Deciphering the genome of *Simplicillium aogashimaense* to understand its mechanisms against the wheat powdery mildew fungus *Blumeria graminis* f. sp. *tritici*

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Abstract

*Simplicillium* spp. are mycoparasites that exert growth-inhibitory effects on phytopathogenic fungi. However, limited studies have examined the effects of *Simplicillium* spp. on powdery mildews. In this study, morphological and molecular analyses revealed that *S. aogashimaense* is a mycoparasite of the wheat powdery mildew fungus, *Blumeria graminis* f. sp. *tritici* (*Bgt*), under field conditions. The inoculation of *Bgt* colonies with *S. aogashimaense* significantly impaired *Bgt* colony formation and conidial distribution and markedly decreased the biomass of *Bgt*. To examine the interaction between *Simplicillium* and *Bgt*, an *S. aogashimaense* strain that constitutively expresses green fluorescent protein (GFP) was constructed using the *Agrobacterium tumefaciens*-mediated transformation (ATMT) method. The hyphae of GFP-expressing *S. aogashimaense* directly penetrated the *B. graminis* structures. These findings indicate that ATMT can be employed to reveal the biocontrol activities of physiologically and phylogenetically diverse *Simplicillium* spp. In vitro, *S. aogashimaense* exudates compromised *Bgt* conidial germination and appressorial formation. Thus, *S. aogashimaense* functions as a potential biological control agent by impeding the development of *Bgt* and can be a viable alternative for controlling the wheat powdery mildew. To gain further insights into the mechanism underlying this mycoparasitism, the genome of *S. aogashimaense* was sequenced and assembled. *S. aogashimaense* harbored seven chromosomes comprising 8963 protein-coding genes. Additionally, two putative effector-coding genes (*Sao008714* and *Sao006491*) were identified. The expression levels of *Sao008714* and *Sao006491* in *S. aogashimaense* were dramatically upregulated during the mycoparasitic interaction. In addition, 41 gene clusters putatively involved in the production of secondary metabolites, which exhibit insecticidal, antifungal and antibacterial activities, were identified using genome-wide identification, annotation and analysis of secondary metabolite biosynthesis gene clusters. These results suggest that *S. aogashimaense* parasitizes *Bgt* and hence, can be considered for phytopathogen management.

**Keywords:** Wheat powdery mildew, Biological control, *Simplicillium aogashimaense*, ITS, Fungal identification

Background

*Blumeria graminis* f. sp. *tritici* (*Bgt*), an obligate biotrophic ascomycete, infests wheat and causes a destructive foliar disease. *Bgt*, which is associated with severe economic losses, is the sixth most crucial fungal phytopathogen worldwide (Dean et al. 2012). Currently, *B. graminis* is most effectively managed by the use of
various chemical fungicides, however, this may pose risks to human health and the environment. Moreover, *B. graminis* has a high risk to develop resistance to several fungicides (FRAC Pathogen Risk List, www.frac.info, 2019). Therefore, some studies have focused on developing sustainable biological alternatives for controlling *B. graminis* (Kiss 2003; Köhl et al. 2019; Zhu et al. 2019). Recently, the potential of antagonists for biological control of powdery mildew diseases in several crops and ornamental plants has been explored owing to their environmentally friendly characteristics (Neveu et al. 2007; Belanger et al. 2012; Matzen et al. 2019; Németh et al. 2019). However, previous studies on biocontrol agents (BCAs) for powdery mildew diseases have mainly focused on the following species: *Verticillium lecanii*, *Pseudopeziza flocculosa*, *Amphelomyces quisqualis* and *Tilletiopsis* spp. (Dik et al. 1998), and no known biological control products have been developed for cereal powdery mildew disease (Köhl et al. 2019). Thus, there is a need to identify novel BCAs.

Conidia and ascospores are important propagules in the pathogenesis of powdery mildew fungi. *B. graminis* mainly undergoes asexual propagation during disease development through repeatedly producing conidia (Zhu et al. 2017). Upon reaching a suitable host leaf surface, the conidia and ascospores of *B. graminis* germinate and then form the appressorium, an infection structure, to penetrate its hosts. After successful infection and haustorium formation, both conidial and ascosporic colonies produce young conidiophores with conidia that can serve as inocula for infection of volunteer plants (Jankovics et al. 2015). Therefore, conidial production and distribution play crucial roles in the pathogenesis of *B. graminis*.

*Simplicillium* species are ecologically and economically valuable due to their broad range of hosts and substrates (such as plants, insects, nematodes, human tissues and fungi), varied lifecycle, bioactive compound production and biocontrol activities (Wei et al. 2019). So far, at least 15 *Simplicillium* species have been identified. Some *Simplicillium* species are reported to be valuable BCAs for fungal phytopathogens. *S. lanosoniveum* exhibits mycophilic and disease-suppressive properties on rust fungus (*Phakopsora pachyrhizi*)-infected soybean leaves (Ward et al. 2012; Gauthier et al. 2014). Similar findings were documented by studies examining the growth-inhibitory effects of *S. aogashimaense* against *Puccinia* rust pathogens (i.e. *P. triticina*, *P. hordei* and *P. coronata* f. sp. *avenae*), *Bipolaris sorokiniana*, *Alternaria alternata* and *Curvularia trifolii* (Teasdale et al. 2018; Wilson et al. 2020). *S. lamellicola* has been demonstrated to decrease the incidence of gray mold disease (*Botrytis cinerea*) in tomato and ginseng (Shin et al. 2017). Meanwhile, *S. obclavatum*, a hyperparasite, affects the infection dynamics of the wheat strip rust fungus, *Puccinia striiformis* f. sp. *tritici* (Wang et al. 2020). Additionally, exudates of *S. lanosoniveum* exert antifungal potencies against pathogens causing brassica dark leaf spot (*Alternaria brassicicola*), rice brown spot (*Cochliobolus miyabeanus*) and the jasmine orange powdery mildew fungus (*Oidium murrayae*) in vitro (Chen et al. 2017). Cyclic peptides isolated from *S. obclavatum* exhibit potent growth-inhibitory activities against *Curvularia australiensis* (Liang et al. 2017). Thus, *Simplicillium* species are potential BCAs as they are capable of efficiently parasitizing and/or suppressing phytopathogens. However, the ability of *Simplicillium* species to parasitize powdery mildew fungi, including *B. graminis*, has not been previously reported.

Next generation sequencing has revolutionized the research on antagonistic microorganisms and phytopathogenic fungi. Previously, the genomes of some biological agents (i.e. *P. flocculosa* and *A. quisqualis*) were sequenced and the molecular mechanisms of antagonistic activities were illustrated (Lefebvre et al. 2013; Siozios et al. 2015; Laur et al. 2018). Although *Simplicillium* species are repeatedly shown antifungal activities against fungal pathogens, the underlying antifungal mechanisms have not been elucidated owing to the limited information on their genomes (Jauregui et al. 2020).

Identification of mycoparasites that are capable of parasitizing cereal pathogenic fungi is critical for developing BCAs against these pathogens. This study characterized a *Simplicillium* species isolated from *Bgt*-infected wheat leaves in a natural environment, which was identified as *Sae*. *aogashimaense* via morphological and molecular analyses. Since limited information is available on the mycophilic and disease-suppressive properties of *S. aogashimaense* in *Bgt* infection, and no studies have examined the interaction between fluorescence protein-transformed *Simplicillium* species and powdery mildew fungi, the characteristics and environmental fate of *Simplicillium* fungi before and after parasitizing powdery mildew pathogens are therefore still underestimated. Thus, in this study, we aimed to determine whether *S. aogashimaense* can efficiently parasitize *Bgt* colonies and to analyze the suppressive effects of *S. aogashimaense* on *Bgt* sporulation by quantifying the biomass during fungal-fungal interactions. Furthermore, the complete chromosome-scale genome of *S. aogashimaense* was elucidated, which is the first assembled genome of *Simplicillium* spp. A green fluorescent protein (GFP)-transformed strain of *S. aogashimaense* was constructed using the *Agrobacterium tumefaciens*-mediated transformation (ATMT) system to gain novel insights into the interactions between this BAC and *Bgt*.
Results

Identification of S. aogashimaense as a mycoparasite in Bgt colony

The morphological characteristics of Bgt colonies on wheat leaves were examined. Compared with Bgt colonies in their natural states on wheat leaves (Fig. 1a–d), aberrant colonies that turned from white to brownish color were observed (Fig. 1e), and the powdery mass significantly decreased in the aberrant colonies (Fig. 1f). To examine if these changes were caused by parasitization, Bgt-infected wheat leaves were examined. Microscopic analysis revealed that Bgt colonies were collapsed and covered by colonies of another fungus (Fig. 1g). Additionally, conidial production of Bgt was suppressed. Furthermore, Bgt conidia were surrounded and trapped by the hyphae of this fungus. Scanning electron microscopy analysis revealed similar findings. The fungus formed colonies, hyphae and conidia on Bgt colonies. The conidiophores of Bgt were collapsed, which impaired sporulation. Bgt conidia were also surrounded by hyphae of the fungus (Fig. 1h). Therefore, this fungus was identified as a mycoparasite of Bgt.

To identify the hyperparasite on Bgt-infected wheat leaves, the morphological characteristics of the purified mycoparasite were examined. The mycoparasite colonies on potato dextrose agar (PDA) exhibited a floccose-white color, while the reverse side exhibited a yellowish-white color (Fig. 2a, b). Conidia in small globose heads were formed at the apex of phialides on the aerial hyphae (Fig. 2c). The length and width of cylindrical conidia were 4.2–6.6 μm and 1.2–2.1 μm, respectively (Fig. 2d–f). Based on these morphological characteristics, this parasitic fungus was initially identified as S. aogashimaense. The internal transcribed spacer (ITS) region of this fungus was then sequenced. The sequence (GenBank accession no. MT936440) exhibited 99.49% identity with that of the previously reported S. aogashimaense. Phylogenetic analysis revealed that S. aogashimaense (AB604002) and the identified fungus clustered in the same branch (Fig. 2g). Therefore, the isolated fungus was confirmed to be S. aogashimaense based on morphological and molecular analyses.

Mycoparasitism of S. aogashimaense on Bgt

To confirm the ability of S. aogashimaense to parasitize Bgt, a mycoparasitism assay was performed. Bgt-infected and healthy wheat leaves were inoculated with S. aogashimaense spore suspension (1 × 10⁶ spores/mL) or water and incubated in a growth chamber. The water-treated Bgt colonies did not exhibit morphological changes at all three time points tested (Fig. 3a–c). However, when inoculated with S. aogashimaense, this parasite formed hyphae and produced spores at 3 days post-inoculation (dpi) on Bgt-infected leaves (Fig. 3d); Bgt colonies were covered with S. aogashimaense at 6 dpi (Fig. 3e); crumbled conidiophores of Bgt were observed upon S. aogashimaense inoculation, and the number of Bgt conidia markedly decreased at 9 dpi (Fig. 3f). In comparison, no S. aogashimaense structures were observed on healthy leaf surfaces at these time points.

Next, the spores of GFP-transformed and wild-type (untransformed) S. aogashimaense were separately inoculated onto Bgt-infected wheat leaves and examined under a fluorescence microscope (Fig. 4). The
results revealed that *S. aogashimaense* infected *Bgt*. Additionally, the mycoparasitism of wild-type and GFP-transformed strains was not markedly different.

**Exudates of *S. aogashimaense* suppress *Bgt* growth**

To determine if *S. aogashimaense* released growth-inhibitory metabolites against *Bgt*, the exudates of *S. aogashimaense* colonies...
aogashimaense produced in potato dextrose broth (PDB) were applied to Bgt conidia in vitro (Fig. 5). In the control group, more than 90% of Bgt conidia germinated and 47% formed an appressorium. However, the proportion of germinated conidia in the S. aogashimaense exudate-treated group was 63%, which was significantly lower than that in the control group. Additionally, only 2% of S. aogashimaense exudate-treated conidia formed an appressorium.
S. aogashimaense decreases the biomass of Bgt on wheat leaves

To quantitatively evaluate the suppressive effects of S. aogashimaense on Bgt, the fungal mass on Bgt-infected wheat leaves inoculated with S. aogashimaense was measured via quantitative real-time polymerase chain reaction (qPCR) analysis (Fig. 6). Compared with that at 0 dpi, the biomasses of S. aogashimaense at 3, 6 and 9 dpi were increased by 26, 28 and 21 times, respectively (Fig. 6d). In contrast, the biomasses of Bgt at 3, 6 and 9 dpi were significantly decreased by 2.0, 5.7 and 3.9 times, respectively, when compared with that at 0 dpi (Fig. 6e).

Complete genome sequence of S. aogashimaense

The genome of S. aogashimaense was sequenced and annotated (Fig. 7). The size of the complete chromosome-scale genome of S. aogashimaense was 30.26 Mb (N50 value = 4.07 Mb) with 48.95% GC content and 98.6% completed benchmarking universal single-copy orthologs (BUSCOs) (Table 1). The number of protein-coding genes was 8963, which comprised 8688 annotated and 275 unannotated genes. The average gene length was 2350.55 bp. The genome comprised 94, 67 and 19 copies of tRNAs, rRNAs and snRNAs, respectively. Additionally, 2094 novel transcripts were annotated using RNA sequencing (RNA-seq) analysis (Additional file 1: Table S1).

Gene mining and Basic Local Alignment Search Tool analyses identified two putative effector-coding genes (Sao008714 and Sao006491) (Additional file 2: Figures S1, S2). These two genes were significantly upregulated during the mycoparasitic interaction (Additional file 2: Figure S3). AntiSMASH (antibiotics & Secondary Metabolite Analysis Shell) analysis identified the gene clusters involved in the putative secondary metabolite biosynthesis in S. aogashimaense. Antibiotics and secondary metabolites produced by S. aogashimaense were also predicted (Additional file 1: Table S2). Among these, nine gene clusters exhibited marked hits (13–100% similarity) to different types of known secondary metabolite biosynthesis gene clusters, including those involved in the biosynthesis of terpene, non-ribosomal peptide synthetase and type I polyketide synthase.

Discussion

Mycoparasitism and antagonistic activity of S. aogashimaense against Bgt

The antagonistic effects of Simplicillium spp. on fungal phytopathogens have been previously reported (Ward et al. 2012; Gauthier et al. 2014; Shin et al. 2017; Teasdale et al. 2018; Wang et al. 2020; Wilson et al. 2020).
However, most studies have focused on the antagonistic effects of *Simplicillium* spp. on rust or gray mold fungi. Limited studies have examined the suppressing effects of *Simplicillium* spp. on other phytopathogenic fungi. In particular, the antagonistic effects of *Simplicillium* spp. on the development of powdery mildew pathogens, including *Bgt* that causes economically and agriculturally important plant diseases, have not been evaluated. This study aimed to identify a BCA for *Bgt*.

A mycoparasite of *Bgt* was identified and characterized under field conditions.

The mycoparasite distinctly interacted with *Bgt* (Fig. 1). Morphological and molecular analyses revealed that the mycoparasite was *S. aogashimaense* (Fig. 2). The color and morphology of the *Bgt* colonies changed upon infection with *S. aogashimaense*. Additionally, the *Bgt* colonies and conidiophores were disrupted upon *S. aogashimaense* infection. Thus, the direct antagonistic effect of *S. aogashimaense* on disrupting *Bgt* colonies and conidiophores was found. These results indicate that *S. aogashimaense* is a potential BCA for powdery mildew diseases. Previously, *S. aogashimaense* was found in soil samples or isolated from *Brachiaria brizantha* as a putative fungal endophyte (Nonaka et al. 2013; Teasdale et al. 2018). This study demonstrated that *S. aogashimaense* can parasitize *Bgt* colonies and efficiently suppressed the development of the wheat powdery mildew fungus. This indicated that *S. aogashimaenseis* is a natural BCA that exerts antagonistic effects on *Bgt*.

*S. aogashimaense* suppresses the growth of *Bgt*

To examine the suppressive effects of *S. aogashimaense* on *Bgt*, the development of this parasite on *Bgt* colonies was examined. *S. aogashimaense* directly wrapped around *Bgt* conidiophores and disrupted conidial distribution (Fig. 3). This further confirmed that *S. aogashimaense* can

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**Table 1** Characteristics of *S. aogashimaense* genome

| Feature                          | Characteristics |
|---------------------------------|-----------------|
| Genome size (Mb)                | 30.26           |
| N50 (Mb)                        | 4.07            |
| Complete BUSCOs (%)             | 99.3            |
| Complete and single-copy BUSCOs (%)| 98.6        |
| GC content (%)                  | 48.95           |
| Protein coding genes            | 8963            |
| Annotated genes                 | 8688            |
| Unannotated genes               | 275             |
| Average gene length (bp)        | 2350.55         |
| tRNA (copy)                     | 94              |
| rRNA (copy)                     | 67              |
| snRNA (copy)                    | 19              |
suppress powdery mildew disease in wheat. To visualize the interactions between mycoparasites and Bgt, GFP was transformed into the BCAs to examine their effect on powdery mildew fungi (Lefebvre et al. 2013; Németh et al. 2019). Previously, S. lanosonivem was transformed with GFP to monitor its infection processes in Phakopsora pachyrhizi (Gauthier et al. 2014). In this study, GFP transformation improved the visualization of mycoparasite structures of S. aogashimaense. S. aogashimaense formed dense mycelia and produced conidiophores on Bgt colonies and consequently inhibited the conidial distribution of Bgt (Fig. 4a–c). In some cases, the Bgt conidia exhibited a fluorescence signal, suggesting that S. aogashimaense can directly penetrate the structures of Bgt (Fig. 4g–i). Similar phenomenon was observed in other Simplicillium spp., which directly penetrate rust pathogens (Gauthier et al. 2014; Wang et al. 2020).

In this study, GFP was stably expressed using the ATMT method (Fig. 4d–f). This indicates that the ATMT method can be applied for transforming physiologically and phylogenetically diverse Simplicillium spp. and for functional analyses of genes and proteins involved in mycoparasitism and metabolism.

In this study, the exudates of S. aogashimaense efficiently inhibited conidial germination and appressorial differentiation (the pre-penetration processes) of Bgt. This suggests that S. aogashimaense produces growth-inhibitory components against Bgt (Fig. 5). These results are consistent with those of previous studies, which demonstrated that S. lanosonivem releases growth-inhibitory substances against various phytopathogens. Previous studies have reported that S. obtusatum produces cyclic peptides with antifungal activities (Chen et al. 2017; Liang et al. 2017). The exudates of S. aogashimaense effectively suppressed the pre-penetration processes that are prerequisite for successful Bgt infection. Thus, the metabolites released from S. aogashimaense can be utilized as bio-fungicides for the management of powdery mildew diseases (Zabka et al. 2008; Hansjakob et al. 2010, 2012).

Secondary metabolite biosynthesis gene cluster analysis revealed 41 gene clusters that were potentially related to antifungal, antibacterial and insecticidal compounds (Additional file 1: Table S2). Three gene clusters were similar to those involved in the biosynthesis of trichodiene (13% similarity), squalestatin 1 (40% similarity) and AbT 1 (100% similarity), which are reported to exhibit antifungal activities (Baxter et al. 1992; Jones et al. 1992; Hasumi et al. 1993; Bills et al. 1994; Slightom et al. 2009; Tijerino et al. 2011; Malmierca et al. 2015a, b). However, the specific antifungal agents in exudates of S. lanosonivem are not known. Further studies are needed to screen the efficient metabolites of S. aogashimaense for fungal phytopathogen control.

Consistent with the microscopic observations, the biomass of S. aogashimaense significantly increased from 3 dpi, while that of Bgt markedly decreased from 3 dpi (Fig. 6). Previous studies also demonstrated the direct colonization and disease suppressive effects of Simplicillium spp. on rust pathogens (Gauthier et al. 2014; Wang et al. 2020). The findings of this study indicate that S. aogashimaense, a natural mycoparasite, can be an effective BCA for powdery mildew. However, the environmental factors affecting the mycoparasitism of S. aogashimaense on phytopathogens have not been completely elucidated. Further studies are needed to determine the optimal conditions for S. aogashimaense development to enable the application of this parasitic fungus for phytopathogen management under field conditions.

**Genome of S. aogashimaense**

At least 15 Simplicillium species have been currently identified. Some members of this genus exhibit growth-inhibitory activities against phytopathogens (Chen et al. 2019). However, the information on the genome of Simplicillium species is limited. Jauregui et al. (2020) reported a draft genome of S. aogashimaense, the only available genome for Simplicillium species, however, the parasitic mechanism and/or the discovery of antifungal secondary metabolites in S. aogashimaense cannot be deeply mined owing to the limited assembly and annotation of the draft genome. Therefore, the genome of S. aogashimaense was sequenced, assembled and annotated at the chromosomal level in this study (Fig. 7). The genome size of the test strain was 30.26 Mb, which is slightly larger than the previously reported draft genome of S. aogashimaense (strain 72–15.1) (Jauregui et al. 2020). Additionally, the genome comprised 8963 protein-coding genes. RNA-seq analysis revealed 2094 novel transcripts. This indicates that the gene expression patterns in S. aogashimaense vary. Genome data provided novel insights into the genomic features of S. aogashimaense, which can be applied for mining key genes related to mycoparasitic processes and antifungal secondary metabolites. Previously, the effectors involved in the interaction between Pseudomonas flocculosa and B. graminis have been elucidated using genome and RNA sequencing (Laur et al. 2018). Sao008714 and Sao006491 were putative effector-coding genes, which were highly similar to their homologs in P. flocculosa and Trichoderma spp. (Additional file 2: Figures S1, S2). The expression levels of Sao008714 and Sao006491 were significantly upregulated from 3 dpi (Additional file 2: Figure S3). Trichoderma spp. are reported to act as BCAs for various phytopathogens (Harman 2006; Moya et al. 2020; Sood et al. 2020; Zin and Badaluddin 2020). Therefore, these two genes, which putatively
code for effectors, may play crucial roles in mycoparasitic interactions. Effectors are reported to play crucial roles in phytopathogen-host and mycoparasitic agent-phytopathogen systems (Laur et al. 2018; Li et al. 2021; Yuan et al. 2021). However, the effectors and their mechanisms in the interaction between S. aogashimaense and Bgt have not been elucidated. The effectors in S. aogashimaense and their functions during mycoparasitism should be elucidated in the future.

Conclusions
This study provides novel insights into the host range and developmental niches of S. aogashimaense that parasitizes Bgt colonies and helps fill knowledge gaps in the understanding of the interaction between S. aogashimaense and B. graminis. The major findings of this study are as follows: (1) S. aogashimaense directly inhibits conidiophore formation and conidial distribution of Bgt; (2) S. aogashimaense decreases the biomass of Bgt during parasitic interaction, demonstrating its potential application in the management of wheat powdery mildew; (3) the visualization of S. aogashimaense is improved with GFP transformation using the ATMT method, which can be applied for genetic modification of Simplicillium spp.; (4) the exudates of S. aogashimaense suppressed conidial germination and appressorial differentiation (the pre-penetration processes) of Bgt; (5) the chromosome-scale genome assembly of S. aogashimaense revealed distinct features that can be used for mining key genes in future studies.

Methods
Fungal and plant materials
Wheat (cv. Aikang 58) was sown in plastic pots (9 cm in diameter) filled with soil collected from the field and maintained in growth chambers at 18 °C, with a light intensity of 200 μmol photons/m²/s and a photoperiod of 16-h light/8-h dark and 70% relative humidity. Bgt was propagated on its host plants under the same conditions. One day before inoculation, heavily infected host leaves were warily shaken to remove older Bgt conidia, and freshly formed spores were then used for further experimentation.

The mycoparasite was isolated from Bgt-infected wheat leaves at a cultivation field and cultured on PDA medium at 25 °C in the dark until fungal colonies appeared. To obtain a pure strain, individual spores of the mycoparasite were isolated and transferred onto PDA medium to allow colony development at 25 °C in the dark. Isolation was conducted twice and the purified strain was used for further assays.

Microscopic observation
To determine morphological characteristics, the mycoparasite was initially imaged under a light microscope (Sunny Optical, EX30, Zhejiang, China) and the images were analyzed using ImageJ software. The fungal structures were examined under a scanning electron microscope (Hitachi TM3030Plus, Japan) following a previously reported method (Zhu et al. 2020a).

DNA extraction and amplification
Total genomic DNA was isolated from the mycoparasite according to a previously reported method (Zhu et al. 2019). The ITS region of rDNA was PCR amplified with ITS1/ITS4 primer pairs (White et al. 1990). PCR was performed using a C1000 TouchTM Thermal Cycler (Bio-Rad, Hercules, California, United States). The PCR conditions were as follows: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and a final elongation at 72 °C for 5 min. The amplicon was sequenced (Invitrogen, Shanghai, China) and the sequence was deposited in GenBank (Accession no. MT936440).

Phylogenetic analysis
The ITS sequences of Simplicillium spp. were retrieved from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) database and aligned using ClustalW in MEGA software (version 10.1.8). The phylogenetic tree was constructed using MEGA software. The maximum likelihood method was used for phylogenetic tree construction with the options of 1000 bootstrap replicates, Tamura-Nei model and 50% site coverage cut off. The ITS sequences of Arthrocladiella mougeotii and Erysiphe sp. were used as the outgroup (Zhu et al. 2020b, c).

Hyperparasitism assays
The leaves of 14-day-old wheat seedlings (cv. Aikang 58) were inoculated with Bgt conidia and incubated in a growth chamber under the conditions described above. At 6 dpi, plants were inoculated with spore suspension (1 × 10⁶ spores/mL) of the identified S. aogashimaense and the inoculated plants were incubated in a growth chamber. Bgt-inoculated plants treated with water served as controls. At 3, 6 and 9 dpi, the leaves were collected for microscopic analysis. The leaves were bleached to observe Bgt and S. aogashimaense under a light microscope (Zhu et al. 2017). The fungal structures were stained with trypan blue in acetic acid/water/glycerol (1:1:1, v/v/v) for 1 h.
Fungal biomass determination
To determine the effect of *S. aogashimaense* on *Bgt* biomass, *Bgt*-infected leaves were treated with water or *S. aogashimaense* spore suspension (1 × 10⁶ spores/mL). At 3, 6 and 9 dpi, DNA was isolated from the samples using TRIzol reagent, following the manufacturer’s instructions (Invitrogen). The DNA was stored at -80 °C until qPCR analysis. The elongation factor 1 (EF1) alpha-encoding genes of wheat, *S. aogashimaense* and *Bgt* were amplified with the following primers: wheat EF1, ATGGTGTGCTTCTCTTCCAA (forward) and GACGATGCGAGTCAACGCCTTT (reverse); *S. aogashimaense* EF1, ATGGGTGCGCTTCTTCCAA (forward) and GACGATGCGAGTCAACGCCTTT (reverse); *Bgt* EF1, AAGCTAAAGGCGCAAGCTGTA (forward) and GCACAGTCAGCTTGAGGT (reverse) (Coram et al. 2008; Hu et al. 2018). To generate the standard curves, the fusion plasmids of wheat EF1, *S. aogashimaense* EF1 were serially diluted and subjected to qPCR analysis with selective primers to obtain the cycle threshold (Ct) values. The biomasses of wheat, *Bgt* and *S. aogashimaense* were calculated according to the corresponding standard curves. All qPCR experiments were conducted using a LightCycler 96 real-time PCR instrument (Roche, Switzerland). The PCR conditions were as follows: 95 °C for 15 min, followed by 45 cycles of 95 °C for 10 s and 60 °C for 30 s.

Pre-penetration processes of *Bgt*
To determine the effect of *S. aogashimaense* exudates on *Bgt* conidial germination and appressorial differentiation in vitro, *S. aogashimaense* was inoculated onto PDA and harvested at 20 dpi. PDA with *S. aogashimaense* (Ø = 1 cm) was then placed in a centrifuge tube with PDB and incubated on a shaker (150 rpm) at 20 °C for 6 days. Next, total PDB with white fungal structures was filtered through a filter paper (Ø = 7 cm, Newstar, Hangzhou, China) and a 0.22 μm syringe filter (Jinteng, Tianjin, China). The resulting solution containing *S. aogashimaense* exudates was stored at 4 °C for further experiments. To observe the prepenetration processes of *Bgt*, Formvar®/wheat wax-coated glass slides were prepared according to a previously described method (Zhu et al. 2017). Briefly, wheat leaf wax was isolated using chloroform and mixed with Formvar® (w/v=0.5%). Cleaned glass slides were dipped into the Formvar®/wheat wax solution for 15 s and dried at room temperature for 24 h. The *S. aogashimaense* exudate (treated group) or filtered PDB (control group) was sprayed onto Formvar® wheat wax-coated slides and dried for at least 12 h. *Bgt* conidia were inoculated onto glass slides and incubated for 18 h according to a previously reported method (Zhu et al. 2019). Individual conidia on the surface of each slide were observed under a light microscope to determine whether the spores remained non-germinated, had formed a primary germ tube, a secondary germ tube, a swollen appressorial germ tube or an appressorium. Only individual, well-separated conidia were counted in each experiment to eliminate the possible inhibition caused by crowding.

Chromosome-scale genome assembly of *S. aogashimaense*
To sequence the *S. aogashimaense* genome, genomic DNA was extracted from the colonies at 25 dpi using the cetyltrimethylammonium bromide method. Single-molecule real-time libraries were constructed and sequenced using a PacBio Sequel II instrument (Pacific Biosciences, Menlo Park, CA, USA) at Frasergen Bioinformatics Co., Ltd. (Wuhan, China). In total, 31.33 G polymerase reads for raw data and 31.1 G subreads for clean data were obtained after the removal of adaptor sequences from the sequencing data. RNA-seq was performed to sequence the full-length transcripts using Illumina® sequencing. *S. aogashimaense* RNA was extracted from the same colonies using TRIzol reagent (Invitrogen) following the manufacturer’s instructions. RNA-seq libraries were prepared using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer’s instructions. The libraries were sequenced on the Novaseq 6000 platform at Frasergen Bioinformatics Co., Ltd. (Wuhan, China).

Pseudochromosomes (superscaffolds) were determined using Hi-C analysis as described previously (Dudchenko et al. 2017). Briefly, 11 Gb of clean read pairs were obtained from the Hi-C library and mapped to the polished *S. aogashimaense* contig assembly using Juicer (version: 1.6) with default parameters (Durand et al. 2016). The LACHESIS tool (Burton et al. 2013) was used to cluster contigs (n=18) into chromosome-level scaffolds using the genomic proximity signal of Hi-C data.

GFP transformation
To transform *S. aogashimaense*, the binary vector pPK2Tgfp (Martinez-Cruz et al. 2016) was transferred into the *A. tumefaciens* strain GV3101. The transformed strain was cultured in 2 mL of YEP medium containing 50 μg/mL kanamycin and 50 μg/mL gentamicin in an orbital shaker at 140 rpm and 28 °C for 24 h. Next, the *A. tumefaciens* strain was directly pipetted onto *S. aogashimaense* culture grown on PDA and incubated for 4 days at room temperature (Németh et al. 2019). Subsequently, the spores of *S. aogashimaense* were inoculated onto PDA medium containing 50 μg/mL carbenzazim and 100 μg/mL cefotaxime and incubated for 10 days at room temperature. Newly formed colonies, which were
transformed *S. aogashimaense* colonies, were observed under a fluorescence microscope (BX63, Olympus, Japan).

**Abbreviations**

Agt: Swollen appressorial germ tube; App: Appressorium; ATMT: Agrobacterium tumefaciens-mediated transformation; BCAs: Biocontrol agents; Bgt: *Blumeria graminis* f. sp. *Triticum*; Ct: Cycle threshold; dpi: Days post-inoculation; GFP: Green fluorescent protein; ITS: Internal transcribed spacer; Ng: Non-germinated conidium; PDA: Potato dextrose agar; PDB: Potato dextrose broth; Pgt: Primary germ tube; Sz: *S. aogashimaense*; Sgt: Secondary germ tube; Ta: *Triticum aestivum*.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s42483-022-00121-5.

Additional file 1: Table S1. Novel transcript annotation of *Simplicillium aogashimaense*. Table S2. AntiSMASH analysis of secondary metabolite biosynthesis gene clusters in *Simplicillium aogashimaense*. The genome-wide identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in *S. aogashimaense* were performed following a previously described method (Blin et al. 2019).

Additional file 2: Figure S1. Alignment (a) and phylogenetic analysis (b) of Sao008714 and its homologs from *Trichoderma* spp. and *Pseudozyma flocculosa* were performed using DNA-MAN and MEGA, respectively. The evolutionary tree was constructed using the maximum likelihood method with the options of 1000 bootstrap replications, Tamura-Nei model and 50% site coverage cut-off (Zhu et al. 2022).

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**Availability of data and materials**

Raw sequencing reads of *Simplicillium aogashimaense* genome reported in this study were deposited into the public database of NCBI (BioProject accession no. PRJNA793154). RNA-seq raw data was also deposited under PRJNA793154.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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