presence or absence of the cluster *sol*, *ooc*, and *zms*.
(XLSX)

S2 Table. Lists of SNPs in *D. solani* IFB0099, D S0432.1, IFB0223, IPO3337 and IPO 2222.
(XLSX)

S3 Table. List of the top 200 putative ArcZ/mRNA *Dickeya solani* D s0432-1 interactions.
(XLSX)

S4 Table. Strains and plasmids used in the study.
(DOCX)

S5 Table. Oligonucleotides used in the study.
(DOCX)

S1 Data. Raw data used to construct the figures, with their statistical analysis. The file can be open with Prism (Graphpad). A free viewer mode is available and can be downloaded online.
(PZFX)

Acknowledgments

We thank Veronique Utzinger for technical assistance, the members of the Membrane Trafficking and Signaling in Bacteria (MTSB) team for discussions, Amélie De Vallée, Nathalie Poussereau and Christophe Bruel for advices on fungi growth and providing the strains, Alexandre Soulard, Marc Lemaire and Jade Ravent for advices on yeast growth and providing the strains and medium.

Author Contributions

Conceptualization: Laetitia Attaiech, Monique Royer, Denis Faure, Nicole Hugouvieux-Cotte-Pattat, Erwan Gueguen.

Formal analysis: Typhaine Brual, Monique Royer, Jérémy Cigna, Denis Faure, Nicole Hugouvieux-Cotte-Pattat, Erwan Gueguen.

Funding acquisition: Laetitia Attaiech, Erwan Gueguen.

Investigation: Typhaine Brual, Géraldine Effantin, Julie Baltenneck, Laetitia Attaiech, Cloé Grosbois, Erwan Gueguen.

Methodology: Erwan Gueguen.

Project administration: Erwan Gueguen.

Resources: Laetitia Attaiech, Jérémy Cigna, Denis Faure, Erwan Gueguen.

Supervision: Laetitia Attaiech, Erwan Gueguen.

Validation: Erwan Gueguen.

Visualization: Erwan Gueguen.

Writing – original draft: Typhaine Brual, Denis Faure, Nicole Hugouvieux-Cotte-Pattat, Erwan Gueguen.

Writing – review & editing: Nicole Hugouvieux-Cotte-Pattat, Erwan Gueguen.

References

1. Hugouvieux-Cotte-Pattat N, Condemine G, Gueguen E, Shevchik VE. *Dickeya* Plant Pathogens. eLS. Pettis G. (ed), Chichester, UK: John Wiley & Sons, 2020. pp. 1–10. https://doi.org/10.1002/9780470015902.a0028932
2. Charkowski A, Blanco C, Condemine G, Expert D, Franza T, Hayes C, et al. The role of secretion systems and small molecules in soft-rot *Enterobacteriaceae* pathogenicity. Annu Rev Phytopathol. 2012; 50: 425–449. https://doi.org/10.1146/annurev-phyto-081211-173013 PMID: 22702350
3. Van Gijsegem F, Toth IK, van der Wolf JM. Soft Rot Pectobacteriaceae: A Brief Overview. In: Van Gijsegem F, van der Wolf JM, Toth IK, editors. Plant Diseases Caused by Dickeya and *Pectobacterium* Species. Cham: Springer International Publishing; 2021. pp. 1–11. https://doi.org/10.1007/978-3-030-61459-1_1
4. Hugouvieux-Cotte-Pattat N, Condemine G, Shevchik VE. Bacterial pectate lyases, structural and functional diversity: Bacterial pectate lyases. Environ Microbiol Rep. 2014; 6: 427–440. https://doi.org/10.1111/1758-2229.12166 PMID: 25646533
5. van der Wolf JM, Nijhuis EH, Kowalewska MJ, Saddler GS, Parkinson N, Elphinstone JG, et al. *Dickeya solani* sp. nov., a pectinolytic plant-pathogenic bacterium isolated from potato (*Solanum tuberosum*). Int J Syst Evol Microbiol. 2014; 64: 768–774. https://doi.org/10.1099/ijs.0.052944–0
6. Blin P, Robic K, Khayi S, Cigna J, Munier E, Dewaegeneire P, et al. Pattern and causes of the establishment of the invasive bacterial potato pathogen *Dickeya solani* and of the maintenance of the resident pathogen *D. dianthicola*. Mol Ecol. 2021; 30: 608–624. https://doi.org/10.1111/mec.15751 PMID: 33226678
7. Garlant L, Koskinen P, Rouhiainen L, Laine P, Paulin L, Auvinen P, et al. Genome Sequence of *Dickeya solani*, a New soft Rot Pathogen of Potato, Suggests its Emergence May Be Related to a Novel Combination of Non-Ribosomal Peptide/Polyketide Synthetase Clusters. Diversity. 2013; 5: 824–842. https://doi.org/10.3390/d5040824
8. Pédron J, Mondy S, des Essarts YR, Van Gijsegem F, Faure D. Genomic and metabolic comparison with *Dickeya dadantii* 3937 reveals the emerging *Dickeya solani* potato pathogen to display distinctive metabolic activities and T5SS/T6SS-related toxin repertoire. BMC Genomics. 2014; 15: 283. https://doi.org/10.1186/1471-2164-15-283 PMID: 24735398
9. Golanowska M, Potrykus M, Motyka-Pomagruk A, Kabza M, Bacci G, Galardini M, et al. Comparison of Highly and Weakly Virulent *Dickeya solani* Strains, With a View on the Pangenome and Panregulon of This Species. Front Microbiol. 2018; 9: 1940. https://doi.org/10.3389/fmicb.2018.01940 PMID: 30233505
10. Motyka-Pomagruk A, Zoledowska S, Misztak AE, Sledz W, Mengoni A, Lojkowska E. Comparative genomics and pangenome-oriented studies reveal high homogeneity of the agronomically relevant enterobacterial plant pathogen *Dickeya solani*. BMC Genomics. 2020; 21: 449. https://doi.org/10.1186/s12864-020-06863-w PMID: 32600255
11. Khayi S, Blin P, Pédron J, Chong T-M, Chan K-G, Moumni M, et al. Population genomics reveals additive and replacing horizontal gene transfers in the emerging pathogen *Dickeya solani*. BMC Genomics. 2015; 16: 788. https://doi.org/10.1186/s12864-015-1997-z PMID: 26467299
12. Cane DE, Walsh CT. The parallel and convergent universes of polyketide synthases and nonribosomal peptide synthetases. Chem Biol. 1999; 6: R319–R325. https://doi.org/10.1016/s1074-5521(00)80001-0 PMID: 10631508
13. Duprey A, Taib N, Leonard S, Garin T, Flandrois JP, Nasser W, et al. The phytopathogenic nature of *Dickeya aquatica* 174/2 and the dynamic early evolution of *Dickeya pathogenicity*. Environ Microbiol. 2019; 21: 2809–2835. https://doi.org/10.1111/1462-2920.14627 PMID: 30969462
14. Matilla MA, Monson RE, Murphy A, Schicketanz M, Rawlinson A, Duncan C, et al. Solanimycin: Biosynthesis and Distribution of a New Antifungal Antibiotic Regulated by Two Quorum-Sensing Systems. Sperandio V, editor. mBio. 2022; e02472–22. https://doi.org/10.1128/mbio.02472-22 PMID: 36214559
15. Matilla MA, Stöckmann H, Leeper FJ, Salmond GPC. Bacterial Biosynthetic Gene Clusters Encoding the Anti-cancer Haterumalide Class of Molecules. J Biol Chem. 2012; 287: 39125–39138. https://doi.org/10.1074/jbc.M112.401026 PMID: 23012376
16. Matilla MA, Leeper FJ, Salmond GPC. Biosynthesis of the antifungal haterumalide, oocydin A, in *Serratia*, and its regulation by quorum sensing, RpoS and Hfq. Environ Microbiol. 2015; 17: 2993–3008. https://doi.org/10.1111/1462-2920.12839 PMID: 25753587
17. Zhou J, Zhang H, Wu J, Liu Q, Xi P, Lee J, et al. A Novel Multidomain Polyketide Synthase Is Essential for Zeamine Production and the Virulence of *Dickeya zeae*. Mol Plant-Microbe Interactions®. 2011; 24: 1156–1164. https://doi.org/10.1094/MPMI-04-11-0087 PMID: 21899437

18. Masschelein J, Mattheus W, Gao L-J, Moons P, Houdt RV, Uytterhoeven B, et al. A PKS/NRPS/FAS Hybrid Gene Cluster from *Serratia plymuthica* RVH1 Encoding the Biosynthesis of Three Broad Spectrum, Zeamine-Related Antibiotics. PLOS ONE. 2013; 8: e54143. https://doi.org/10.1371/journal.pone.0054143 PMID: 23349809

19. Effantin G, Brual T, Rahbé Y, Hugouvieux-Cotte-Pattat N, Gueguen E. *Dickeya solani* D s0432-1 produces an arsenal of secondary metabolites with anti-prokaryotic and anti-eukaryotic activities against bacteria, yeasts, fungi, and aphids. bioRxiv; 2021. p. 2021.07.19.452942. https://www.biorxiv.org/content/10.1101/2021.07.19.452942v1

20. Strobel G, Li J-Y, Sugawara F, Koshino H, Harper J, Hess WM. Oocydin A, a chlorinated macrocyclic lactone with potent anti-oomycete activity from *Serratia marcescens*. Microbiology. 1999; 145: 3557–3564. https://doi.org/10.1099/00221287-145-12-3557 PMID: 10627053

21. Zhou J, Cheng Y, Lv M, Liao L, Chen Y, Gu Y, et al. The complete genome sequence of *Dickeya zeae* EC1 reveals substantial divergence from other *Dickeya* strains and species. BMC Genomics. 2015; 16: 571. https://doi.org/10.1186/s12864-015-1545-x PMID: 26239726

22. Wang X, He S-W, Guo H-B, Han J-G, Thin KK, Gao J-S, et al. *Dickeya oryzae* sp. nov., isolated from the roots of rice. Int J Syst Evol Microbiol. 2020; 70: 4171–4178. https://doi.org/10.1099/ijsem.0.004265 PMID: 32552985

23. Masschelein J, Jenner M, Challis GL. Antibiotics from Gram-negative bacteria: a comprehensive overview and selected biosynthetic highlights. Nat Prod Rep. 2017; 34: 712–783. https://doi.org/10.1039/c7np00010c PMID: 28650032

24. Hellberg JEEU Matilla MA, Salmond GPC. The broad-spectrum antibiotic, zeamine, kills the nematode worm *Caenorhabditis elegans*. Front Microbiol. 2015; 6. https://doi.org/10.3389/fmicb.2015.00137 PMID: 25767467

25. Loria R, Kers J, Joshi M. Evolution of Plant Pathogenicity in *Streptomyces*. Annu Rev Phytopathol. 2006; 44: 469–487. https://doi.org/10.1146/annurev.phyto.44.032905.091147 PMID: 16719719

26. Rodicio R, Heinisch JJ. Yeast on the milky way: genetics, physiology and biotechnology of *Kluyveromyces lactis*. Yeast. 2013; 30: 165–177. https://doi.org/10.1002/yea.2954 PMID: 23576126

27. Valentin-Hansen P, Eriksen M, Udesen C. MicroReview: The bacterial Sm-like protein Hfq: a key player in RNA transactions. Mol Microbiol. 2004; 51: 1525–1533. https://doi.org/10.1111/j.1365-2958.2003.03935.x PMID: 15009882

28. Vogel J, Luisi BF. Hfq and its constellation of RNA. Nat Rev Microbiol. 2011; 9: 578–589. https://doi.org/10.1038/nrmicro2615 PMID: 21760622

29. Dutta T, Srivastava S. Small RNA-mediated regulation in bacteria: A growing palette of diverse mechanisms. Gene. 2018; 656: 60–72. https://doi.org/10.1016/j.gene.2018.02.068 PMID: 29501814

30. Papenfort K, Said N, Welsink T, Lucchini S, Hinton JCD, Vogel J. Specific and pleiotropic patterns of mRNA regulation by ArcZ, a conserved, Hfq-dependent small RNA. Mol Microbiol. 2009; 74: 139–158. https://doi.org/10.1111/j.1365-2958.2009.06857.x PMID: 19732340

31. Wright PR, Georg J, Mann M, Sorescu DA, Richter AS, Lott S, et al. CopraRNA and IntaRNA: predicting small RNA targets, networks and interaction domains. Nucleic Acids Res. 2014; 42: W119–123. https://doi.org/10.1093/nar/gku359 PMID: 24838564

32. Argaman L, Hershberg R, Vogel J, Bejerano G, Wagner EGH, Margalit H, et al. Novel small RNA-encoding genes in the intergenic regions of *Escherichia coli*. Curr Biol. 2001; 11: 941–950. https://doi.org/10.1016/S0960-9822(01)00270-6 PMID: 11448770

33. Neubacher N, Tobias NJ, Huber M, Cai X, Glatter T, Pidot SJ, et al. Symbiosis, virulence and natural-product biosynthesis in entomopathogenic bacteria are regulated by a small RNA. Nat Microbiol. 2020; 5: 1481–1489. https://doi.org/10.1038/s41564-020-00797-5 PMID: 33139881

34. Liang Z, Huang L, He F, Zhou X, Shi Z, Zhou J, et al. A Substrate-Activated Efflux Pump, DesABC, Confers Zeamine Resistance to *Dickeya zeae*. mBio. 2019; 10. https://doi.org/10.1128/mBio.00713-19 PMID: 31138747

35. Royet K, Parisot N, Rodrigue A, Gueguen E, Condemine G. Identification by Tn-seq of *Dickeya dadantii* genes required for survival in chicory plants. Mol Plant Pathol. 2019; 20: 287–306. https://doi.org/10.1111/mpp.12754 PMID: 30267562

36. Chao Y, Li L, Girodat D, Förstner KU, Said N, Corcoran C, et al. In Vivo Cleavage Map Illuminates the Central Role of RNase E in Coding and Non-coding RNA Pathways. Mol Cell. 2017; 65: 39–51. https://doi.org/10.1016/j.molcel.2016.11.002 PMID: 28061332

37. Mandin P, Gottesman S. Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. EMBO J. 2010; 29: 3094–3107. https://doi.org/10.1038/emboj.2010.179 PMID: 20683441

38. Yuan X, Zeng Q, Khokhani D, Tian F, Severin GB, Waters CM, et al. A feed-forward signalling circuit controls bacterial virulence through linking cyclic di-GMP and two mechanistically distinct sRNAs, ArcZ and RsmB. Environ Microbiol. 2019; 21: 2755–2771. https://doi.org/10.1111/1462-2920.14603 PMID: 30895662

39. Battesti A, Majdalani N, Gottesman S. The RpoS-mediated general stress response in *Escherichia coli*. Annu Rev Microbiol. 2011; 65: 189–213. https://doi.org/10.1146/annurev-micro-090110-102946 PMID: 21639793

40. Wilf NM, Salmond GPC. The stationary phase sigma factor, RpoS, regulates the production of a carbapenem antibiotic, a bioactive prodigiosin and virulence in the enterobacterial pathogen *Serratia* sp. ATCC 39006. Microbiology. 2012; 158: 648–658. https://doi.org/10.1099/mic.0.055780–0

41. Zeng Q, McNally RR, Sundin GW. Global Small RNA Chaperone Hfq and Regulatory Small RNAs Are Important Virulence Regulators in *Erwinia amylovora*. J Bacteriol. 2013; 195: 1706–1717. https://doi.org/10.1128/JB.02056-12 PMID: 23378513

42. De Lay N, Gottesman S. A complex network of small non-coding RNAs regulate motility in *Escherichia coli*. Mol Microbiol. 2012; 86: 524–538. https://doi.org/10.1111/j.1365-2958.2012.08209.x PMID: 22925049

43. Schachterle JK, Zeng Q, Sundin GW. Three Hfq-dependent small RNAs regulate flagellar motility in the fire blight pathogen *Erwinia amylovora*. Mol Microbiol. 2019; 111: 1476–1492. https://doi.org/10.1111/mmi.14232 PMID: 30821016

44. Fraser GM, Hughes C. Swarming motility. Curr Opin Microbiol. 1999; 2: 630–635. https://doi.org/10.1016/s1369-5274(99)00033-8 PMID: 10607626

45. Lisicka W, Fikowicz-Krosko J, Jafra S, Narajczyk M, Czaplewska P, Czajkowski R. Oxygen Availability Influences Expression of *Dickeya solani* Genes Associated With Virulence in Potato (*Solanum tuberosum* L.) and Chicory (*Cichorium intybus* L.). Front Plant Sci. 2018; 9: 374. https://doi.org/10.3389/fpls.2018.00374 PMID: 29619040

46. Pritchard L, Humphris S, Baeyen S, Maes M, Van Vaerenbergh J, Elphinstone J, et al. Draft Genome Sequences of Four *Dickeya dianthicola* and Four *Dickeya solani* Strains. Genome Announc. 2013;1. https://doi.org/10.1128/genomeA.00087-12 PMID: 23887905

47. Fikowicz-Krosko J, Czajkowski R. Systemic Colonization and Expression of Disease Symptoms on Bittersweet Nightshade (*Solanum dulcamara*) Infected with a GFP-Tagged *Dickeya solani* IPO2222 (IPO2254). Plant Dis. 2018; 102: 619–627. https://doi.org/10.1094/PDIS-08-17-1147-RE PMID: 30673477

48. Czajkowski R, de Boer WJ, Velvis H, van der Wolf JM. Systemic Colonization of Potato Plants by a Soilborne, Green Fluorescent Protein-Tagged Strain of *Dickeya* sp. Biovar 3. Phytopathology®. 2010; 100: 134–142. https://doi.org/10.1094/PHYTO-100-2-0134 PMID: 20055647

49. Wésolowski-Louvel M. An efficient method to optimize *Kluyveromyces lactis* gene targeting. FEMS Yeast Res. 2011; 11: 509–513. https://doi.org/10.1111/j.1567-1364.2011.00741.x PMID: 21627769

50. Clamp M, Cuff J, Searle SM, Barton GJ. The Jalview Java alignment editor. Bioinforma Oxf Engl. 2004; 20: 426–427. https://doi.org/10.1093/bioinformatics/btg430 PMID: 14960472

51. Edgar RC. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics. 2004; 5: 113. https://doi.org/10.1186/1471-2105-5-113 PMID: 15318951