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ORIGINAL PAPER

Penicillium roqueforti conidia induced by L-amino acids can germinate without detectable swelling

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Abstract *Penicillium roqueforti* is used for the production of blue-veined cheeses but is a spoilage fungus as well. It reproduces asexually by forming conidia. Germination of these spores can start the spoilage process of food. Germination is typically characterized by the processes of activation, swelling and germ tube formation. Here, we studied nutrient requirements for germination of *P. roqueforti* conidia. To this end, > 300 conidia per condition were monitored in time using an oCelloScope imager and an asymmetric model was used to describe the germination process. Spores were incubated for 72 h in $NaNO_3$, Na_2HPO_4/NaH_2PO_4, $MgSO_4$ and KCl with 10 mM glucose or 10 mM of 1 out of the 20 proteogenic amino acids. In the case of glucose, the maximum number of spores (P_{max}) that had formed germ tubes was 12.7%, while time needed to reach 0.5 P_{max} (τ) was about 14 h. Arginine and alanine were the most inducing amino acids with a P_{max} of germ tube formation of 21% and 13%, respectively, and a τ of up to 33.5 h. Contrary to the typical stages of germination of fungal conidia, data show that *P. roqueforti* conidia can start forming germ tubes without a detectable swelling stage.

Keywords Conidia · Fungus · Germination · Germ tube · Penicillium · Swelling

Introduction

Penicillium roqueforti is known for its use in production of blue-veined cheeses but is a food spoilage fungus as well of for instance rye bread and dairy products (Coton et al. 2020; Samson et al. 2010). The ability of *P. roqueforti* to grow at high CO_2 concentrations (up to 84.8%), low O_2 levels (as low as 0.3%), and at low temperature (as low as 0 °C) distinguishes this species from other filamentous fungi (Kalai et al. 2017; Nguyen Van Long, et al. 2017b) and makes it a common spoilage fungus. Spoilage is facilitated by the fact that *P. roqueforti* disperses high numbers of stress resistant asexual spores called conidia via air, water or other vectors (Dijksterhuis 2017; Punt et al. 2020; Wyatt et al. 2013).

Germination of fungal conidia is characterized by the stages of activation, swelling and germ tube formation (d'Enfert 1997). Activation of resting

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conidia results in swelling of the spores. This period of isotropic growth is followed by polarized growth resulting in the production of a germ tube that grows out into a hypha. Conidia of *Cladosporium halotolerans* and *Penicillium rubens* germinate in pure water (Segers et al. 2017), while most other conidia, including those of *Aspergillus niger* (Ijadpanahsaravi et al. 2021) require certain nutrients. Until now, the nutrient requirements of *P. roqueforti* conidia are not known. Yet, the impact of water activity (a_w), temperature and pH on germination of these spores has been studied (Kalai et al. 2017). Modelling shows that *P. roqueforti* conidia germinate at temperatures between − 0.2 and 33 °C with an optimum at 26.9 °C. The optimum pH is close to 5.6 and ranges between 2.84 and 13.8, while the lower limit of water activity (a_w) is 0.83 with an optimum of 0.98. Germination starts after 10 h when exposed to optimal conditions. Germination of conidia is also impacted by conditions during the production of the conidia. For instance, a low temperature and low a_w, but not pH, during sporulation significantly reduces the germination time (Nguyen Van Long et al. 2017a).

Here, we studied medium requirements for germination of conidia of *P. roqueforti*. Data show that glucose, alanine and arginine activate 12–21% of the conidia to germinate. Other amino acids are much less, if at all, effective in inducing germination. In all cases, swelling of conidia was minimal or even undetectable before germ tube formation.

Material and methods

Strains and culture conditions

P. roqueforti strain DTO377G3 (Westerdijk Fungal Biodiversity Institute) was routinely grown at 25 °C on malt extract agar (MEA; Oxoid, Hampshire, UK). Plates (20 mL MEA) were inoculated by spreading 10^7 conidia over the agar surface and conidia were harvested with ACES buffer (10 mM N(2-acetamido)-2-aminoethanesulfonic acid, 0.02% Tween 80, pH 6.8) after seven days of growth. The spores were filtered using sterilized glass wool and washed twice with ACES buffer with intermittent centrifugation for 5 min at 4 °C and 1700 g. Conidia concentration was determined using a Bürker-Türk haemocytometer.

Germination analysis

Conidia were used directly after harvesting. A total number of 6 10^3 spores was seeded per well of a 96 wells suspension culture plate (Greiner Bio-One, Cellstar 655,185, www.gbo.com) in 150 µL NPS (70.6 mM $NaNO_3$, 11 mM Na_2HPO_4/NaH_2PO_4 (pH 6.0), 2 mM $MgSO_4$) either or not containing 6.71 mM KCl and / or 10 mM carbon source (i.e. glucose or 1 of the 20 proteogenic L-amino acids). Milli-Q was used as a baseline condition. Germination of conidia was monitored on line at 25 °C using an oCelloScope (Biosense Solutions, www.biosensesolutions.dk) (Fredborg et al. 2013) with UniExplorer software version 8.1.0.7682-RL2. Measurements (using biological triplicates and technical duplicates) started after 1 h of incubation, enabling settling of the conidia at the bottom of the well. Objects were scanned every hour during the first 24 h and every 2 h during the next 48 h. A subsection of the well was selected for analysis. Scan area length was set at 405, the object area (min–max) at 70–1500 pixels, and the maximum number of objects at 1500. Individual objects were followed over time using oCelloScope XY coordinates and a custom R script. Conidial aggregates, and non-conidial objects at t = 1 h were manually removed from the data set. Conidia were followed in time based on their X and Y coordinates using the fast k-nearest neighbour (KNN) searching algorithm from the R package 'FNN' (Beygelzimer et al. 2019). This was done from t = x to t = x + 1 and vice versa. In addition, neighbour distance of an object was not allowed to exceed 27.5 µm (i.e. 50 pixels) between 2 adjacent time points. The lineage was discontinued if these conditions were no longer met. The objects were classified as resting, swelling or germinating conidia. At t = 1 h, resting conidia had a surface area < 68 µm^2 (225 pixels) and a circularity ≥ 0.83, swollen conidia were defined as objects with a ≥ twofold increased surface area when compared to t = 1 h with a circularity ≥ 0.83. Conidia with germ tubes had a circularity < 0.83 and had a ≥ twofold increased surface area when compared to t = 1 h. Conidia that met the parameter settings of germ tube formation but that had not been classified as swollen conidia in preceding time points were nonetheless classified as swollen at the moment germ tube formation had started.

Modelling of germination kinetics

The asymmetric model (Dantigny et al. 2011) was used to describe swelling and germ tube formation (P) and germination time τ (h) as a function of time (Eq. 1).

$$P = P_{max}\left(1 - \frac{1}{1 + \left(\frac{t}{\tau}\right)^{d}}\right) \quad (1)$$

P_{max} is the maximal percentage of swollen conidia or spores that form a germ tube (the asymptotic value of P at $t \rightarrow +\infty$). Germination time τ (h) is the time at which $P = 0.5 \cdot P_{max}$, while d is a shape parameter that can be correlated to the heterogeneity of the population. A low d reflects a population where conidia have more variable individual germination times. To estimate the model parameters of the asymmetric model, three biological replicates (≥ 300 conidia per condition) were fitted together with the R package GrowthRates (Petzoldt, 2019) using the Levenberg–Marquardt algorithm. Parameters were limited to $P \geq 0$ and ≤ 120%, $\tau \geq 1$ and ≤ 72 h, $d \geq 1$ and ≤ 30 to support the fitting process. Objects that had decreased in size during the analysis were excluded from the data set. Missing objects represent resting spores (R) that are lost during the analysis (i.e. that were no longer detected at $t \geq 2$ h because the object had moved or was obscured for instance by germlings of other spores) before they reached the swelling (S) or germination (G) stage. Size and circularity data of all objects were used for the parameter estimation until the time point when hyphal growth started to obscure resting spores.

Results

The effect of L-amino acids on germination

Preliminary experiments with *P. roqueforti* strains 013F2 and 377G3 (Westerdijk Fungal Biodiversity Institute) showed that up to 90% of the conidia can germinate in the oCelloScope set up when grown in malt extract for 72 h (Data not shown). Here, we assessed the impact of the presence of inorganic medium components, glucose, and amino acids on germination. To this end, monitoring of the germination process was started after 1 h, allowing conidia to settle at the bottom of the 96-wells plate. P_{max} of swelling and germ tube formation in Milli-Q was 0.66% (Table 1) and 0.41% (Fig. 1A; Table 2), respectively. Similar results were obtained with NPS (data not shown) or KNPS (Tables 1,2). The addition of KCl to NPS medium, however, did increase P_{max} of swelling and germ tube formation in the presence of alanine or arginine about threefold (data not shown). The same was observed when $NaNO_3$ or Na_2HPO_4/NaH_2PO_4 was replaced for either KNO_3 or K_2HPO_4/KH_2PO_4 (data not shown) showing that potassium facilitates *P. roqueforti* germination. Therefore, the impact of glucose or one of the proteogenic amino acids on swelling and germ tube formation was assessed in the presence of KNPS. P_{max} of swelling and germ tube formation was 12.72% (Table 1) and 12.75% (Fig. 1B; Table 2), respectively, in the case of glucose, while τ was 13.89 h for swelling and 14.05 h for germ tube formation. In the case of the proteogenic L-amino acids, P_{max} of swelling ranged between 0.2% and 21.3% (Table 1). P_{max} of swelling in the presence of arginine or alanine was 21.3% and 13.11%, respectively, with a τ of 25.01 h and 30.94 h (Table 1). P_{max} of germ tube formation of these two L-amino acids was 21.18% and 12.45%, while τ was 25.24 h and 33.5 h, respectively (Table 2; Fig. 1CD). In the case of the other amino acids, P_{max} ranged between 0.2 and 5.8% and, as a consequence, the predicted model parameters were often inaccurate (e.g. represented by a > 20 h variance in τ) despite the low (< 0.1) RMSE values. Together, glucose, arginine and alanine induce germination of *P. roqueforti* conidia, while other amino acids hardly, if at all, induce germination within the 72 h period. Yet, some hyphal growth was observed by microscopy in every condition (including Milli-Q) after seven days. This shows that a small part of the *P. roqueforti* conidia do germinate in absence of nutrients added to the medium.

P_{max} and τ of swelling and germ tube were distinct in the case of *A. niger* (Ijadpanahsaravi et al. 2021), showing that these stages are separated in time. Notably, these parameters were similar in the case of swelling and germination of *P. roqueforti* conidia. Yet, swelling in our data analysis was defined as a ≥ twofold increase of the surface area. Therefore, aata obtained by incubating conidia in the presence of arginine, alanine and glucose were re-analysed to exclude that germination was preceded by an increase of surface area > 1 but < 2. This showed that the

Table 1 Parameter estimates of the asymmetrical model used to describe swelling of spores in the presence of 10 mM glucose (G) or 10 mM of one of the 20 proteinogenic amino acids (AA).

AA	G	Medium	P_{max} (%)		τ (h)		d (−)		RMSE	N	M
Ala	0	KNPS	13.11	[12.47; 13.74]	30.94	[29.6; 32.27]	6.16	[4.78; 7.55]	0.14	405	290
Arg	0	KNPS	21.3	[20.53; 22.06]	25.01	[23.98; 26.03]	4.41	[3.75; 5.06]	0.16	389	263
Asn	0	KNPS	0.2	[0.15; 0.26]	53.07	[49.3; 56.84]	30	[− 25.74; 85.74]	0.01	402	34
Asp	0	KNPS	3.29	[− 3.31; 9.89]	72	[− 65.85; 209.85]	1.97	[0.25; 3.68]	0.05	362	70
Cys	0	KNPS	4.51	[− 2.46; 11.49]	72	[20.55; 123.45]	4.11	[1.21; 7]	0.05	427	41
Gln	0	KNPS	0.55	[0.35; 0.75]	18.89	[9.54; 28.25]	3.49	[− 2.22; 9.2]	0.05	451	83
Glu	0	KNPS	6.16	[− 4.56; 16.88]	72	[− 164.6; 308.6]	1	[0.18; 1.82]	0.08	305	69
Gly	0	KNPS	1.6	[1.41; 1.8]	24.83	[21.54; 28.13]	6.39	[2; 10.77]	0.05	393	59
His	0	KNPS	0.6	[0.5; 0.71]	31.55	[27.23; 35.87]	10.39	[− 2.59; 23.36]	0.03	389	12
Ile	0	KNPS	2.11	[− 4.75; 8.98]	72	[− 251.26; 395.25]	1.36	[− 0.6; 3.33]	0.05	414	46
Leu	0	KNPS	3.48	[2.99; 3.96]	33.04	[29.04; 37.04]	4.34	[2.56; 6.11]	0.07	462	104
Lys	0	KNPS	1.85	[1.47; 2.23]	30.81	[25.02; 36.59]	5.37	[0.9; 9.84]	0.07	421	57
Met	0	KNPS	1.1	[− 2.23; 4.42]	72	[− 74.94; 218.94]	2.8	[− 0.98; 6.59]	0.02	466	50
Phe	0	KNPS	1.43	[− 1.42; 4.28]	49.98	[− 51.93; 151.89]	2.16	[− 1.23; 5.55]	0.06	449	88
Pro	0	KNPS	0.97	[0.56; 1.39]	38.22	[25.54; 50.9]	3.95	[0.35; 7.56]	0.04	434	133
Ser	0	KNPS	2.26	[1.92; 2.61]	25.46	[21.06; 29.86]	4.29	[1.7; 6.89]	0.07	421	74
Thr	0	KNPS	1.37	[0.95; 1.8]	37.08	[29.47; 44.69]	8.7	[− 5.12; 22.53]	0.09	459	71
Trp	0	KNPS	1.2	[0.97; 1.42]	20.83	[16.5; 25.16]	6.83	[− 2.07; 15.73]	0.07	376	41
Tyr	0	KNPS	0.57	[0.26; 0.89]	29.87	[10.75; 49]	2.41	[0.11; 4.7]	0.02	348	33
Val	0	KNPS	1.79	[1.42; 2.16]	43.92	[38.56; 49.27]	5.15	[2.81; 7.48]	0.03	422	54
–	0	Milli-Q	0.66	[0.59; 0.74]	7.78	[5.43; 10.14]	2.25	[0.75; 3.74]	0.02	342	339
–	0	KNPS	0.2	[0.15; 0.25]	23.01	[19.26; 26.76]	30	[− 68.57; 128.57]	0.02	375	50
–	10	KNPS	12.72	[12.32; 13.13]	13.89	[13.21; 14.57]	4.73	[3.72; 5.74]	0.14	426	302

Spores were seeded in KNPS (6.71 mM KCl, 70.6 mM NaNO3, 11 mM Na2HPO4/NaH2PO4 (pH 6.0), 2 mM MgSO4) or Milli-Q. Confidence intervals are indicated between brackets, N represents the number of objects at t = 1 h, while M represents the number of objects that were no longer detected at $t \geq 2$ h because the object had moved or the object was obscured. RMSE represents the root mean square error of the modelled data and is a measure for the goodness of fit (Dantigny et al., 2011, Ratkowsky, 2004)

surface area of most *P. roqueforti* conidia had not increased from the start of the experiment up to the moment germ tubes had formed (Fig. 2; Figure S1). The fact that spores can form germ tubes in the absence of swelling is also indicated by the small difference in τ for swelling and germ tube formation ($\tau_{germ\ tube\ formation}$—$\tau_{swelling}$) being 0.23, 2.56 and 0.16 h in the presence of arginine, alanine and glucose, respectively.

Discussion

Here, we assessed germination of *P. roqueforti* conidia in water or defined medium consisting of $NaNO_3$, Na_2HPO_4/NaH_2PO_4, $MgSO_4$ and KCl (KNPS) either or not supplemented with glucose or one of the 20 proteogenic amino acids. Out of these carbon sources only glucose, arginine and alanine induced germination > 10% during a 72 h period according to the asymmetrical model. The overall low germination percentage of spores (a maximum percentage of 21.18% was obtained in the presence of arginine) can be explained by the low nutrient conditions since germination in malt extract resulted in germination of up to 90% of the spores, which is similar to the germination percentage on malt extract agar (Nguyen Van Long et al., 2017). Notably in all cases, but in particular in the presence of arginine and alanine, part of the spores formed germ tubes without

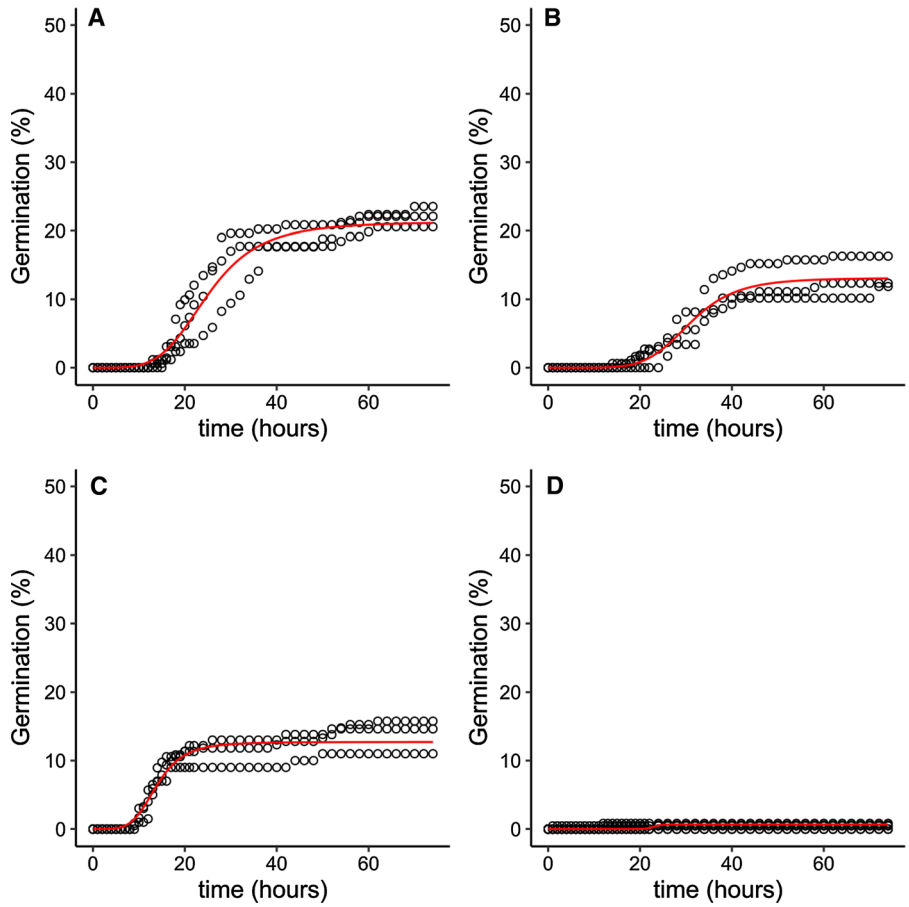

Fig. 1 The asymmetrical model used to describe germ tube formation by *P. roqueforti* conidia in KNPS (6.71 mM KCl, 70.6 mM $NaNO_3$, 11 mM Na_2HPO_4/NaH_2PO_4 (pH 6.0), 2 mM $MgSO_4$) supplemented with 10 mM arginine (**A**), alanine (**B**), glucose (**C**), Milli-Q (**D**) serving as a control. Open dots represent the average of two technical replicates

detectable swelling. This strongly indicates that swelling is not a pre-requisite for conidia to germinate.

Potassium salts were shown to increase germination of *P. roqueforti* conidia about threefold, while up to 100% germination was observed in their absence in the case of *A. niger* conidia (Ijadpanahsaravi et al. 2021). Potassium salts are considered essential for cellular development, implying that *A niger* spores have an internal storage. This suggests that this fungus has a selective advantage to *P. roqueforti* in K-depleted substrates such as soil (Benito et al. 2004). An inducing effect of potassium on germination has also been reported for the nematophagous fungus *Hirsutella rhossiliensis.* Addition of 0.4 M KCl increased germination of this fungus from 66 to 86% in a 24 h period (Eayre et al. 1990). In contrast, germination of *Penicillium frequentans* and *Septoria tritici* was reduced by 70% as result of the addition of 0.5 and 0.75 M KCl, respectively (Guijarro et al. 2007; Mann et al. 2004).

With the exception of alanine and arginine, germination was only $\leq$ 5.8% when conidia were exposed for 72 h to an amino acid. In fact, the predicted model parameters were often inaccurate showing large confidence intervals because of the low number of germinating spores that served as input for the model. Indeed, arginine and alanine did show low confidence intervals because in these cases the number of germinating spores was higher with a P_{max} of germ tube formation of 21.18% and 12.45%, respectively. Arginine and alanine were also among the most inducing amino acids in the case of *A. niger* but its conidia responded strongest to proline with 55% of the spores germinating. The response of *A. niger* to

Table 2 Parameter estimates of the asymmetrical model used to describe germ tube formation of spores in the presence of 10 mM glucose (G) or 10 mM of one of the 20 proteogenic amino acids (AA).

AA	G	medium	P_{max} (%)		τ (h)		d (−)		RMSE	N	M
Ala	0	KNPS	12.45	[11.89; 13.01]	33.5	[32.28; 34.72]	6.3	[5.05; 7.54]	0.11	405	290
Arg	0	KNPS	21.18	[20.4; 21.97]	25.24	[24.18; 26.3]	4.27	[3.65; 4.9]	0.16	389	263
Asn	0	KNPS	0.2	[0.15; 0.26]	53.07	[49.3; 56.84]	30	[− 25.74; 85.74]	0.01	402	34
Asp	0	KNPS	3.29	[− 3.31; 9.89]	72	[− 65.85; 209.85]	1.97	[0.25; 3.68]	0.05	362	70
Cys	0	KNPS	2.47	[1.07; 3.87]	61.57	[51.74; 71.39]	9.12	[2.39; 15.84]	0.05	427	41
Gln	0	KNPS	0.55	[0.34; 0.76]	19.62	[9.79; 29.45]	3.46	[− 2.13; 9.06]	0.05	451	83
Glu	0	KNPS	6.16	[− 4.56; 16.88]	72	[− 164.6; 308.6]	1	[0.18; 1.82]	0.08	305	69
Gly	0	KNPS	1.6	[1.4; 1.8]	24.97	[21.54; 28.41]	6.11	[1.94; 10.27]	0.05	393	59
His	0	KNPS	0.6	[0.5; 0.71]	31.55	[27.23; 35.87]	10.39	[− 2.59; 23.36]	0.03	389	12
Ile	0	KNPS	2.11	[− 4.75; 8.98]	72	[− 251.26; 395.25]	1.36	[− 0.6; 3.33]	0.05	414	46
Leu	0	KNPS	3.51	[3; 4.01]	33.76	[29.61; 37.9]	4.33	[2.54; 6.12]	0.07	462	104
Lys	0	KNPS	1.83	[1.48; 2.19]	30.93	[25.51; 36.36]	5.79	[0.86; 10.72]	0.07	421	57
Met	0	KNPS	1.1	[− 2.23; 4.42]	72	[− 74.94; 218.94]	2.8	[− 0.98; 6.59]	0.02	466	50
Phe	0	KNPS	1.57	[− 2.29; 5.43]	55.53	[− 85.81; 196.87]	2.03	[− 1.24; 5.3]	0.06	449	88
Pro	0	KNPS	1.06	[0.44; 1.68]	41.79	[22.7; 60.87]	3.44	[0.31; 6.57]	0.04	434	133
Ser	0	KNPS	2.22	[1.92; 2.53]	25.69	[21.83; 29.55]	5.06	[1.93; 8.2]	0.07	421	74
Thr	0	KNPS	1.31	[0.89; 1.73]	36.89	[28.87; 44.92]	8.55	[− 5.61; 22.71]	0.09	459	71
Trp	0	KNPS	1.2	[0.97; 1.42]	20.83	[16.5; 25.16]	6.83	[− 2.07; 15.73]	0.07	376	41
Tyr	0	KNPS	0.57	[0.26; 0.89]	29.87	[10.75; 49]	2.41	[0.11; 4.7]	0.02	348	33
Val	0	KNPS	2.01	[1.36; 2.67]	48.83	[39.52; 58.13]	4.48	[2.32; 6.65]	0.03	422	54
-	0	Milli-Q	0.41	[0.33; 0.5]	11.49	[9.49; 13.49]	30	[− 83.7; 143.7]	0.04	342	339
-	0	KNPS	0.2	[0.15; 0.25]	23.01	[19.26; 26.76]	30	[− 68.57; 128.57]	0.02	375	50
-	10	KNPS	12.75	[12.34; 13.16]	14.05	[13.36; 14.74]	4.6	[3.63; 5.57]	0.13	426	302

Spores were seeded in KNPS (6.71 mM KCl, 70.6 mM NaNO3, 11 mM Na2HPO4/NaH2PO4 (pH 6.0), 2 mM MgSO4) or Milli-Q. Confidence intervals are indicated between brackets, N represents the number of objects at $t = 1$ h, while M represents the number of objects that were no longer detected at $t \geq 2$ h because the object had moved or the object was obscured. RMSE represents the root mean square error of the modelled data and is a measure for the goodness of fit (Dantigny et al., 2011, Ratkowsky, 2004)

arginine was similar to *P. roqueforti* but alanine-induced germination was significantly reduced in *P. roqueforti* compared to *A. niger* conidia, the latter showing 37% spore germination (Ijadpanahsaravi et al. 2021). Glucose is not a superior inducer of germination in *P. roqueforti* and *A. niger* when compared to the amino acids. However, the time frame within which *P. roqueforti* conidia formed germ tubes (τ) was almost 10 h lower in glucose when compared to the amino acids. This implies that germ tube formation in the presence of glucose occurs faster in the case of *P. roqueforti.* This effect was not observed in the case of *A. niger* (Ijadpanahsaravi et al. 2021).

Although germination was hardly, if at all, observed in most of the culturing conditions during the first 72 h, growth was observed in most cases after seven days of growth, even in pure water. To estimate germination rates in Milli-Q (or other conditions with slow and poor germination rates) the measuring time must be extended. Germination of *P. rubens* has also been observed in pure water (Segers et al. 2017). Possibly, conidia do not need an external trigger to start germination but triggers simply speed up the process. We can also not exclude that nutrients are released from the cell wall or due to lysis of some of the conidia of the population. In fact, this may be a strategy for *P. roqueforti* conidia to initiate

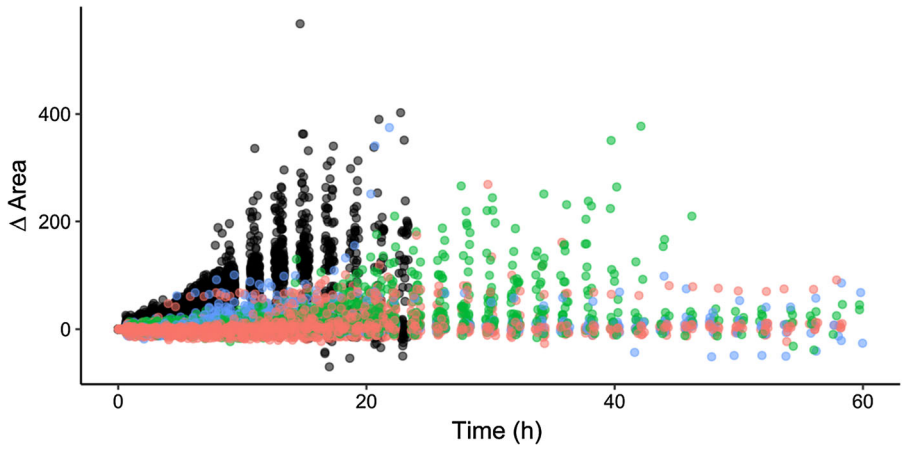

Fig. 2 ΔArea ($Area_{t=x}$-$Area_{t=1}$) in pixels of individual germinating conidia of *P. roqueforti* incubated in KNPS + arginine (red), alanine (green) or glucose (blue) over time. Black dots show germinating *A. niger* conidia in NPS + alanine

germination irrespective of the nutrient conditions in the environment.

Conidia diameter increases more than twofold during swelling of several aspergilli (Baltussen et al. 2018; Ijadpanahsaravi et al. 2021; Van Leeuwen et al. 2010). In contrast, swelling of *P. roqueforti* conidia was absent in part of the germinating spores in our defined media. Preliminary data show that *P. roqueforti* spores do show pronounced swelling in malt extract broth (Figure S2). Possibly, swelling in our defined medium is limited by nutrient availability. For instance, *Fusarium culmorum* conidia show reduced swelling in the absence of a nitrogen source (Marchant and White 1966). Under such conditions, conidia may consume their internal compatible solute content at a higher rate, leading to a lower osmotic pressure. This would reduce the water uptake and, as a consequence, the swelling of the spores. Together, data suggest that germination can take place in fungi without a swelling stage.

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Declarations

Conflict of interest The authors declare not to have any conflict of interest.

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