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ORIGINAL ARTICLE

Multicellular growth of the Basidiomycota phytopathogen fungus *Sporisorium reilianum* induced by acid conditions

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Abstract

Fungi are considered model organisms for the analysis of important phenomena of eukaryotes. For example, some of them have been described as models to understand the phenomenon of multicellularity acquisition by different unicellular organisms phylogenetically distant. Interestingly, in this work, we describe the multicellular development in the model fungus *S. reilianum*. We observed that *Sporisorium reilianum*, a Basidiomycota cereal pathogen that at neutral pH grows with a yeast-like morphology during its saprophytic haploid stage, when incubated at acid pH grew in the form of multicellular clusters. The multicellularity observed in *S. reilianum* was of clonal type, where buds of "stem" cells growing as yeasts remain joined by their cell wall septa, after cytokinesis. The elaboration and analysis of a regulatory network of *S. reilianum* showed that the putative zinc finger transcription factor CBQ73544.1 regulates a number of genes involved in cell cycle, cellular division, signal transduction pathways, and biogenesis of cell wall. Interestingly, homologous of these genes have been found to be regulated during *Saccharomyces cerevisiae* multicellular growth. In adddition, some of these genes were found to be negatively regulated during multicellularity of *S. reilianum*. With these data, we suggest that *S. reilianum* is an interesting model for the study of multicellular development.

Keywords *Sporisorium reilianum* · Multicellular growth · Acid conditions · Fungal development

Introduction

Multicellularity is the developmental process by which unicellular organisms became pluricellular during evolution, possibly by two mechanisms: (i) cell aggregation and binding to constitute a single structure; or (ii) a mechanism involving the attachment of descendents of a stem cell after cell division, all sharing the same genotype (Grosberg and Strathmann 2007; Rensing 2016; Rivera-Yoshida et al. 2018). In the literature it has been described that multicellularity occurred early and repeatedly during the evolution of life, and that its development involved environmental, ecological, and genetic factors (Rokas 2008). Accordingly, multicellularity has different levels of complexity, going from a simple aggregation or

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union of cells forming sheets, films, pseudofilaments, filaments, or clusters, to the most complex organisms (Knoll 2011; Oud et al. 2013; Ratcliff et al. 2015; Nagy et al. 2018; Rivera-Yoshida et al. 2018). Under this principle, the capacity of acquiring a multicellular phenotype has been described even in microorganisms phylogenetically distant including bacteria, algae, and fungi that are normally considered as unicellular organisms (Bonner 1998; Grosberg and Strathmann 2007; Smukalla et al. 2008; Knoll 2011; Koschwanez et al. 2011; Oud et al. 2013; Ratcliff et al. 2015; Arias Del Angel et al. 2017). This capacity has been described as a mechanism for tolerance to environmental stress, to increase affinity for substrates and obtainment of nutrients, for defense against predators, and possibly to ensure offspring by production of a protected internal environment (Kessin et al. 1996; Bonner 1998; Boraas et al. 1998; Koschwanez et al. 2011).

In the case of fungi, acquisition and loss of multicellularity has occurred several times, being periodically lost during evolution. The mechanisms for these processes has shown to be difficult to analyze, although some of them have been proposed (Grosberg and Strathmann 2007; Rensing 2016; Bonner 1998). For these reasons, the idea to search for simple models that even today might acquire (or lose) this capacity under different conditions has been found to be attractive to different investigators. Interestingly, through the analysis of the effect of different growing conditions on the Basidiomycota phytopathogenic fungus *Sporisorium reilianum*, we observed that its cultivation under acidic conditions led the fungus to form multicellular clusters. *S. reilianum* is a dimorphic biotropic fungus with a parasitic mycelial phase, and a saprobiotic yeast-like phase during its life cycle (Bhaskaran and Smith 1993; Martinez et al. 2002). *S. reilianum* infects maize (*Zea mays*) and sorghum (*Sorghum bicolor*) causing the disease known as head smut (Bhaskaran and Smith 1993; Martinez et al. 2002; Poloni and Schirawski 2016). In nature, there are two formae speciales of this fungus that infect sorghum or maize [*S. reilianum* f. sp. *reilianum* (SRS) and *S. reilianum* f. sp. *zeae* (SRZ), respectively (Zuther et al. 2012)]. The preference of the fungus to infect one or the other host plant is apparently due to the ability of each formae speciale to deal with the defense mechanisms of their corresponding host plant (Poloni and Schirawski 2016). Recently, the ability of *S. reilianum* to infect *Arabidopsis thaliana* plants has been described and shown that in this experimental host the fungus induces severe physiological changes such as, size reduction, and overproduction of roots and anthocyanins (Martínez-Soto et al. 2019).

S. reilianum presents different characteristics that make it an attractive model of study for different types of phenomena. Among them, we may cite the following: small genome, short life cycle, easy handling in the laboratory, different lifestyles (saprophytic or pathogenic), different morphologies of growth (yeast-like or mycelium), and that fact that its sequenced genome has been partially annotated. Considering these characteristics, in the present work we have proceeded to describe and analyze the multicellular growth capacity of this model fungus.

Materials and methods

Sporisorium reilianum strain, culture media, and culture conditions

The wild type haploid strain of *Sporisorium reilianum* SRZ2 (*a2b2*) used in this study was kindly provided by Prof. Dr. Jan Schirawski (Institute of Applied Microbiology, RWTH Aachen University, Germany). The cells were maintained in 50% glycerol at − 70 °C, recovered, and incubated in liquid complete medium (MC (Holliday 1974)) at 28 °C and 200 r.p.m. for 24 h, and used as inocula for the experiments described below.

Determination of the growth of *S. reilianum*

Cells from cultures were recovered by centrifugation at 2500*g* for 10 min. Sedimented cells were washed three times with SDW by centrifugation, and the protein content was measured by the Bradford method (Kruger 1994), using bovine serum albumin as a standard.

Induction of multicellularity in *S. reilianum*

The haploid strains of the fungus were treated by the protocol described for mycelium induction in *Ustilago maydis* by acid pH (Ruiz-Herrera et al. 1995). Briefly, 10^6 cells/mL (counted using a Neubauer cytometer (Hausser Scientific, Horsham PA, US)) was inoculated in liquid complete medium (CM (Holliday 1974)) and grown for 24 h. Cells were sedimented and washed by centrifugation in sterile distilled water (SDW) and shaken in SDW for 3 h, centrifuged, washed, sauspended in SDW and incubated at 4 °C. Cells (10^6 cells/mL) were then inoculated in minimal medium (MM (Holliday 1974)) of different pH values (from 2 to 9), and incubated at 28 °C and 200 r.p.m. for 3 days. At intervals, aliquots were recovered to determine growth and morphology of the cells. Formation of aggregates from single cells was followed on solid MM and observed by phase contrast microscopy (see below).

Alkaline treatment of multicellular clusters

In order to determine whether the cells forming multicellular clusters were bound or merely adsorbed, these were recovered by centrifugation, washed three times with SDW, and treated with 1 M NaOH at 100 °C for 10 min. The treated cells were recovered by centrifugation, washed, suspended in with SDW, and observed by bright field and epifluorescence microscopy as described below.

Microscopy

Yeast-like cells or cell clusters were either observed by phase contrast microscopy, or stained with cotton blue-lactophenol (Sigma-Aldrich, 61335) or calcofluor white (Sigma-aldrich, 18909), and observed by bright field or epifluorecence respectively with a Leica DMRE microscope (Leica microsystems; Wetzlar, DE), and photographed with a Leica camera DFC450 C (Leica microsystems; Wetzlar, DE).

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were performed according to protocols described by Chávez-Munguía and Martínez-Palomo (2011). Briefly, TEM cells were fixed at room temperature during 1 h with 2.5% (v/v) glutaraldehide in 0.1 M sodium cacodylate buffer pH 7.2, followed by dehydratation periods in increasing concentrations of ethanol and propylene oxide. Samples were embedded in polybed epoxy resins and polymerized at 60 °C during 24 h; thin sections were made and contrasted with uranyl acetate and lead citrate. Finally, the samples were observed in a transmission electron microscope JOEL JEM 1011 (Peabody, MA). For SEM, cells were fixed and dehydrated as described above, and critical drying point was performed in a Samdri 780 (Rockville, MD). Finally, the samples were covered with gold ions in a JOEL JFC-1100 sputtering device, and observed in a scanning electron microscope Gemini DSM982 (Carl Zeiss, Peabody, MA).

Bioinformatic analyses and development of a transcriptional network

Genes of *S. reilianum* [forma specialis SRZ2 (taxid:999809)] with homology or similar functions to those described during the multicellular growth of *S. cerevisiae* (Oud et al. 2013; Ratcliff et al. 2015) were used for the design and analysis of the transcriptional network. Amino acid sequence of the *S. cerevisiae* proteins were obtained from *Saccharomyces* Genome Database (SGD) (https://www.yeastgenome.org/), and protein BLAST analysis was performed in the online page of National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/).

The accession numbers of the *S. reilianum* sequences were obtained from NCBI. The gene network was made using as model the transcriptional network of *S. cerevisiae* (Teixeira et al. 2018), and displayed using the Cytoscape 3.6.1 program (Shannon et al. 2003).

Expression of genes possibly involved in multicellular growth

Expression of some genes of *S. reilianum* analyzed or identified in the transcriptional network was analyzed by qRT-PCR of cells grown for 24 and 72 h under conditions that induced multicellular development in *S. reilianum*. Cells were recovered from the culture media by centrifugation, total RNA was extracted with TRIzol (Invitrogen, CA, USA) using EZNA Plant RNA Kit (OMEGA Bio-Tek), and treated with DNAse I (Invitrogen, CA, USA). RNA integrity was determined by electrophoresis in agarose gels, and the concentration was measured with a Nanodrop (Thermo Scientific, MA, USA). KAPA SYBR FAST qPCR Master Mix kit with ROX (Kapa Biosystems, Basilea, Suiza) used according to the instructions of manufacturer in StepOne Real-Time PCR Systems (Applied Biosystems, CA, USA) for qRT-PCR analysis. The expression levels were calculated according to Palomeros-Suárez et al. (2017), using the conditions described by Zhao (2015), and Zhao et al. (2015), to normalize the expression levels of target genes in cluster of cells. For qRT-PCR analyses, three biological replicates with the mixture of two technical replicas of the fungus grown in MM pH 3 or pH 7 were performed. The genes analyzed and their respective primers are listed in the Supplementary Table S1.

Results

Growth and morphology of *S. reilianum* under different pH values in the culture medium

S. reilianum was able to grow at pH 3 and 7, although a slightly better growth was observed at neutral pH (Fig. 1a). During growth at either pH, the fungus only produced a weak acidification after about 18 h of growth (Fig. 1b). However, the morphology was dramatically different when grown at acid pH values. Interestingly, under acid conditions (pH 3 or less), the fungus grew in the form of large clusters of spheroidal cells (Fig. 2a and b; Table 1), which easily sedimented in the culture medium (Fig. 2c; Table 1). In culture medium of pH values of 7 or higher, the fungus grew in the form of yeast-like spheroidal cells (Fig. 2d and e), that did not sediment in the culture medium (Fig. 2c; Table 1).

Interestingly, it was observed that the multicellular growth observed in *S. reilianum* under acid conditions was reversible. Cells initially grown as cell clusters in culture medium at pH 3, showed offspring yeast-like growth when they were re-inoculated in culture medium of pH 7, where the new buds no longer remained attached to the "stem" cells after cytokinesis; and those cells initially grown as yeasts in culture medium at pH 7 showed multicellular growth when they were re-inoculated under acidic conditions (Table 2).

Monitoring of the multicellular growth of *S. reilianum* under acid conditions

The multicellular development of *S. reilianum* was followed by observation of single cells. Accordingly, it was observed that under acid conditions the "stem" cell growing yeast-like proceeded to form a bud-like daughter cell that remained bound to the mother cell, and in due time also generated their own daughter cells by a

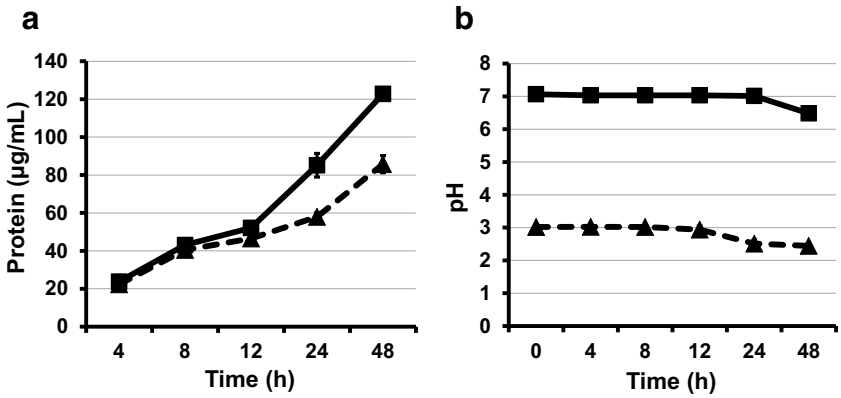

Fig. 1 Growth of *S. reilianum* in MM at different pH values. **a**, Growth of *S. reilianum* at pH 3 or 7 expressed as cell protein. **b**, pH determination in the culture medium. Squares and solid lines, pH 7 MM. Triangles and dotted lines, pH 3 MM. Results from three independent experiments with two replicas in each one. Bars represent standard error values

process that was repeated consecutively without cellular separation giving rise to the formation of large cellular clusters. One representative example of this behavior is shown in Fig. 3.

S. reilianum cells in the clusters are separated by cell wall septa

To determine if the cells in the clusters were bound by some secreted sticky material, or really bound and separated by cell wall septa, samples of cell clusters were heated in 1 M NaOH, washed, stained with calcofluor white, and observed under fluorescence microscopy. It was observed that after this treatment the cells remained bound in the original cell clusters (Fig. 4). To observe in more detail how the cells were linked, cell clusters were analyzed by scanning (SEM) and transmission (TEM) electron microscopy. The results obtained by SEM (Fig. 5a and b) offer a better three-dimensional perspective of the organization of the clusters, or yeast-like cells

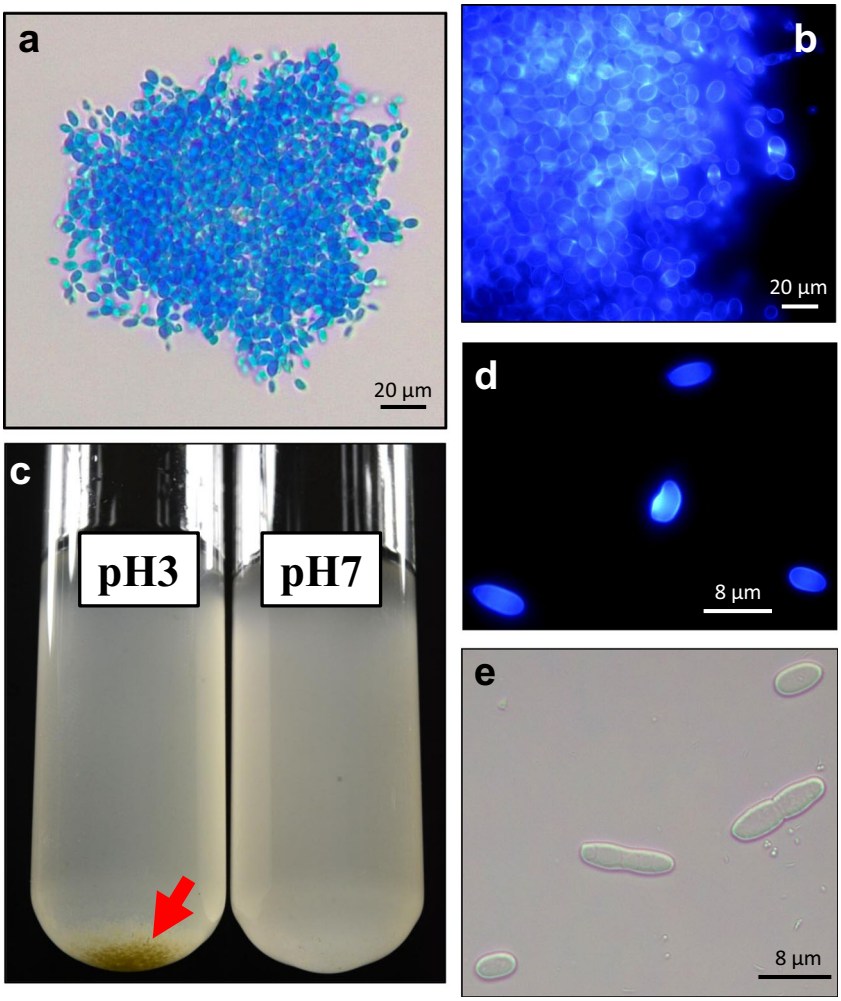

Fig. 2 Phenotype of *S. reilianum* grown at different pH values. **a** and **b**, multicellular phenotype of cells grown at pH 3 (brigth field or fluorescence microscopy respectively). **c**, photograph showing sedimentation (red arrow) of cells grown only at acid pH values, almost immediately after stop of shaking. **d** and **e**, yeast-like morphology of cells grown at pH 7 (fluorescence or phase contrast microscopy respectively)

Table 1 Multicellular growth of *S. reilianum* at different pH values

MM pH	Percent of multicellularity*	Observation of multicellular sediment
2	93.5 (± 2.3)	+
3	99.1 (± 0.5)	+
4	83.7 (± 3.0)	+/−
5	56.2 (± 1.4)	−
6	5.6 (± 1.3)	−
7	0.4 (± 0.1)	−
8	0.4 (± 0.2)	−
9	26.4 (± 7.4)	−
10	11.9 (± 1.4)	−

MM, minimal medium culture

* Percentage of multicellular clusters observed. The cells grown yeast-like or clusters were counted under a Bright-field microscopy in five different fields, and in nine independent repetitions (n = 9) for each pH value analyzed

grown at pH 3 or pH 7 respectively. In Fig. 5c and d, the details of the wall septa that separate and bind the cells forming the clusters are clearly shown; and in Fig. 5e, the aspect of the starting budding of the mother cell can be appreciated. All these data clearly show that the cells forming the aggregates are really bound, and not merely adsorbed.

Transcriptional interaction of genes involved in the multicellular growth of *S. reilianum*

We analyzed whether *S. reilianum* has putative homologs for some of the *S. cerevisiae* genes described to be involved in its multicellular phenotype. We found that some of them were related to cell división, cell cycle, biogenesis and cell wall structure, and signal transduction (Supplementary Table S2). Interestingly, the network analysis showed that many genes putatively involved in the *S. reilianum* multicellular growth are putatively regulated by the zinc finger transcription factor CBQ73544.1 (Table 3; Fig. 6). Also, our analysis showed the non-existence in *S. reilianum* of homologs for the *FLO* genes described in *S. cerevisiae* as involved in yeast flocculation (Soares 2011).

Expression of genes involved in the multicellular growth of *S. reilianum*

We proceeded to analyze the expression levels of some genes of *S. reilianum* identified and analyzed by the transcriptional network presented in this work, at 24 and 72 h of growth under conditions of multicellularity induction. Among the genes analyzed were the zinc finger transcription factor CBQ73544.1, genes involved in the cell cycle and cell division, as well as genes related to cell wall biogenesis. These genes were chosen considering their importance in cellular phenomena related with the multicellular growth reported in other model organisms. Interestingly, all the genes analyzed were negatively regulated during the multicellular growth of *S. reilianum* (Table 4).

Discussion

Undoubtedly, one of the major transition points during the evolution of complex organisms was the appearence of multicellularity (Maynard-Smith and Szathmary 1995; Bonner 1998; Pfeiffer and Bonhoeffer 2003; Grosberg and Strathmann 2007). This process required the convergence of ecological, genetic, and environmental factors (Rokas 2008; Mora van Cauwelaert et al. 2016; Rivera-Yoshida et al. 2018); and was an adaptation mechanism to respond to different environmental conditions: response to stress (Smukalla et al. 2008), improvement to obtain and utilize nutrients

Table 2 Reversibility of the multicellular phenotype of *Sporisorium reilianum*

Hours of growth in MM pH 3		Morphology in MM pH 7 after 3 dpi	Hours of growth in MM pH 7		Morphology in MM pH 3 after 3 dpi
2	After the aforementioned hours, 1 mL of *S. reilianum* culture was changed to grow in MM pH 7	Yeast	2	After the aforementioned hours, 1 mL of *S. reilianum* culture was changed to grow in MM pH 3	Multicellular
4		Yeast	4		Multicellular
8		Yeast	8		Multicellular
12		Yeast	12		Multicellular
24		Yeast	24		Beginning of multicellular growth
48		Yeast	48		Beginning of multicellular growth
72		Yeast	72		Beginning of multicellular growth

MM, minimal medium culture; *dpi*, days post inoculation

n = 3, with 3 quantifications per treatment

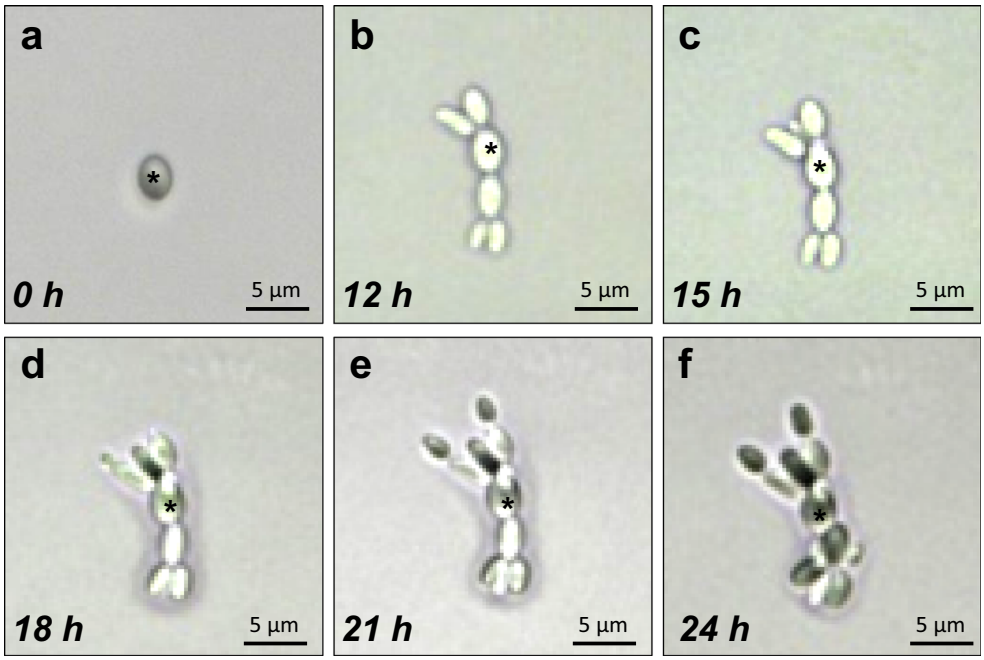

Fig. 3 Multicellular development of *S. reilianum* from a single cell. Photographs were sequentially obtained from the same parental cell (asterisk) grown under microculture conditions on solid MM pH 3. Numbers and letter h in italics in each image, represent the hours of cultivation

(Koschwanez et al. 2011; Biernaskie and West 2015), and protection from predation (Kessin et al. 1996; Boraas et al. 1998). In nature, this process involved the formation of cell clusters by an incomplete cell division (clonal multicellularity), or cell aggregation (aggregative multicellularity) (Bonner 1998; Grosberg and Strathmann 2007; Rensing 2016; Rivera-Yoshida et al. 2018).

Fungi have been recently considered useful model organisms for the study of multicellularity in eukaryotes phylogenetically distant (Oud et al. 2013; Ratcliff et al. 2015; Koschwanez et al. 2011). Fungi can display multicellularity at several stages of their development or under different environmental conditions. Examples of this phenomenon are the formation of pseudomycelium, truely septated mycelium (Du et al. 2015; Nagy et al. 2018; Rivera-Yoshida et al. 2018; Mowat et al. 2019), and even more elaborate structures like fruiting bodies (see Busch and Braus 2007; Kües and Navarro-González 2015; León-Ramírez et al. 2017; Nagy et al. 2018). In fact, other systems such as the yeast-to-mycelium dimorphic transition of different fungi are known, for example *C. albicans* (Saporito-Irwin et al. 1995; Kelly et al. 2004), *U. maydis* (Banuett and Herskowitz 1994; Ruiz-Herrera et al. 1995; Klose et al. 2004), *S. reilianum* (Bhaskaran and Smith 1993), *Y. lipolytica* (Szabo 1999; Palande et al. 2014), and others. Nevertheless, these later studies were focused as morphological changes during the virulence processes developed by some of these fungi, or merely as response mechanisms to different environmental conditions, and not exactly as multicellular phenomena.

One interesting aspect of the multicellularity phenomenon of *S. reilianum* is that it occurred by its cultivation under acid conditions without requiring long periods of culture in bioreactors, or by mutation or deletion of specific genes, as has been reported in *C. albicans* (Kelly et al. 2004) or *S. cerevisiae* (Oud et al. 2013; Ratcliff et al. 2015). The multicellularity phenomenon in *S. reilianum* is a clonal

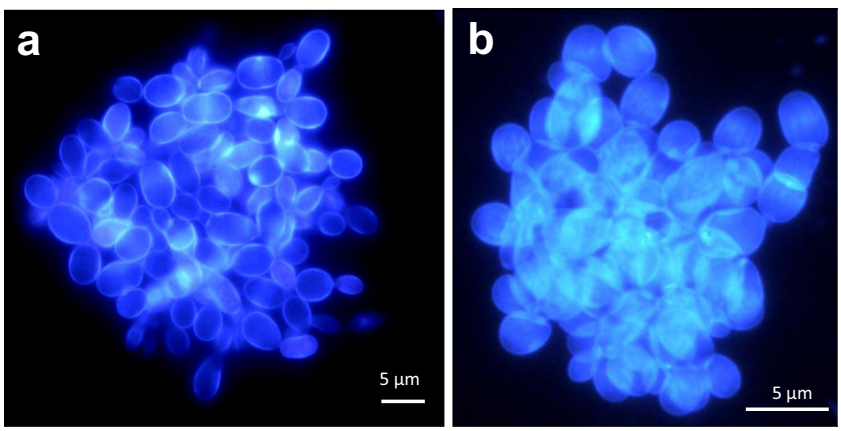

Fig. 4 Effect of hot alkaline treatment on multicellular clusters of *S. reilianum*. Microphotographs of untreated multicellular clusters of the fungus (**a**), or after treatment with 1 M NaOH at 100 °C for 10 min (**b**)

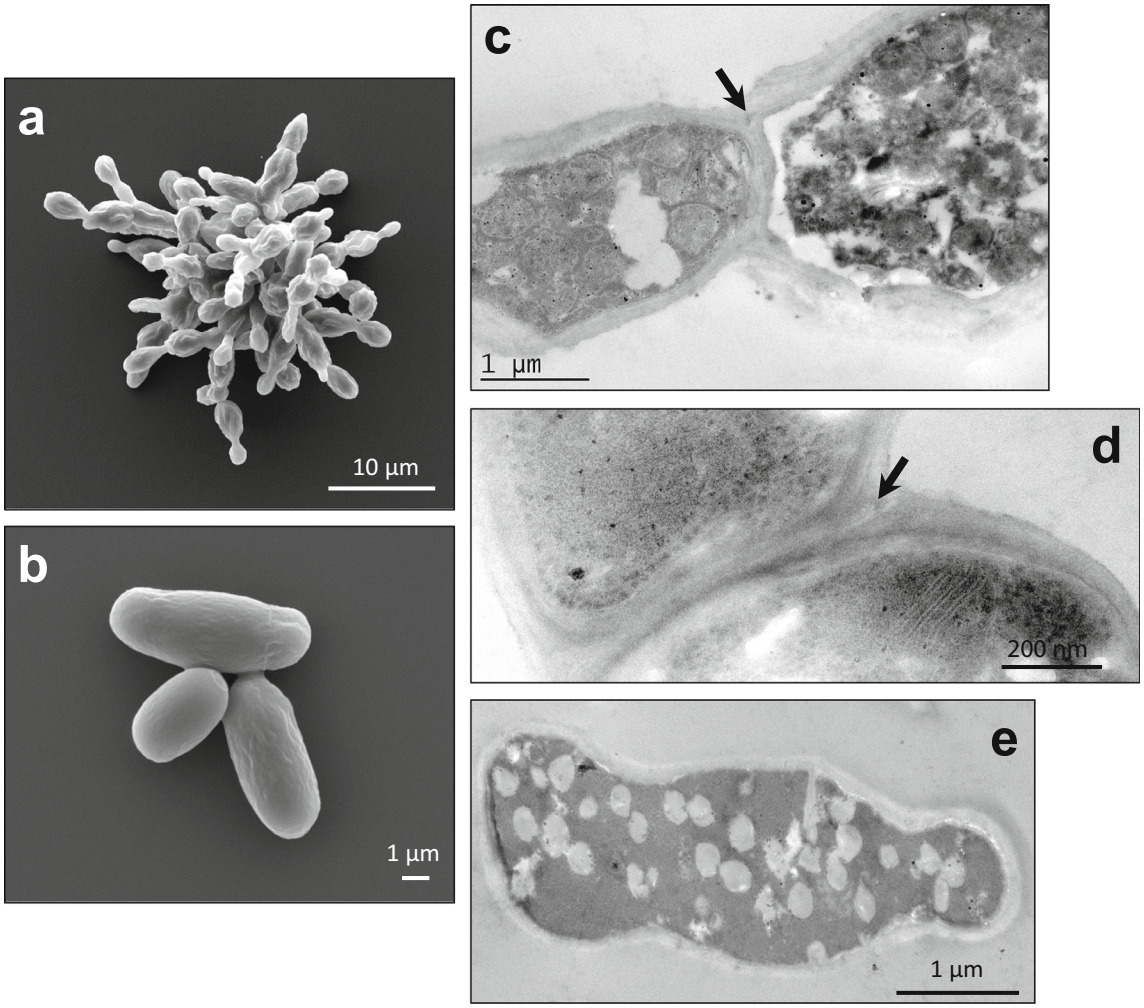

Fig. 5 Electron microscopy of the cellular morphology of *S. reilianum* grown in MM at different pH values. **a** and **b**, scanning electron microphotographs of the fungus growing as a cluster of cells, or yeast-like at pH 3 or pH 7 respectively. **c** and **d**, transmission electron microphotographs of the fungus growing as a cluster of cells at pH 3; and **e**, transmission electron microphotographs of the fungus growing yeast-like at pH 7. Notice in **c** and **d** the cell wall septa that both separates and binds the cells (black arrows) in multicellular clusters

phenomenon, where cells are not separated after cytokinesis, and continue physically joined by cell wall septa. This phenotype allows to keep together a group of cells with the same genetic information (clonal development), avoiding posible conflicts between the cells within the cluster. This process allows them to have a collective fitness which could have been a critical step during the origins of multicellularity (Bonner 1998; Koschwanez et al. 2011; Rivera-Yoshida et al. 2018).

It is also important to note that the multicellular phenotype of *S. reilianum* is reversible. Thus, a unicellular phenotype is initiated when the multicellular aggregates are changed to a neutral médium. Accordingly, new buds no longer remain attached to the "stem" cells after cytokinesis. This characteristic might be an important experimental advantage for the study of this model, since it allows the analysis of the genes differentially expressed during the processes of multicellularity and its dissipation.

We observed that during the multicellular growth of *S. reilianum* occurred the negative regulation of the zinc finger transcription factor CBQ73544.1, as well as genes under their putative regulation, according to the analysis of the network (see above). Among these genes we may cite some involved in the cell cycle, cell division, and cell wall biogénesis. According to these data, it may be hypothesized that under acid environmental conditions the transcription of the CBQ73544.1 gene is inhibited, thus preventing the transcription of the genes that it regulates, giving rise to the multicellular phenotype observed in *S. reilianum*.

These data suggest that the genetic control of multicellularity in phylogenetically separated fungi may involve similar mechanisms. This similarity has been hypothesized by different authors interested in the study of the origins of multicellularity on earth (Rokas 2008; Niklas 2014; Nagy et al. 2018).

Table 3 *Sporisorium reilianum* genes putatively involved in multicellular growth and putatively regulated by CBQ73544.1

ID gene	Description in National Center for Biotechnology Information (NCBI)
CBQ67444.1	Related to HMF1-heat-shock induceable inhibitor of cell growth
CBQ67486.1	Related to YTP1, probable type-III integral membrane protein of unknown function
CBQ67499.1	Probable DAK2-dihydroxyacetone kinase
CBQ67520.1	Related to glucan 1,3-beta-glucosidase precursor
CBQ67611.1	Related to GPI1-required for N-acetylglucosaminyl phosphatidylinositol synthesis
CBQ67731.1	Related to UTR2-cell wall protein, Chitin transglycosylase
CBQ67838.1	Probable TPS1, trehalose-6-phosphate synthase
CBQ67865.1	Related to MCD4-sporulation protein, morphogenesis checkpoint-dependent
CBQ68014.1	Probable SCH9-serine/threonine protein kinase involved in stress response and nutrient-sensing signaling pathway
CBQ68144.1	Related to MAPKK kinase
CBQ68592.1	Related to serine/threonine protein kinase
CBQ68853.1	Chitin synthase 2
CBQ68966.1	Related to protein kinase lkh1
CBQ69181.1	Related to EXG1-Exo-1,3-beta-glucanase precursor
CBQ69228.1	Related to CEF1-required during G2/M transition, essential splicing factor
CBQ69274.1	Protein kinase A, catalytic subunit
CBQ69286.1	Related to SPA2 protein, functions in actin cytoskeletal organization during polarized growth
CBQ69439.1	Related to PKH1-ser/thr protein kinases
CBQ69712.1	Prrobable MBF1-multiprotein bridging factor mediates GCN4-dependent transcriptional activation
CBQ70529.1	Probable cAMP-dependent protein kinase catalytic subunit
CBQ70570.1	Related to CDC50-cell division protein
CBQ70627.1	Related to SSD1 protein, translational repressor with a role in polar growth and wall integrity
CBQ70778.1	Related to GIS2-putative zinc finger protein, proposed to be involved in the RAS/cAMP signaling pathway
CBQ70856.1	Related to KRE6-glucan synthase subunit
CBQ71031.1	Related to C2H2-type zinc finger protein
CBQ71112.1	Related to serine/threonine protein kinase
CBQ71192.1	Related to transcription activator amyR
CBQ71356.1	Conserved hypothetical protein
CBQ71393.1	Related to spindle assembly checkpoint protein
CBQ71401.1	Conserved hypothetical protein
CBQ71675.1	Related to ECM4-involved in cell wall biogenesis and architecture
CBQ71851.1	Probable 1,3-beta-D-glucan synthase subunit
CBQ72260.1	Probable chitin deacetylase
CBQ72493.1	Related to YAK1-ser/thr protein kinase
CBQ72668.1	Related to serine/threonine-protein kinase
CBQ73003.1	Related to YRO2-putative plasma membrane protein, transcriptionally regulated by Haa1p
CBQ73514.1	Related to pH-regulated antigen pra1 precursor
CBQ73648.1	Related to trehalose-6-phosphate phosphatase
CBQ73835.1	Related to BDF1-sporulation protein, protein involved in transcription initiation

In summary, our results are evidence that *S. reilianum* is able to develop in a multicellular form. They also show that it possesses experimental advantages as a model organism to study the phenomenon of multicellularity. Among them we may cite the short period of time necessary to observe the development of a multicellular phenotype, the fact that this

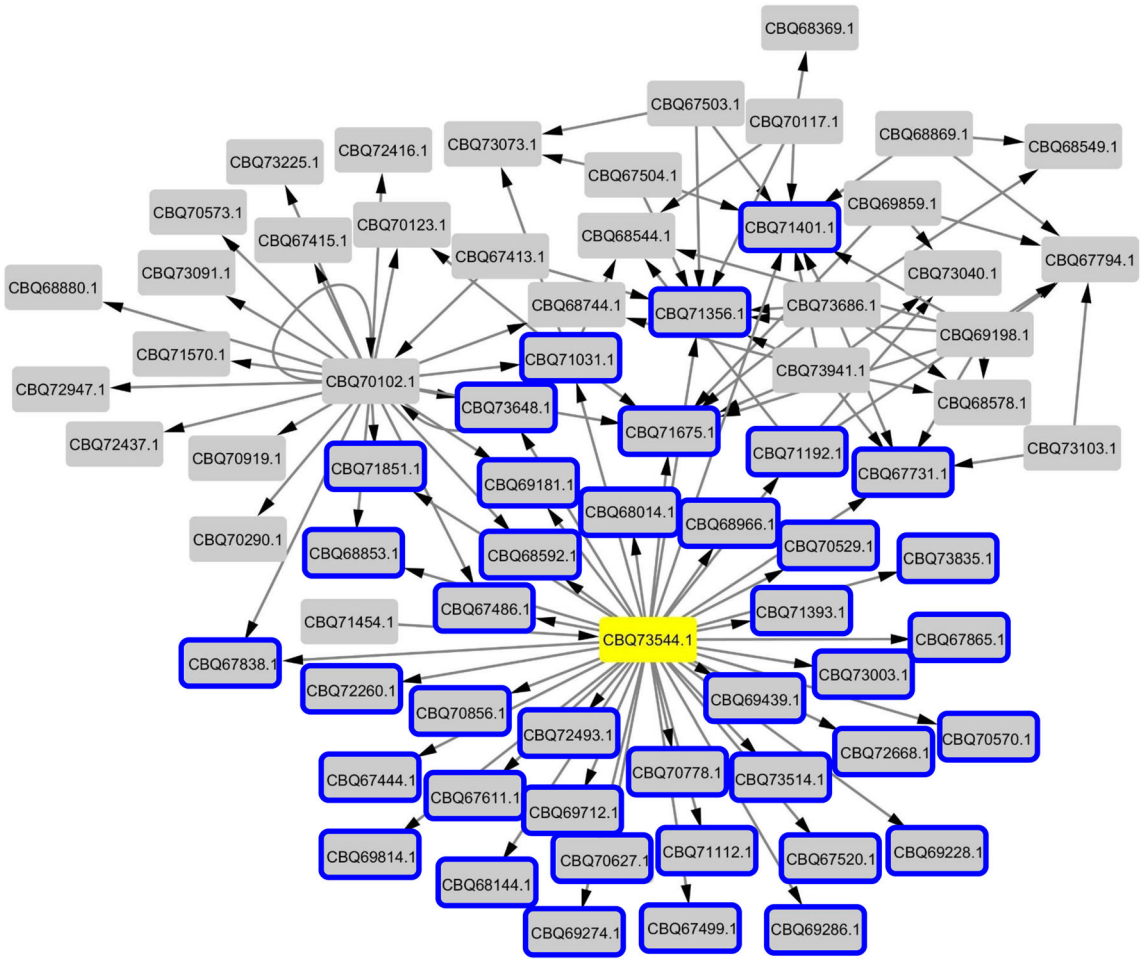

Fig. 6 Putative transcriptional network of the multicellular growth of *Sporisorium reilianum*. The genes framed in blue, are genes putatively regulated directly by the putative transcription factor CBQ73544.1 (highlighted in yellow)

Table 4 Relative expression of some genes represed during the multicellular growth of *Sporisorium reilianum*

ID gene (NCBI)	Description (NCBI)	Relative expression*	
		24 h of multicellular growth	72 h of multicellular growth
CBQ73544.1	Related to Zinc finger protein	− 1.15	− 4.55
CBQ67731.1	Related to UTR2-cell wall protein	− 2.99	− 7.75
CBQ68369.1	Probable Clb1-B-type cyclin 1	− 2.55	− 1.44
CBQ70570.1	Related to CDC50-cell division protein	− 7.16	− 1.31
CBQ68549.1	Related to SKT5-activator of chitin synthase III	− 4.03	− 4.37
CBQ71675.1	Related to ECM4-involved in cell wall biogenesis and architecture	− 1.94	− 1.09
CBQ72260.1	Probable chitin deacetylase	− 2.51	− 3.22

*The data were calculated by comparing gene expression levels during *S. reilianum* multicellular growth, against yeast-like growth

phenomernon is induced by a simple change in pH, not requiring a mutation or deletion, and the observation that this phenomenon is reversible.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interests.

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