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Abstract

Filamentous fungi are used in the dairy industry as adjunct cultures in fermented products, but can also lead to food spoilage, waste and economic losses. The control of filamentous fungi with abiotic factors contributes to longer food shelf life and prevention of fungal spoilage. One of the main abiotic factors for controlling fungal growth in foods is water activity (a_w). This study aimed to evaluate radial growth as a function of a_w for sixteen fungal adjuncts and/or spoilers isolated from dairy products or a dairy environment. Glycerol (a nonionic compound) and sodium chloride (NaCl, an ionic compound) were used to adjust the a_w of culture media. This study showed significant diversity in the responses of the tested fungal strains as a function of medium a_w. The growth response of *Penicillium bialowiezense* and *Sporendonema casei* was binary, with no clear decrease of growth rate until the growth limit, when the a_w was reduced. For the strains of *Bisifusarium domesticum*, *Mucor circinelloides* and *Penicillium camemberti,* a decrease of a_w had the same impact on radial growth rate regardless of the a_w belonging to their growth range. For the strains of *Aspergillus flavus*, *Cladosporium herbarum*, *Geotrichum candidum, Mucor lanceolatus*, *Penicillium expansum*, *Penicillium fuscoglaucum, Penicillium nalgiovense*, *Paecilomyces niveus, Penicillium roqueforti*, *Penicillium solitum* and *Scopulariopsis asperula,* the impact of a decrease in a_w was more pronounced at high a_w than at low a_w. A mathematical model was suggested to describe this impact on the radial growth rate. For all tested species, radial growth was more sensitive to NaCl than glycerol. The ionic strength of NaCl mainly explains the difference in the effects of the two solutes.

Keywords

Mycelial growth, predictive microbiology, sodium chloride, glycerol, ionic strength

1. Introduction

In the food industry, filamentous fungi and yeasts are used as adjunct cultures to make fermented products, including sausages (Berwal and Dincho, 1995), cheeses (Nielsen et al., 1998) and beverages (Malbaša et al., 2011). Some species, however, can also spoil a large range of foods such as dairy (Garnier et al., 2017) or bakery goods (Dagnas and Membré, 2013). Indeed, the quality of food products can be affected by fungi, which can alter the colour, odour and taste of products, leading to economic losses and food waste. In addition, safety concerns exist concerning their ability to produce mycotoxins in food products (Sengun et al., 2008).

In the dairy industry, fungal contamination of products mainly originates from the production environment, in which spores are highly dispersed i.e., air, work surfaces, equipment, workers, ingredients for food production (Bernardi et al., 2019; Kure and Skaar, 2019). Cheeses are excellent substrates for fungal growth, and can be spoiled during production, ripening, storage, distribution in food shops or consumers' homes (Garnier et al., 2017). While some mycotoxins are unstable in a cheese matrix, others can persist under processing conditions (Kure and Skaar, 2019). The food industry, and especially the dairy industry, now aims to replace traditional preservation techniques by alternative methods in order to reduce the use of chemical compounds in food, in line with developments in food legislation and new consumer demands.

The control of abiotic factors contributes greatly to the prevention of fungal spoilage and shelf-life extension. Water activity (a_w) is generally assumed to be one of the most important factors impacting fungal growth; it may also contribute to reducing mycotoxin production (Camardo Leggieri et al., 2016; Dagnas and Membré, 2013; Gibson et al., 1994; Kosegarten et al., 2017). Salts and sugars can be added to foods to improve the organoleptic properties of products and to limit the development of spoilage microorganisms by reducing a_w. The nature

of the solute used to reduce a_w is known to influence the relationship between the medium a_w and fungal proliferation (Pitt and Hocking, 1977; Rosso and Robinson, 2001). The a_w of dairy products depends on the type of product and on the production stages (drying, ripening, salting) and techniques: 0.988–0.995 a_w for whole milk , 0.961–0.980 a_w for butter (unsalted), 0.979 a_w for cream (40% fat), 0.970–0.980 a_w for Camembert, Brie and Emmental cheeses, 0.945–0.950 a_w for Cheddar and Gruyère cheeses and 0.917–0.920 a_w for Parmesan cheese (Fox, 1997; Guinee and Fox, 2017; Schmidt and Fontana, 2008). Most fungi are able to grow at low a_w compared with bacteria (Guinee and Fox, 2017), and the main species responsible for cheese spoilage belong to the genera *Aspergillus* and *Penicillium*. The latter are tolerant to a_w reduction but other abiotic factors can also have an impact on the fungal population dynamics by favouring the growth of certain species at the expense of others (Kure and Skaar, 2019; Marín et al., 2014; Musa et al., 2018). Although fungi can spoil all types of cheeses, fungal contamination occurs mainly in soft cheeses with high a_w (Kure and Skaar, 2019). Nonetheless, little is known concerning this effect in terms of risk management by predictive mycology, especially for dairy products. It was previously reported that cardinal values obtained with NaCl could be an appropriate method to develop predictive models for fungal cheese spoilage, although further studies would be needed to better understand and model the effects of NaCl on fungal growth (Nguyen Van Long et al., 2017).

In the current study, the effect of a_w on the radial growth of sixteen fungal adjuncts and/or spoilers encountered in the dairy industry was studied by using glycerol (a non-ionic compound) and sodium chloride (NaCl, an ionic compound) as solutes in culture media to modulate a_w.This study aims at assessing the diversity in physiological responses to variation of water activity adjusted with ionic or non-ionic solute of the prominent fungal species encountered in the dairy environment in order to control the spoilage of different dairy products or to improve the colonization of cheeses by adjuncts. A predictive mycology

approach was used to estimate the a_w cardinal values of fungal growth for strains belonging to sixteen different species. The models obtained for both solutes were compared to highlight the diversity of fungal physiological responses and the impact of the solute choice for a_w reduction. The data obtained with glycerol and NaCl were also used to suggest a model of the radial growth response to ionic strength.

2. Material and methods

2.1. Fungal strains

Sixteen fungal strains belonging to different species were selected for their significance in dairy products or the dairy environment (Table 1). All strains and informations about them were provided by the Université de Bretagne Occidentale Culture Collection (University of Western Brittany [UBOCC], Plouzané, France). Each strain was genetically identified by the UBOCC. Additional sequencing of the cyclopropane fatty-acyl-phospholipid synthase (CFS) encoding gene was done to identify UBOCC-A-109153, since the *Mucor circinelloides* complex was recently resolved (Wagner et al., 2020) (GenBank accession number: OK560012, data not shown).

2.2. Conidia production

Conidia were harvested from cultures obtained on potato dextrose agar (PDA, Difco, Becton Dickinson Sparks, MD, USA, pH 5.6, 0.995 a_w) after a 7-day incubation at 25°C in the dark. For spore collection, the cultures were flooded with sterile water containing Tween 80 at 0.01% (v/v). Conidia concentrations were estimated using a haemocytometer (Malassez, Preciss, Paris, France), and conidia were diluted prior to storage (suspended in 15% glycerol at -80°C) or inoculation. In specific situations, different incubation/harvest parameters were used in order to maximize the conidium concentration: (1) the incubation temperature was increased to 30°C for the *P. niveus* strain, and (2) the incubation period was extended to 14

days for the *P. niveus*, *B. domesticum* and *S. asperula* strains. We verified that freezing step of conidial suspensions did not impact the radial growth of fungi. The growth rates obtained with fresh and frozen conidia were not significantly different.

2.3. Growth media

Radial growth assessments were performed on potato dextrose broth (PDB, Difco, Becton Dickinson Sparks, MD, USA) supplemented with agar (Biomérieux, Marcy-l'Etoile, France); the pH of the culture medium was 5.3. Glycerol (Thermo Fischer Scientific, Waltham, MA, USA) was added according to the Langmuir equation (Nevarez et al., 2009). Ten a_w levels were tested, from 0.820 to 0.995 a_w (0.820, 0.880, 0.900, 0.920, 0.940, 0.950, 0.960, 0.970, 0.980 and 0.995 a_w). NaCl (Sigma-Aldrich, Saint-Louis, MO, USA) was added according to the Stokes-Robinson equation (Robinson and Stokes, 1965). Eleven a_w levels were tested, ranging from 0.815 to 0.995 a_w (0.815, 0.840, 0.862, 0.902, 0.919, 0.935, 0.950, 0.964, 0.977, 0.988 and 0.995 a_w). In order to prevent agar polymerization issues, the culture medium and water activity depressor were double-concentrated in the same bottle, while agar was double-concentrated in another bottle. The solutions were sterilised separately by autoclaving (20 min at 121°C). Before use, the culture medium was mixed with the depressor and agar at 55°C and poured into Petri dishes.

2.4. Medium inoculation and radial growth assessment

Conidium suspensions were diluted down to 10^6 conidia/mL in a solution containing Tween 80 at 0.01% (v/v), and were adjusted, using glycerol or NaCl, to the a_w of the agar medium to be inoculated. Then, 10 µL of a 10^6 conidia/mL suspension were deposited on the medium in the centre of Petri dishes (4 technical replicates) containing 20 mL of tested medium. The Petri dishes were sealed with parafilm and incubated in plastic boxes (34 x 25 x 12 cm) containing 200 mL of a solution at the same a_w of the tested medium in order to avoid desiccation of the agar plate. The petri dishes were incubated at 25°C. Previous studies were

performed on the selected strains to evaluate the impact of temperature on the fungal growth (unpublished data). The optimal growth temperature was always found in the 20°C-30°C range depending on the strain tested. Thallus diameters were regularly measured in two directions using a precision decimetre during a maximum storage period of 70 days.

2.5. Data treatment and statistical analyses

2.5.1. Radial growth modelling

Radial growth was modelled as a function of time with a two-phase primary model (Equation 1) (Dagnas and Membré, 2013; Nguyen Van Long et al., 2017) to estimate the primary parameters, namely the latency and radial growth rate:

$$r_{(t)} = \begin{cases} r_{(0)} + \varepsilon i \; if \; t \leq \lambda \\ r_{(0)} + \mu.(t - \lambda) + \varepsilon i \; if \; t > \lambda \end{cases} \quad (1)$$

in which $r_{(t)}$ is the thallus radius (mm), t is the incubation time (days), λ is the latency (days) and μ is the radial growth rate (mm.day^{-1}), and εi the residual error on the estimated radius. In this study, the radius $r_{(0)}$ was fixed at 3 mm.

2.5.2. Modelling the effect of water activity on the radial growth primary parameters

For the glycerol data, the radial growth rate (μ, mm.day^{-1}) was modelled as a function of water activity (a_w) with a monophasic model developed to demonstrate the effect of a_w on mould growth (Equation 2) (Rosso and Robinson, 2001):

$$\sqrt{\mu} = \sqrt{\mu_{opt}.CMR_{n(aw)}} + \varepsilon ii$$

$$CMR_{n\,(aw)} = \begin{cases} \dfrac{(a_w - a_{w\,min})^n.(a_w - a_{w\,max})}{(a_{w\,opt} - a_{w\,min})^{n-1}.\{(a_w - a_{w\,min}).(a_w - a_{w\,opt}) - (a_{w\,opt} - a_{w\,max}).[(n-1).a_{w\,opt} + a_{w\,min} - n.a_w]\}}, & \begin{matrix} a_w \leq a_{wmin} \\ a_{wmin} < a_w < a_{wmax} \\ a_w > a_{wmax} \end{matrix} \\ 0 \end{cases} \quad (2)$$

where $a_{w\,min}$, $a_{w\,opt}$ and $a_{w\,max}$ are the cardinal a_w values corresponding to the minimum, optimum and maximum a_w values respectively and εii is the residual error on the estimated value. In this study, the $a_{w\,max}$ parameter was fixed at 1.000. The radial growth rate, μ, was estimated with Equation (1); μ_{opt} is the value of μ when a_w is equal to $a_{w\,opt}$. n is a shape parameter, equal to 2 in our case (Rosso and Robinson, 2001). When the data did not allow the estimation of an optimal value, the $a_{w\,opt}$ parameter was fixed at the average value between the two a_w levels for which the highest growth rates were observed. This model describes a regular reduction of growth rate by a_w decrease.

For the NaCl data, two different equations were used to model the radial growth rate (μ, mm.day^{-1}) as a function of a_w. For some strains, the Cardinal Model Rosso (CMR) model (Equation 2) was used. For other strains, a biphasic model adapted from a model developed for growth modelling of *Listeria monocytogenes* as a function of temperature was used (Equation 3) (Le Marc et al., 2002):

$$\sqrt{\mu} = \sqrt{\mu_{opt}.CMLM_n(a_w)} + \varepsilon ii$$

$$CMLM_{n\,(aw)} = \begin{cases} \dfrac{(a_w - a_{w\,1})^2.(a_w - a_{w\,max})}{(a_{w\,opt} - a_{w\,1}).[(a_{w\,opt} - a_{w\,1}).(a_w - a_{w\,opt}) - (a_{w\,opt} - a_{w\,max}).(a_{w\,opt} + a_{w\,1} - 2.a_w)]}, if\, a_w \geq a_{w\,c} \\ \dfrac{(a_{w\,c} - a_{w\,1})^2.(a_{w\,c} - a_{w\,max})}{(a_{w\,opt} - a_{w\,1}).[(a_{w\,opt} - a_{w\,1}).(a_{w\,c} - a_{w\,opt}) - (a_{w\,opt} - a_{w\,max}).(a_{w\,opt} + a_{w\,1} - 2.a_{w\,c})]}.\left(\dfrac{a_w - a_{w\,min}}{a_{w\,c} - a_{w\,min}}\right)^2, if\, a_w < a_{w\,c} \end{cases} \quad (3)$$

where the parameters are the same as those described for Equation 2, with two additional parameters: $a_{w\,c}$, the a_w at which the switch between two different growth phases is observed, and $a_{w\,1}$, the point of intersection between the first linear part and the abscissa straight line. When the data did not allow the $a_{w\,opt}$ to be obtained, this parameter was fixed at the average value between the two a_w levels for which the highest growth rates were observed, and εii is the residual error on the estimated value. This model describes the irregular evolution of the

growth rate as a function of a_w, with a lower sensitivity to a_w variations at the lowest a_w range. The latter model was called Cardinal Model Le Marc (CMLM) in the present work.

The models were fitted by minimizing the sums of squares of the residuals (*lsqcurvefit* function, MATLAB 2018, The Mathworks Inc., USA). Linear approximations and calculations were performed to estimate the confidence intervals with a risk $\alpha = 0.05$ (*nlparci* function of MATLAB). The Akaike information criterion (AIC) was used to evaluate the fitting performances of the tested models and to determine which model had the best fit with the data.

2.5.3. Theoretical impact of ionic strength on radial growth rates

The effect of NaCl concentration on radial growth rates differs from that of glycerol concentration. Glycerol is a nonionic solute that penetrates the microbial cells and limits growth in a way that could be influenced by water availability. The impact of a_w could therefore be modelled by the effects of these solutes, as follows:

$$\mu = \mu_{opt} * \mathrm{CMR}_{n\,(aw)}$$

where $CMR_{n\,(aw)}$ is the water activity associated with the use of glycerol.

When NaCl is used in formulations of food or growth media, we hypothesize that its impact on the growth rate involves both a_w and ionic strength. The growth rate in NaCl experiments should, therefore, be expressed as follows:

$$\mu = \mu_{opt} * \mathrm{CMR}_{n\,(aw)} * \gamma_{n\,(is)}$$

where $\mathrm{CMR}_{n\,(aw)}$ could be associated with the impact of the water activity and $\gamma_{n(is)}$is the impact of the ionic strength.

Assuming that the effect of glycerol on the radial growth rate is only due to the effect of water activity, the effect of ionic strength could be expressed as:

$$\mu_{opt} * \gamma_{n\,(is)} = \frac{\mu}{\mathrm{CMR}_{n\,(aw)}}$$

The effect of ionic strength on radial growth rate can be assessed by making a graphical representation of $\frac{\mu}{CMR_{n(aw)}}$ as a function of ionic strength (*is*).

3. Results

3.1. Effect of a_w reduction on radial growth kinetics

For all tested strains, a latency phase was observed prior to the formation of a visible thallus. The duration of this latency phase varied among the tested strains and a_w levels. After the initial latency phase, the growth of the thallus radius was linear. This phenomenon was observed in all conditions except when the tested a_w was below the minimum a_w required for the fungus to produce a visible mycelium within 70 days. This level was called MINa_w in our study and can serve a rough indicator of sensitivity to a_w reduction for a given strain. If the lag phase was longer than the experiment duration (i.e. no visible growth at the end of incubation period), the radial growth rate (μ) was not estimated. However, the lag phase was not always satisfactorily estimated, this could bias estimations of secondary modelling. For these reasons, only the radial growth rate values were used for secondary modelling.

3.2. Effect of a_w reduction on radial growth rate (μ)

The reduction of a_w in the culture medium led to a lag phase increase and a radial growth rate decrease for both solutes. The CMR model (Equation 2) was used to represent a linear evolution of the growth rate in suboptimal conditions, while the CMLM model (Equation 3) was used to represent the lower sensitivity to a_w variations at low a_w. For all strains, the data from the glycerol experiments were fitted with the CMR model, whereas the CMLM model was needed to fit data obtained from the NaCl experiments for eleven out of the sixteen strains.

The different strains were classified into three groups depending on their response to a_w reduction when NaCl was used as the solute: 1) strains for which a linear evolution of the growth rate was observed in suboptimal conditions for both solutes (CMR); 2) strains for which a linear evolution of the growth rate for glycerol experiments (CMR) and a non-linear evolution of the growth rate for NaCl experiments (CMLM) were observed in suboptimal conditions; and 3) strains for which none of the models tested in the current study were satisfactory to fit the data.

The *B. domesticum*, *M. circinelloides* and *P. camemberti* strains had a linear evolution of growth rate in suboptimal a_w conditions for both solutes (Fig. 1A–C) and were classified into the first group. The CMR model was the best fitting model for these data (Table 2). Of these three strains, *B. domesticum* and *M. circinelloides* were more sensitive to a_w reduction than *P. camemberti*. For both these species, the MINa_w corresponding to the low a_w with visible growth were above or equal to 0.900 a_w for both solutes. The MINa_w for the *P. camemberti* strain could not be determined for either solute because it grew at the lowest tested a_w (i.e., 0.820 and 0.815 a_w for the glycerol and NaCl experiments, respectively). Moreover, the *B. domesticum*, *M. circinelloides* and *P. camemberti* strains grew faster on glycerol media than on NaCl media. These results suggest that these species were more sensitive to NaCl than glycerol. Regardless of the solute, *M. circinelloides* grew faster than the other species of this group. The μ_{opt} were estimated above 11 mm.day^{-1}, whereas they were estimated between 2.6 and 3.4 mm.day^{-1} and under 1 mm.day^{-1} for *P. camemberti* and *B. domesticum*, respectively, for both solutes.

Eleven strains were classified into the second group *(A. flavus*, *C. herbarum*, *M. lanceolatus*, *P. fuscoglaucum, P. nalgiovense*, *P. roqueforti*, *P. solitum*, *S. asperula*, *G. candidum*, *P. niveus* and *P. expansum*). For these strains, in suboptimal conditions, a constant evolution of the growth rate as a function of the a_w of the medium was observed using glycerol, whereas a

non-linear evolution of the growth rate was observed using NaCl (Fig. 1D–H and Fig. 2A–F). For that reason, the CMR model and the CMLM model had the best fit for the glycerol and NaCl data, respectively. Consequently, two supplementary secondary parameters had to be estimated for NaCl (Table 2 and Table 3). The MINa_w of the *A. flavus*, *P. fuscoglaucum*, *P. nalgiovense*, *P. roqueforti* and *P. solitum* strains could not be determined for either of the solutes because the fungi grew at the lowest tested a_w. Of the eleven strains, *M. lanceolatus, G. candidum* and *P. niveus* were the most sensitive to a_w reduction, with the MINa_w above 0.900 a_w for both solutes. The *Aspergillus* and *Penicillium* strains were the most tolerant to a_w reduction. For the majority of these strains, the μ_{opt} were estimated between 2.7 and 5.5 mm.day^{-1}. Nevertheless, *M. lanceolatus* grew faster, with estimations of μ_{opt} above 6.5 mm.day^{-1}, while *S. asperula* grew slower, with estimations under 1.2 mm.day^{-1} for both solutes. The estimations of a_{w1} and $a_{w\,c}$ depended on the species and the extent of the biphasic effect phenomenon.

For the *P. bialowiezense* and *S. casei* strains, which were in the third group, the radial growth rate changes were not fitted by the secondary models tested (Fig. 2G–H). They grew very slowly, the estimated growth rates were under 1 mm.day^{-1}for all tested conditions as for *B. domesticum* (in the first group). The MINa_w of *P. bialowiezense* could not be determined (Table 3) because it grew at the lowest tested a_w. The MINa_w for *S. casei* were equal or close to 0.900 a_w for both solutes (Table 3). None of the tested models were able to satisfactorily fit the data.

3.3. Effect of ionic strength on radial growth rate (μ) for NaCl experiments

Our proposed hypothesis for the glycerol experiments was that the impact on the growth rate was only due to a_w reduction, while for NaCl experiments the impact on the growth rate was due to the effects of a_w reduction and the ionic strength of the solute. The a_w effect observed on the growth rate for glycerol was removed to NaCl data in order to showed the effect of

ionic strength on the growth rate. A linear decrease in the growth rate was observed for *M. circinelloides*, *G. candidum* and *P. roqueforti* (Fig. 3A-C) between 0 and 0.95 mol·L^{-1} (corresponding to 0.995 and 0.935 a_w, respectively). This suggests a linear effect of ionic strength on the growth rate in this ionic strength range. In contrast, for *P. nalgiovense* (Fig. 4D), ionic strength seemed to have no clear effect on the growth rate.

4. Discussion

This study aimed to compare the physiological responses of filamentous fungi found in cheeses to a_w reduction, using either a nonionic compound (glycerol) or an ionic compound (NaCl). Numerous studies on the growth or mycotoxin production of *A. flavus*, *P. roqueforti* or *P. expansum* as a function of a_w are available in the literature. However, little or no data are available for *S. casei*, *B. domesticum* and other less emblematic species, although their occurrence and function in the dairy industry have become increasingly evident (Bachmann et al., 2005; Dupont et al., 2017; Irlinger et al., 2015).

According to a previous study (Marín et al., 2014), *Aspergillus* and *Penicillium* are generally more tolerant of reduced a_w than *Mucor* species, and the present data support this observation. Indeed, *Mucor* species do not belong to the phylum Ascomycota like the other species of the present study but to the Mucoromycota. They also appear to be very different regarding their ecological strategy: they are not S-selected species, competitive in a stressful environment, but rather ruderal or R-selected species (Cooke and Rayner, 1984; Magan and Aldred, 2007) with rapid growth, rapid commitment to reproduction, utilization of easily assimilable resources and non-persistence (Cooke and Rayner, 1984). In accordance with other studies, lowering the a_w of the medium limits the radial growth of a fungus. It is noteworthy that some differences can be observed between the various published studies due to intraspecific variability or experimental conditions, such as the composition of culture media and nature of the solute, which makes it difficult to compare the a_w values obtained from different studies.

Regarding intraspecific variability, strain choice can have a significant impact on the growth parameters. One study showed that only 80% of *P. nalgiovense* strains isolated from regional sausages were able to grow at 0.90 a_w (the lowest a_w level tested) and 25°C on malt extract agar containing glycerol (Ludemann, 2004). In the current study, the adjunct *P. nalgiovense* strain isolated from a cheese crust was able to grow at the lowest tested a_w level, which corresponded to 0.820 a_w for the glycerol experiments. It was also previously reported that the temperature and a_w cardinal values varied significantly among 29 *P. roqueforti* strains isolated from different cheeses and environments (Nguyen Van Long et al., 2021). For *P. expansum*, when NaCl was used as the solute in PDA medium incubated at 25°C, the MINa_w for a strain isolated from decayed apple was between 0.93 and 0.96 a_w (Lahlali et al., 2005), whereas this value was between 0.89 and 0.92 a_w for a strain isolated from fresh cheese (Nguyen Van Long et al., 2017). The value in the present study was between 0.815 and 0.840 a_w.

Previous studies have shown that culture media composition could also influence the growth response to a_w reduction. The MINa_w for a strain of *P. camemberti* (CBS 122399) was 0.90 a_w on Czapek yeast agar (Camardo Leggieri et al., 2016), whereas it was below 0.83 a_w on malt extract autolysate agar (Camardo Leggieri et al., 2018). Both media had been incubated at 20°C, and NaCl was used as a water activity depressor. The MINa_w for NaCl experiments of the *Penicillium camemberti* strain in our study was closer to the one obtained in the malt extract autolysate agar than those obtained in Czapek yeast agar because the strain grew at 0.815 a_w when NaCl was used as the solute. In addition, the MINa_w was higher for low nutrient medium compared with a rich nutrient culture medium as observed on three strains of *A. flavus* grown on corn extract medium (low nutrient) or CYA medium (nutrient rich) at 30°C (Astoreca et al., 2012). Moreover, the nature of the solute (sugar, salt) added to the culture media could have an effect on the a_w values. For *P. expansum,* grown at 25°C on PDA, the MINa_w was equal to or below 0.890 a_w when glycerol, sorbitol or glucose was used

as a depressor, while the MIN a_w was between 0.930 and 0.960 a_w when NaCl was used as a depressor (Lahlali et al., 2005). Similar results were observed for *A. flavus* (Pitt and Hocking, 1977).

In addition, the maximum incubation time set in a study can bias the minimum a_w level for growth when the growth latency is longer than the duration of the experimental incubation. For *P. nalgiovense*, the observed MINa_w was 0.90 a_w when the experiments were monitored for 14 days, whereas it was 0.87 a_w when the incubation time was extended to 56 days (Camardo Leggieri et al., 2016). This observation suggested that experiments with a short incubation period, such as 7 days (Ludemann, 2004) or 10 days (Camardo Leggieri et al., 2018), could underestimate the growth latency and potentially overestimate the MINa_w for growth in comparison with studies with longer incubation times such as 30 days (Nguyen Van Long et al., 2017), 70 days (Morin-Sardin et al., 2016) or 100 days (Pitt and Hocking, 1977; Pitt and Miscamble, 1995). Naturally, longer incubation times can lead to culture medium stability issues that could also cause technical bias.

Regarding *M. lanceolatus*, there were differences in the estimated values of the secondary parameters for NaCl experiments in two previous studies. In the first study (Morin-Sardin et al., 2016), the $a_{w\,min}$ and $a_{w\,opt}$ were estimated at 0.928 and 0.996 a_w, while in the second study (Nguyen Van Long et al., 2017) these parameters were estimated at 0.870 and 0.969 a_w. These differences could be due to differences in the experimental conditions. Morin-Sardin et al. (2016) used PDB to dilute the spores, and Nguyen Van Long et al. (2017) used culture media buffered at pH 4.2 with a mixture of citric acid monohydrate and dibasic sodium phosphate. All data from both of these studies were fitted with the CMR model. In our study, the spores were diluted in a water and Tween solution, the culture media were not buffered, and the NaCl data were fitted with the CMLM model. Consequently, all experimental parameters

(temperature, pH, culture medium), but also the intraspecific variability must be taken into account for data comparison because they could impact the apparentes values of the a_w limit.

In the literature, the most common model used to fit the data and estimate the cardinal a_w values of fungi is the cardinal model (CMR model) (Rosso and Robinson, 2001). However, this model was not always suitable in our study because the μ_{opt} could be underestimated and the $a_{w\,min}$ could be overestimated as the growth rate for some strains was less impacted by a_w variations at low a_w using NaCl. Therefore, the CMLM model, derived from a temperature cardinal model (Le Marc et al., 2002), was tested.

When glycerol was used, the a_w reduction had a consistent impact on the radial growth rate for all tested strains. In this case, the CMR model could be used. When a shift in the growth rate sensitivity was observed between low and high a_w changes with NaCl, the CMLM model could be used. This model is an extension of the cardinal model with two supplementary parameters, and allowed a good description of fungal growth for NaCl experiments. However, the use of the CMLM model could be limited in specific situations since this model may require optimization of the experimental design or additional data.

A simplification of the CMLM model was tested to reduce the number of parameters by studying the correlation of $a_{w\,min}$, $a_{w\,opt}$, $a_{w\,1}$ and $a_{w\,c}$ (data not shown). This model simplification was not used for data fitting because it was not possible to set a constant a_w value for the shift, as its position is species dependent. For *A. flavus*, *P. nalgiovense* and *S. asperula* (Table 2 and Table 3), the estimated $a_{w\,min}$ seemed to be underestimated because the values were very low. The confidence intervals of $a_{w\,min}$ for *A. flavus* and *P. fuscoglaucum* were very wide, with values above 0.100 a_w compared with those of the other fungi which indicates a lower precision of estimation in these two cases (Table 2). The estimations of $a_{w\,min}$ were lower than the MINa_w when the growth / no growth interface was observed for the majority of the experiments. This phenomenon was probably due to the limited number of

experimental data close to MINa_w. The acquisition of more data could lead to more accurate estimatation of $a_{w\ min}$, although these results will probably not be very different from those presented in this study. To keep food safety conditions, the use of a model which underestimated $a_{w\ min}$ was better compared to one which overestimated this parameter.

Tolerance mechanisms to a_w reduction were more often described in yeasts than in filamentous fungi (this occurred in two halotolerant yeasts : *Hortaea werneckii*, which can sometimes contaminate salted foods (Cabañes et al., 2012; Musa et al., 2018; Zalar et al., 2019), and *Debaryomyces hansenii*, which can be an adjunct (cheeses, sausages) or a spoiler in foods (Garnier et al., 2017; Musa et al., 2018)). The addition of sugars or salts to the culture media leads to hyperosmotic shock. One of the main strategies used by different fungal species is the accumulation of compatible solutes to limit the loss of turgor pressure and cell volume (Luxo et al., 1993). Compatible solutes are generally uncharged organic molecules with a low molecular weight. They protect the cells under osmotic stress without inhibiting enzymatic activities. Glycerol is generally the main compatible solute accumulated under water stress (Gunde-Cimerman et al., 2018; Kogej et al., 2007; Zidan and Abdel-Mallek, 1987), but several polyols (arabinitol, erythritol, mannitol and others) (Adler et al., 1982) and other compounds, such as trehalose and proline (Davis et al., 2000; Jennings and Burke, 1990; Welsh, 2000), can be accumulated by fungi. *G. candidum* does not have an effective retention system to maintain a high glycerol concentration within its cells (Hocking, 1986; Luxo et al., 1993), but it is able to accumulate arabinitol, which might act as a compatible solute under salt stress (Luxo et al., 1993). A strain of *A. flavus* was identified as a high producer of sugar alcohols and was able to accumulate D-arabinitol, D-mannitol and glycerol after 15 days at 28°C on Czapek medium containing 15% NaCl (El-Kady et al., 1995). Regarding other strategies described in *H. werneckii*, cell melanisation promotes glycerol retention by decreasing cell porosity (Gunde-Cimerman et al., 2018; Kejžar et al., 2013; Kogej et al.,

2007; Musa et al., 2018) and mycosporine-like amino acids might contribute to osmoregulation as compatible solutes for a particular range of salinity (Kogej et al., 2007, 2006). The plasma membrane composition (sterol-to-phospholipid ratios, unsaturated fatty acids, fluids domains) could modify membrane fluidity (Gunde-Cimerman et al., 2009; Turk et al., 2007, 2004). Several shifts in the transcription of genes involved in plasma membrane and cell wall composition were also observed under salt stress. This suggests that the plasma membrane and cell wall could be modified under high salt content, as for *Aspergillus sydowii*, which has rarely been found in dried foods (Pérez Llano et al., 2020). In addition, different strategies have been identified concerning the intracellular tolerance to Na^+ ions: *D. hansenii* relies on the accumulation of Na^+ ions without toxic effect, while *H. werneckii* relies on the exclusion of Na^+ and the maintenance of Na^+ intracellular concentrations under the toxic level (Gunde-Cimerman et al., 2018, 2009; Kogej et al., 2005; Musa et al., 2018). These results suggest that several mechanisms could be involved in salt tolerance.

Significant differences between glycerol and NaCl have been previously reported (Giorni et al., 2011; Lahlali et al., 2005; Morin-Sardin et al., 2016; Nguyen Van Long et al., 2017), with $a_{w\ min}$ significantly affected by the solute used (Nguyen Van Long et al., 2017; Rosso and Robinson, 2001). Another study showed that the species *P. expansum* and *M. circinelloides* were more sensitive to ionic stress than nonionic stress (Lahlali et al., 2005; Marín et al., 2014; Morin-Sardin et al., 2016; Nguyen Van Long et al., 2017). High concentrations of salt may lead to oxidative stress, with the production of reactive oxygen species (ROS) that could damage biomolecules or cells. This phenomenon could explain the growth differences between these solutes. For nonionic osmolytes, ATP synthesis and lipid peroxidation decreased in *H. werneckii*, while at high concentrations of NaCl, gene expression linked to energy production and oxidative damage protection increased (Gunde-Cimerman et al., 2018). Another study conducted on strains of *H. werneckii*, *D. hansenii* and *A. flavus* showed that the

number of genes encoding antioxidant enzymes was correlated with the maximum tolerated salinity (Gostinčar and Gunde-Cimerman, 2018). A different study focusing on *Aspergillus sydowii* showed that hydroperoxides, which provide an indication about the oxidation of biomolecules, were three-fold higher in the mycelium at 5.13 M compared with 1.0 M NaCl, and the activity of catalase significantly increased in the mycelium at the higher tested salinity (Jiménez-Gómez et al., 2020). All of these observations suggest that high salt concentrations could induce oxidative stress.

The impact of ionic strength in our study was investigated by representing the $\mu_{opt} * \gamma_{n\,(is)}$ as a function of ionic strength (Fig. 3). Ionic strength seemed to have an effect on the growth rate of *M. circinelloides*, *G. candidum* and *P. roqueforti*. Increasing concentrations of Na^+ and Cl^- ions might affect the cellular system of fungi by modifying enzymatic activity. The decreased growth rate related to ionic strength could be linear until ionic saturation of the cellular system is achieved; beyond that, the impact of ionic strength could become less significant on the growth rate. This hypothesis might explain why the impact of NaCl on the growth rate for *P. solitum* and *P. roqueforti* in a previous study became less than that of glycerol for the lowest a_w (Marín et al., 2014). On the contrary, no effect was observed on the growth rate of *P. nalgiovense*, suggesting that this strain was not sensitive to ionic strength. This species could, therefore, be used to make fermented products containing high amounts of salt, such as cured sausages, as it seems to have developed specific physiological strategies to grow at high ionic strength. Fungal sensitivity to ionic strength seems to be species-dependent. Indeed, it was reported that a moderate increase of NaCl concentration (< 10%) could improve the growth of some fungi as for *Penicillium nalgiovense* for which an increase of 4% NaCl produced a significant stimulus on growth (García-Rico et al., 2011). A previous study showed that the optimal a_w for growth of *Penicillium nalgiovense* and other *Penicillium* and *Aspergillus* species was 0.950 a_w (corresponding to 8% NaCl) compared to 0.990 a_w and

0.910 a_w which corresponding to 0 and around 13% NaCl respectively (Camardo Leggieri et al., 2018). In another study, the growth rate of *Penicillium roqueforti* and *Penicillium solitum* were higher at 4% NaCl than 0 and around 8% NaCl. Likewise, the growth rate of *Penicillium olsonii* and *Aspergillus pseudoglaucus* were higher at around 8% NaCl than 0 and around 12% NaCl (Marín et al., 2014). Nevertheless, additional studies are needed. The data transformation used in this study is impacted by the experimental variability. Consequently, the impact of ionic strength does not show a clear trend for some strains. Futher studies could be performed by combining several abiotic factors (temperature, pH, organic acids, a_w and modified atmospheres) in culture media then in food matrices to validate the growth rates predicted in culture media. Similar methods to those described in previous studies could be used, the results of them in cheese matrices were hopeful (Morin-Sardin et al., 2016; Nguyen Van Long et al., 2017).

To conclude, this study helps to estimate the development of food technology and spoilage strains by taking into account a large diversity of fungi found in a dairy environment as a function of reduced a_w by solutes of different types. Fungal growth as a function of a_w reduction by ionic compounds suggests that ionic strength may impact fungal growth. Predictive mycology tools may be of interest in understanding the physiology of the selected fungi as a function of a_w variation in food or industrial environments, such as *B. domesticum*, which is widely used in the production of Saint Nectaire and raclette cheeses (Bachmann et al., 2005), and *C. herbarum*, which has been reported as a spoiler of soft cheeses (Bailly & Brugère, 1999; Jacquet & Desfleurs, 1966).

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References

Adler, L., Pedersen, A., Tunblad-Johansson, I., 1982. Polyol accumulation by two filamentous fungi grown at different concentrations of NaCl. Physiologia Plantarum 56, 139–142. https://doi.org/10.1111/j.1399-3054.1982.tb00315.x

Astoreca, A., Vaamonde, G., Dalcero, A., Ramos, A.J., Marín, S., 2012. Modelling the effect of temperature and water activity of Aspergillus flavus isolates from corn. International Journal of Food Microbiology 156, 60–67. https://doi.org/10.1016/j.ijfoodmicro.2012.03.002

Bachmann, H.P., Bobst, C., Bütikofer, U., Casey, M.G., Dalla Torre, M., Fröhlich-Wyder, M.T., Fürst, M., 2005. Occurrence and significance of Fusarium domesticum alias Anticollanti on smear-ripened cheeses. LWT - Food Science and Technology 38, 399–407. https://doi.org/10.1016/j.lwt.2004.05.018

Bailly, J.D., Brugère, H.,1999. Accidents de fabrication dus aux moisissures en fromagerie. Revue de médecine vétérinaire 150, 413–430.

Bernardi, A.O., Garcia, M.V., Copetti, M.V., 2019. Food industry spoilage fungi control through facility sanitization. Current Opinion in Food Science, Trends in sustainable food processing • Food Mycology 29, 28–34. https://doi.org/10.1016/j.cofs.2019.07.006

Berwal, J.S., Dincho, D., 1995. Molds as Protective Cultures for Raw Dry Sausages. Journal of Food Protection 58, 817–819. https://doi.org/10.4315/0362-028X-58.7.817

Cabañes, F.J., Bragulat, M.R., Castellá, G., 2012. Hortaea werneckii isolated from silicone scuba diving equipment in Spain. Medical Mycology 50, 852–857. https://doi.org/10.3109/13693786.2012.679628

Camardo Leggieri, M., Decontardi, S., Battilani, P., 2018. Modelling the sporulation of some fungi associated with cheese, at different temperature and water activity regimes. International Journal of Food Microbiology 278, 52–60. https://doi.org/10.1016/j.ijfoodmicro.2018.04.023

Camardo Leggieri, M., Decontardi, S., Bertuzzi, T., Pietri, A., Battilani, P., 2016. Modeling Growth and Toxin Production of Toxigenic Fungi Signaled in Cheese under Different Temperature and Water Activity Regimes. Toxins (Basel) 9. https://doi.org/10.3390/toxins9010004

Cooke, R.C., Rayner, A.D.M., 1984. Ecology of saprotrophic fungi. Longman, London; New York.

Dagnas, S., Membré, J.-M., 2013. Predicting and preventing mold spoilage of food products. Journal of Food Protection 76, 538–551. https://doi.org/10.4315/0362-028X.JFP-12-349

Davis, D.J., Burlak, C., Money, N.P., 2000. Osmotic pressure of fungal compatible osmolytes. Mycological Research 104, 800–804. https://doi.org/10.1017/S0953756299002087

Dupont, J., Dequin, S., Giraud, T., Le Tacon, F., Marsit, S., Ropars, J., Richard, F., Selosse, M.-A., 2017. Fungi as a Source of Food. Microbiology Spectrum 5. https://doi.org/10.1128/microbiolspec.FUNK-0030-2016

El-Kady, I., Moubasher, H., Mostafa, M., 1995. Accumulation of sugar alcohols by filamentous fungi. Folia Microbiologica 40, 481–486. https://doi.org/10.1007/BF02814727

Fox, P.F., 1997. Advanced Dairy Chemistry Volume 3: Lactose, water, salts and vitamins, 2nd ed. Springer US. https://doi.org/10.1007/978-1-4757-4409-5

Garcia-Rico, R., Chavez, R., Fierro, F., Laich, F., 2011. Mold-Fermented Foods: Penicillium spp. as Ripening Agents in the Elaboration of Cheese and Meat Products, in: Mycofactories. pp. 73-98. https://doi.org/10.2174/978160805223311101010073

Garnier, L., Valence, F., Mounier, J., 2017. Diversity and Control of Spoilage Fungi in Dairy Products: An Update. Microorganisms 5. https://doi.org/10.3390/microorganisms5030042

Gibson, A.M., Baranyi, J., Pitt, J.I., Eyles, M.J., Roberts, T.A., 1994. Predicting fungal growth: the effect of water activity on Aspergillus flavus and related species. International Journal of Food Microbiology 23, 419–431. https://doi.org/10.1016/0168-1605(94)90167-8

Giorni, P., Magan, N., Pietri, A., Battilani, P., 2011. Growth and aflatoxin production of an Italian strain of Aspergillus flavus: influence of ecological factors and nutritional substrates. World Mycotoxin Journal 4, 425–432. https://doi.org/10.3920/WMJ2011.1300

Gostinčar, C., Gunde-Cimerman, N., 2018. Overview of Oxidative Stress Response Genes in Selected Halophilic Fungi. Genes 9, 143. https://doi.org/10.3390/genes9030143

Guinee, T.P., Fox, P.F., 2017. Chapter 13 - Salt in Cheese: Physical, Chemical and Biological Aspects, in: McSweeney, P.L.H., Fox, P.F., Cotter, P.D., Everett, D.W. (Eds.), Cheese (Fourth Edition). Academic Press, San Diego, pp. 317–375. https://doi.org/10.1016/B978-0-12-417012-4.00013-2

Gunde-Cimerman, N., Plemenitaš, A., Oren, A., 2018. Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations. FEMS Microbiology Reviews 42, 353–375. https://doi.org/10.1093/femsre/fuy009

Gunde-Cimerman, N., Ramos, J., Plemenitaš, A., 2009. Halotolerant and halophilic fungi. Mycological Research 113, 1231–1241. https://doi.org/10.1016/j.mycres.2009.09.002

Hocking, A.D., 1986. Effects of Water Activity and Culture Age on the Glycerol Accumulation Patterns of Five Fungi. Microbiology, 132, 269–275. https://doi.org/10.1099/00221287-132-2-269

Irlinger, F., Layec, S., Hélinck, S., Dugat-Bony, E., 2015. Cheese rind microbial communities: diversity, composition and origin. FEMS Microbiology Letters 362, 1–11. https://doi.org/10.1093/femsle/fnu015

Jacquet, J., Desfleurs, M., 1966. Cladosporium herbarum link, agent d'accidents tardifs de "bleu" sur les fromages a pâte molle, et specialement le Camembert. https://doi.org/10.1051/LAIT:196645820

Jennings, D.H., Burke, R.M., 1990. Compatible solutes – the mycological dimension and their role as physiological buffering agents. New Phytologist 116, 277–283. https://doi.org/10.1111/j.1469-8137.1990.tb04715.x

Jiménez-Gómez, I., Valdés-Muñoz, G., Moreno-Perlin, T., Mouriño-Pérez, R.R., Sánchez-Carbente, M. del R., Folch-Mallol, J.L., Pérez-Llano, Y., Gunde-Cimerman, N., Sánchez, N. del C., Batista-García, R.A., 2020. Haloadaptative Responses of Aspergillus sydowii to Extreme Water Deprivation: Morphology, Compatible Solutes, and Oxidative Stress at NaCl Saturation. Journal of Fungi 6, 316. https://doi.org/10.3390/jof6040316

Kejžar, A., Gobec, S., Plemenitaš, A., Lenassi, M., 2013. Melanin is crucial for growth of the black yeast Hortaea werneckii in its natural hypersaline environment. Fungal Biology 117, 368–379. https://doi.org/10.1016/j.funbio.2013.03.006

Kogej, T., Gostinčar, C., Volkmann, M., Gorbushina, A., Gunde-Cimerman, N., 2006. Mycosporines in Extremophilic Fungi—Novel Complementary Osmolytes? https://doi.org/10.1071/EN06012

Kogej, T., Ramos, J., Plemenitas, A., Gunde-Cimerman, N., 2005. The halophilic fungus Hortaea werneckii and the halotolerant fungus Aureobasidium pullulans maintain low intracellular cation concentrations in hypersaline environments. Applied and Environmental Microbiology 71, 6600–6605. https://doi.org/10.1128/AEM.71.11.6600-6605.2005

Kogej, T., Stein, M., Volkmann, M., Gorbushina, A.A., Galinski, E.A., Gunde-Cimerman, N., 2007. Osmotic adaptation of the halophilic fungus Hortaea werneckii: role of osmolytes and melanization. Microbiology (Reading, Engl.) 153, 4261–4273. https://doi.org/10.1099/mic.0.2007/010751-0

Kosegarten, C.E., Ramírez-Corona, N., Mani-López, E., Palou, E., López-Malo, A., 2017. Description of Aspergillus flavus growth under the influence of different factors (water activity, incubation temperature, protein and fat concentration, pH, and cinnamon essential oil concentration) by kinetic, probability of growth, and time-to-detection models. International Journal of Food Microbiology 240, 115–123. https://doi.org/10.1016/j.ijfoodmicro.2016.04.024

Kure, C.F., Skaar, I., 2019. The fungal problem in cheese industry. Current Opinion in Food Science, Trends in sustainable food processing • Food Mycology 29, 14–19. https://doi.org/10.1016/j.cofs.2019.07.003

Lahlali, R., Serrhini, M.N., Jijakli, M.H., 2005. Studying and modelling the combined effect of temperature and water activity on the growth rate of P. expansum. International Journal of Food Microbiology 103, 315–322. https://doi.org/10.1016/j.ijfoodmicro.2005.02.002

Le Marc, Y., Huchet, V., Bourgeois, C.M., Guyonnet, J.P., Mafart, P., Thuault, D., 2002. Modelling the growth kinetics of Listeria as a function of temperature, pH and organic acid concentration. International Journal of Food Microbiology 73, 219–237. https://doi.org/10.1016/S0168-1605(01)00640-7

Ludemann, V., 2004. Determination of growth characteristics and lipolytic and proteolytic activities of Penicillium strains isolated from Argentinean salami. International Journal of Food Microbiology 96. https://doi.org/10.1016/S0168-1605(04)00132-1

Luxo, C., Nobre, M.F., Costa, M.S. da, 1993. Intracellular polyol accumulation by yeastlike fungi of the genera Geotrichum and Endomyces in response to water stress (NaCl). Canadian Journal of Microbiology 39, 868–873. https://doi.org/10.1139/m93-130

Magan, N., Aldred, D., 2007. Post-harvest control strategies: minimizing mycotoxins in the food chain. International Journal of Food Microbiology119, 131–139. https://doi.org/10.1016/j.ijfoodmicro.2007.07.034

Malbaša, R.V., Lončar, E.S., Vitas, J.S., Čanadanović-Brunet, J.M., 2011. Influence of starter cultures on the antioxidant activity of kombucha beverage. Food Chemistry 127, 1727–1731. https://doi.org/10.1016/j.foodchem.2011.02.048

Marín, P., Palmero, D., Jurado, M., 2014. Effect of solute and matric potential on growth rate of fungal species isolated from cheese. International Dairy Journal 36, 89–94. https://doi.org/10.1016/j.idairyj.2014.01.012

Morin-Sardin, S., Rigalma, K., Coroller, L., Jany, J.-L., Coton, E., 2016. Effect of temperature, pH, and water activity on Mucor spp. growth on synthetic medium, cheese analog and cheese. Food Microbiology 56, 69–79. https://doi.org/10.1016/j.fm.2015.11.019

Musa, H., Kasim, F.H., Nagoor Gunny, A.A., Gopinath, S.C.B., 2018. Salt-adapted moulds and yeasts: Potentials in industrial and environmental biotechnology. Process Biochemistry. 69, 33–44. https://doi.org/10.1016/j.procbio.2018.03.026

Nevarez, L., Vasseur, V., Le Madec, A., Le Bras, M.A., Coroller, L., Leguérinel, I., Barbier, G., 2009. Physiological traits of Penicillium glabrum strain LCP 08.5568, a filamentous fungus isolated from bottled aromatised mineral water. International Journal of Food Microbiology 130, 166–171. https://doi.org/10.1016/j.ijfoodmicro.2009.01.013

Nguyen Van Long, N., Rigalma, K., Coroller, L., Dadure, R., Debaets, S., Mounier, J., Vasseur, V., 2017. Modelling the effect of water activity reduction by sodium chloride or glycerol on conidial germination and radial growth of filamentous fungi encountered in dairy foods. Food Microbiology 68, 7–15. https://doi.org/10.1016/j.fm.2017.06.014

Nguyen Van Long, N., Rigalma, K., Jany, J.-L., Mounier, J., Vasseur, V., 2021. Intraspecific variability in cardinal growth temperatures and water activities within a large diversity of Penicillium roqueforti strains. Food Research International 148, 110610. https://doi.org/10.1016/j.foodres.2021.110610

Nielsen, M.S., Frisvad, J.C., Nielsen, P.V., 1998. Protection by fungal starters against growth and secondary metabolite production of fungal spoilers of cheese. International Journal of Food Microbiology 42, 91–99. https://doi.org/10.1016/S0168-1605(98)00070-1

Pérez Llano, Y., Rodríguez-Pupo, E., Druzhinina, I., Chenthamara, K., Cai, F., Gunde-cimerman, N., Zalar, P., Gostinčar, C., Kostanjsek, R., Folch-Mallol, J., Batista García, R., Sánchez, M., 2020. Stress Reshapes the Physiological Response of Halophile Fungi to Salinity. Cells 9, 525. https://doi.org/10.3390/cells9030525

Pitt, J.I., Hocking, A.D., 1977. Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. Journal of General Microbiology 101, 35–40. https://doi.org/10.1099/00221287-101-1-35

Pitt, J.I., Miscamble, B.F., 1995. Water Relations of Aspergillus flavus and Closely Related Species. Journal of Food Protection 58, 86–90. https://doi.org/10.4315/0362-028X-58.1.86

Robinson, R.A., Stokes, R.H., 1965. Electrolyte Solutions 2d ed. Butterworth and Co., Ltd, London, p. 158.

Rosso, L., Robinson, T.P., 2001. A cardinal model to describe the effect of water activity on the growth of moulds. International Journal of Food Microbiology 63, 265–273. https://doi.org/10.1016/s0168-1605(00)00469-4

Schmidt, S.J., Fontana, A.J., 2008. Appendix E: Water Activity Values of Select Food Ingredients and Products, in: Water Activity in Foods. John Wiley & Sons, Ltd, pp. 407–420. https://doi.org/10.1002/9780470376454.app5

Sengun, I., Yaman, D., Gonul, S., 2008. Mycotoxins and mould contamination in cheese: a review. World Mycotoxin Journal 1-3, 291-298. https://doi.org/info:doi/10.3920/WMJ2008.x041

Turk, M., Abramović, Z., Plemenitaš, A., Gunde-Cimerman, N., 2007. Salt stress and plasma-membrane fluidity in selected extremophilic yeasts and yeast-like fungi. FEMS Yeast Research 7, 550–557. https://doi.org/10.1111/j.1567-1364.2007.00209.x

Turk, M., Méjanelle, L., Sentjurc, M., Grimalt, J., Gunde-cimerman, N., Plemenitas, A., 2004. Salt-induced changes in lipid composition and membrane fluidity of halophilic yeast-like melanized fungi. Extremophiles : life under extreme conditions 8, 53–61. https://doi.org/10.1007/s00792-003-0360-5

Wagner, L., Stielow, J.B., de Hoog, G.S., Bensch, K., Schwartze, V.U., Voigt, K., Alastruey-Izquierdo, A., Kurzai, O., Walther, G., 2020. A new species concept for the clinically relevant Mucor circinelloides complex. Persoonia - Molecular Phylogeny and Evolution of Fungi 44, 67–97. https://doi.org/10.3767/persoonia.2020.44.03

Welsh, D.T., 2000. Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. FEMS Microbiology Reviews 24, 263–290. https://doi.org/10.1111/j.1574-6976.2000.tb00542.x

Zalar, P., Zupančič, J., Gostinčar, C., Zajc, J., de Hoog, G.S., De Leo, F., Azua-Bustos, A., Gunde-Cimerman, N., 2019. The extremely halotolerant black yeast Hortaea werneckii - a model for intraspecific hybridization in clonal fungi. IMA Fungus 10, 10. https://doi.org/10.1186/s43008-019-0007-5

Zidan, M.A., Abdel-Mallek, A.Y., 1987. Effect of NaCl on the accumulation of glycerol by three Aspergillus species. Journal of Basic Microbiology 27, 393–397. https://doi.org/10.1002/jobm.3620270713

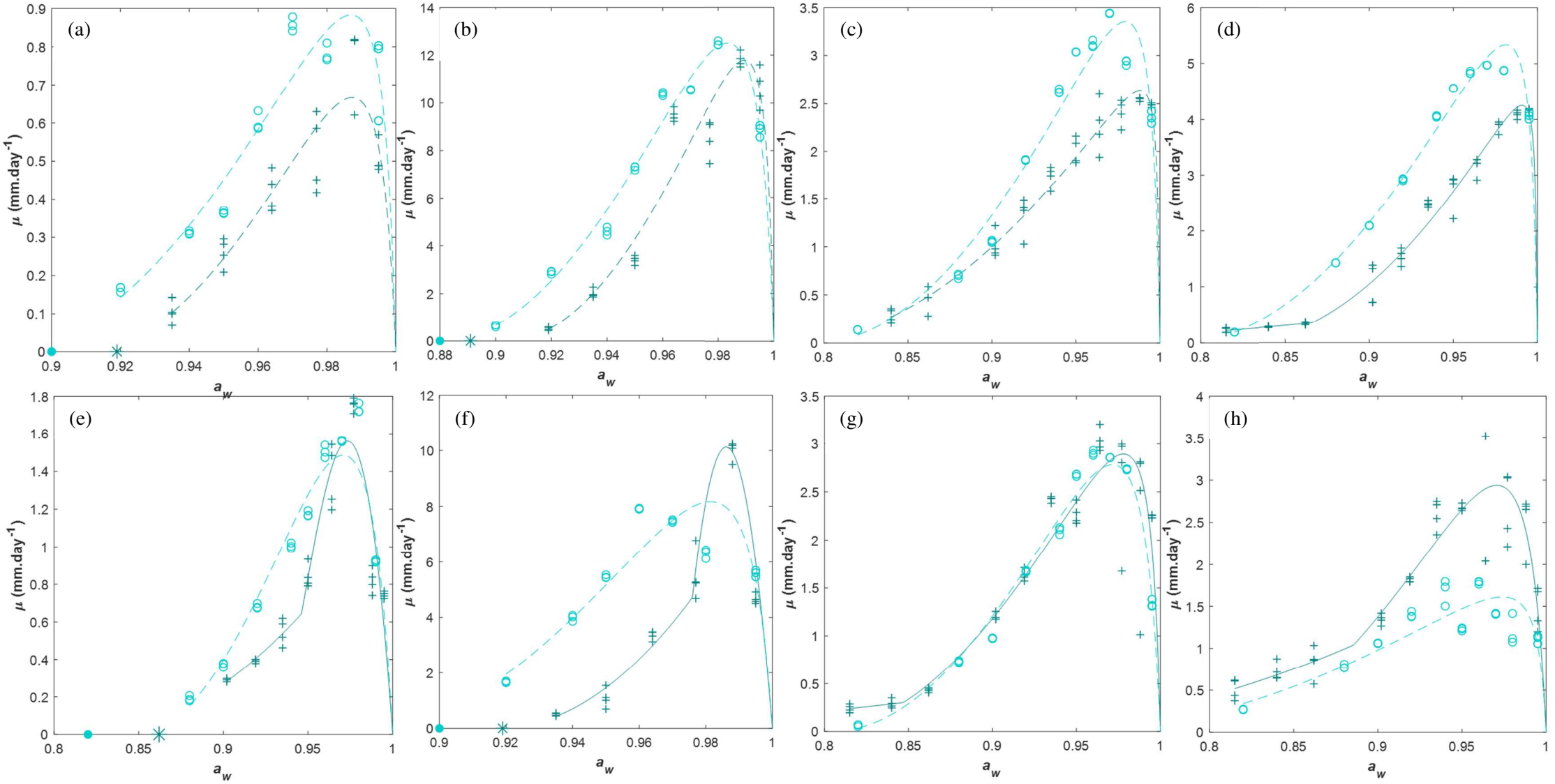


Fig. 1. Influence of a_w reduction of PDA medium at 25°C on the growth rate of *B. domesticum* (a), *M. circinelloides* (b), *P. camemberti* (c), *A. flavus* (d), *C. herbarum* (e), *M. lanceolatus* (f), *P. fuscoglaucum* (g) and *P. nalgiovense* (h). Water activity was reduced either with glycerol: (-), no growth observed after 70 days (●), μ_{max} observed (○) and fitted value with CMR model values (--); or NaCl: (-), no growth observed after 70 days (∗), μ_{max} observed (+) and fitted with CMR model (--) or CMLM model values (—).

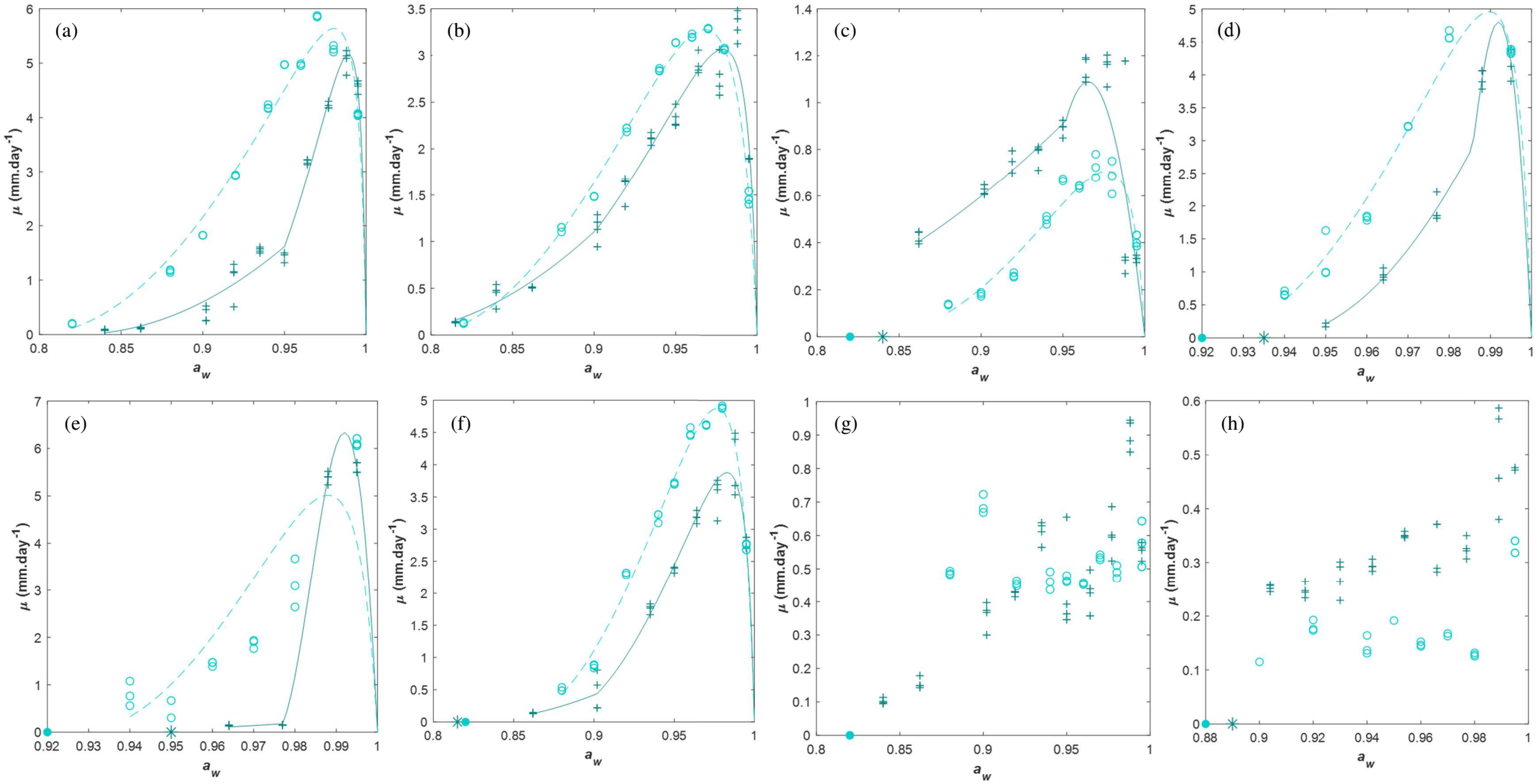


Fig. 2. Influence of a_w reduction of PDA medium at 25°C on the growth rate of *P. roqueforti* (a), *P. solitum* (b), *S. asperula* (c), *G. candidum* (d), *P. niveus* (e), *P. expansum* (f), *P. bialowiezense* (g) and *S.casei* (h). Water activity was reduced either with glycerol: (-), no growth observed after 70 days (●), μ_{max} observed (○) and fitted with CMR model values (--); or NaCl (-): no growth observed after 70 days (∗), μ_{max} observed (+) and fitted with CMR model (--) or CMLM model values (—).

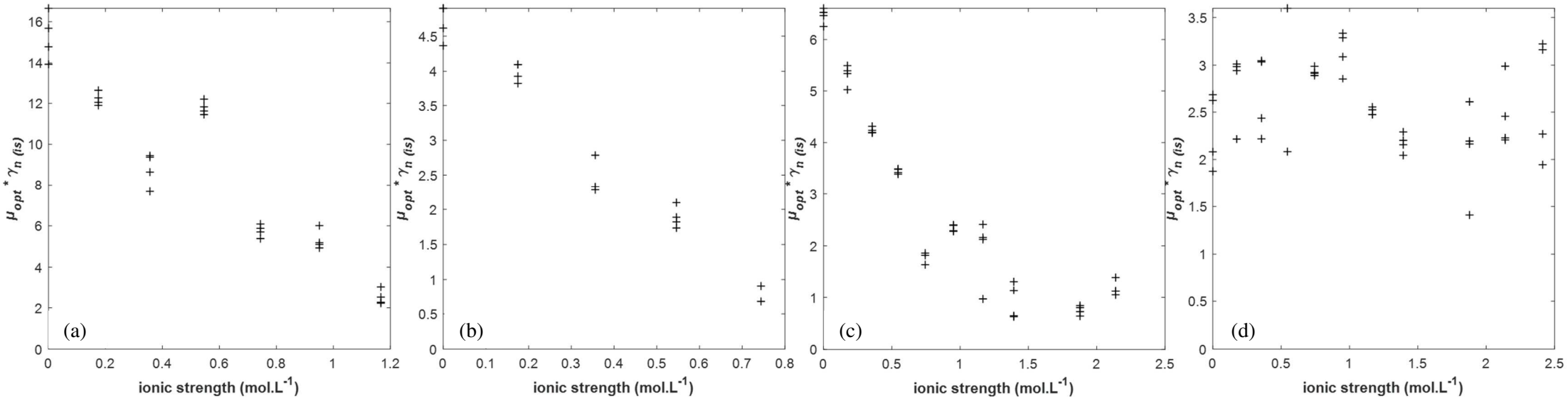


Fig. 3. Influence of the ionic strength of PDA medium at 25°C on $\mu_{opt\,N} * \gamma_{n\,(is)}$ (+) of *M. circinelloides* (a), *G. candidum* (b), *P. roqueforti* (c) and *P. nalgiovense* (d).

Table 1. List of the strains used in this study.

Fungal species	Strain Collection number	Substrate of origin	Type of isolate
Aspergillus flavus	UBOCC-A-108066	butter	Spoiler
Cladosporium herbarum	UBOCC-A-108074	cheese	Spoiler
Bisifusarium domesticum	UBOCC-A-113010	cheese (crust)	Adjunct
Geotrichum candidum	UBOCC-A-113018	dairy environment	Adjunct
Mucor circinelloides	UBOCC-A-109182	dairy environment	Spoiler
Mucor lanceolatus	UBOCC-A-109153	dairy environment	Adjunct
Paecilomyces niveus	UBOCC-A-110204	cow milk	Spoiler
Penicillium bialowiezense	UBOCC-A-110007	fresh dairy product	Spoiler
Penicillium camemberti	UBOCC-A-113011	cheese (crust)	Adjunct
Penicillium expansum	UBOCC-A-110032	fresh cheese	Spoiler
Penicillium fuscoglaucum	UBOCC-A-113012	dairy environment	Spoiler
Penicillium nalgiovense	UBOCC-A-113013	cheese (crust)	Adjunct
Penicillium roqueforti	UBOCC-A-113014	cheese	Adjunct
Penicillium solitum	UBOCC-A-113015	dairy environment	Spoiler
Scopulariopsis asperula	UBOCC-A-113016	dairy environment	No information
Sporendonema casei	UBOCC-A-113017	dairy environment	No information

Table 2

Estimation of secondary model parameters (and confidence intervals, α = 0.05) obtained by fitting the CMR (Eq. (2)) or CMLM (Eq. (3)) models to radial growth rate (μ_{max}) of *B. domesticum*, *M. circinelloides*, *P. camemberti*, *A. flavus*, *C. herbarum*, *M. lanceolatus*, *P. fuscoglaucum* and *P. nalgiovense* at 25°C as a function of PDA medium containing either glycerol or NaCl to adjust a_w. The accuracy of models was evaluated by using the Akaike Information criterion (AIC). (MIN_{aw}): the lowest a_w with observed growth. When growth was observed at the lowest tested aw, the MIN_{aw} was considered inferior or equal to this value. (-): model not adjusted or parameter not estimated.

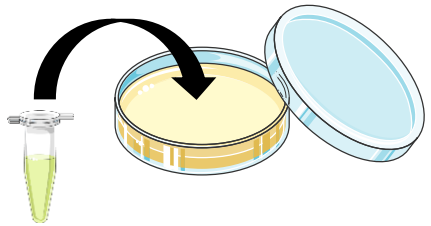
Species	*B. domesticum*		*M. circinelloides*		*P. camemberti*		*A. flavus*	
Group	1		1		1		2	
Solute	Glycerol	NaCl	Glycerol	NaCl	Glycerol	NaCl	Glycerol	NaCl
MIN_{aw}	0.920	0.935	0.900	0.902	≤ 0.820	≤ 0.815	≤ 0.820	≤ 0.815
Model	CMR	CMR	CMR	CMR	CMR	CMR	CMR	CMLM
$a_{w\ min}$	0.883 ± 0.006	0.908 ± 0.005	0.880 ± 0.002	0.903 ± 0.003	0.794 ± 0.005	0.776 ± 0.005	0.786 ± 0.002	0.619 ± 0.113
$a_{w\ opt}$	0.987 ± 0.001	0.987 ± 0.001	0.983 ± 0.001	0.989 ± 0.001	0.979 ± 0.001	0.988 ± 0.001	0.981 ± 0.000	0.991 ± 0.002
μ_{opt}	0.88 ± 0.03	0.67 ± 0.03	12.50 ± 0.23	11.79 ± 0.46	3.35 ± 0.07	2.64 ± 0.05	5.34 ± 0.05	4.25 ± 0.10
$a_{w\ 1}$	-	-	-	-	-	-	-	0.818 ± 0.007
$a_{w\ c}$	-	-	-	-	-	-	-	0.866 ± 0.007
AIC	-62.48	-57.91	-27.53	12.86	-47.46	-68.53	-85.14	-51.23
Species	*C. herbarum*		*M. lanceolatus*		*P. fuscoglaucum*		*P. nalgiovense*	
Group	2		2		2		2	
Solute	Glycerol	NaCl	Glycerol	NaCl	Glycerol	NaCl	Glycerol	NaCl
MIN_{aw}	0.880	0.902	0.920	0.935	≤ 0.820	≤ 0.815	≤ 0.820	≤ 0.815
Model	CMR	CMLM	CMR	CMLM	CMR	CMLM	CMR	CMLM
$a_{w\ min}$	0.849 ± 0.004	0.815 ± 0.038	0.876 ± 0.007	0.917 ± 0.005	0.804 ± 0.003	0.549 ± 0.631	0.709 ± 0.020	0.645 ± 0.072
$a_{w\ opt}$	0.971 ± 0.001	0.973 ± 0.002	0.981 ± 0.001	0.986 ± 0.006	0.972 ± 0.001	0.978 ± 0.002	0.974 ± 0.002	0.971 ± 0.002
μ_{opt}	1.49 ± 0.04	1.56 ± 0.08	8.17 ± 0.34	10.09 ± 3.44	2.78 ± 0.04	2.89 ± 0.12	1.61 ± 0.06	2.94 ± 0.11
$a_{w\ 1}$	-	0.927 ± 0.009	-	0.972 ± 0.470	-	0.794 ± 0.012	-	0.795 ± 0.017
$a_{w\ c}$	-	0.946 ± 0.004	-	0.976 ± 0.003	-	0.847 ± 0.015	-	0.886 ± 0.016
AIC	-62.71	-38.11	-3.53	2.73	-73.16	-1.13	-35.74	-30.68

Table 3

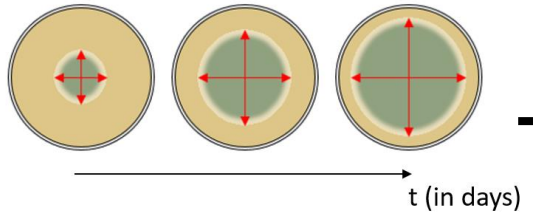
Estimation of secondary model parameters (and confidence intervals, α = 0.05) obtained by fitting the CMR (Eq. (2)) or CMLM (Eq. (3)) models to radial growth rate (μ_{max}) of *P. roqueforti*, *S. asperula, G. candidum*, *P. niveus*, *P. expansum, P. bialowiezense* and *S.casei* at 25°C as a function of PDA medium containing either glycerol or NaCl to adjust a_w. The accuracy of models was evaluated using the Akaike information criterion (AIC). (MIN_{aw}): the lowest a_w with an observed growth. When a growth was observed at the lowest tested a_w, the MIN_{aw} was considered inferior or equal to this value. (-): model not adjusted or parameter not estimated.

Species	*P. roqueforti*		*P. solitum*		*S. asperula*		*G. candidum*	
Group	2		2		2		3	
Solute	Glycerol	NaCl	Glycerol	NaCl	Glycerol	NaCl	Glycerol	NaCl
MIN_{aw}	≤ 0.820	≤ 0.815	≤ 0.820	≤ 0.815	0.880	0.862	0.940	0.950
Model	CMR	CMLM	CMR	CMLM	CMR	CMLM	CMR	CMLM
$a_{w\ min}$	0.796 ± 0.004	0.822 ± 0.006	0.792 ± 0.001	0.763 ± 0.013	0.833 ± 0.005	0.686 ± 0.050	0.919 ± 0.002	0.937 ± 0.002
$a_{w\ opt}$	0.980 ± 0.001	0.989 ± 0.001	0.968 ± 0.000	0.979 ± 0.001	0.977 ± 0.001	0.965 ± 0.017	0.990 ± 0.001	Fixed at 0.992
μ_{opt}	5.64 ± 0.10	5.16 ± 0.18	3.28 ± 0.02	3.06 ± 0.07	0.71 ± 0.02	1.09 ± 0.10	4.96 ± 0.13	4.77 ± 0.18
$a_{w\ 1}$	-	0.912 ± 0.006	-	0.809 ± 0.009	-	0.932 ± 0.904	-	0.978 ± 0.004
$a_{w\ c}$	-	0.950 ± 0.004	-	0.902 ± 0.027	-	0.952 ± 0.100	-	0.986 ± 0.001
AIC	-46.84	-18.67	-123.06	-55.11	-86.03	-31.24	-38.76	-51.27
Species	*P. niveus*		*P. expansum*		*P. bialowiezense*		*S. casei*	
Group	3		3		4		4	
Solute	Glycerol	NaCl	Glycerol	NaCl	Glycerol	NaCl	Glycerol	NaCl
MIN_{aw}	0.940	0.964	0.880	0.840	0.880	≤ 0.815	0.900	0.904
Model	CMR	CMLM	CMR	CMLM	-	-	-	-
$a_{w\ min}$	0.927 ± 0.007	0.916 ± 0.019	0.848 ± 0.002	0.814 ± 0.017	-	-	-	-
$a_{w\ opt}$	Fixed at 0.988	Fixed at 0.992	0.977 ± 0.001	Fixed at 0.983	-	-	-	-
μ_{opt}	5.01 ± 0.66	6.31 ± 0.19	4.87 ± 0.07	3.88 ± 0.12	-	-	-	-
$a_{w\ 1}$	-	0.975 ± 0.004	-	0.869 ± 0.008	-	-	-	-
$a_{w\ c}$	-	0.977 ± 0.004	-	0.902 ± 0.009	-	-	-	-
AIC	26.27	-56.62	-66.95	-35.39	-	-	-	-

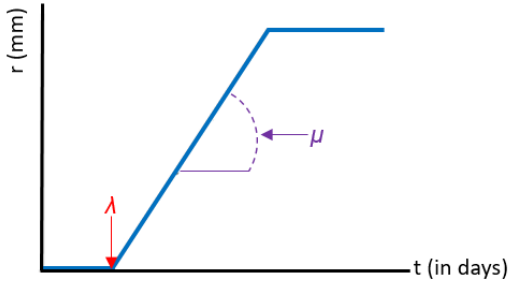
Fungal conidia inoculated on culture media with several a_w levels



Regular measures of thallus diameters



Estimation of growth rates (primary modelling)



Estimation of a_w cardinal values (secondary modelling)