Evaluation of radial growth dynamics variability of *Geotrichum candidum*: A quantitative approach

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Summary

Geotrichum candidum is a fungus dominant in most smear and mould-ripened cheese varieties. Its representatives display tremendous amount of morphological and genetic variability and a marked polymorphism. In this work, 18 isolates and 6 collection strains of *G. candidum* were compared with respect to their radial growth behaviour at 15 °C. Our findings did not indicate considerable strain-dependent growth potential of this dairy fungus based on values of lag phase and exponential growth. In general, growth rate (*Gr*) and lag phase durations (λ) were normally distributed. The growth rate values ranged from 0.114 mm·h^{-1} to 0.197 mm·h^{-1} (average Gr = 0.152 mm·h^{-1} ± 0.021 mm·h^{-1}; coefficient of variation CV = 13.8 %) and estimated average λ = 31.2 h ± 10.1 h). Collected research data and statistics provided an interesting insight into variability in the specific growth rate, and variability between different strains. The obtained knowledge is important for *G. candidum* growth prediction and food quality management.

Keywords

Geotrichum candidum; surface growth potential; strain diversity; predictive mycology

In foods, two main roles of *Geotrichum candidum* Link or other microscopic fungi may be considered. First, yeasts and moulds are used as important adjunct cultures in food products such as bread, beer, wine or other fermented foods, and these microscopic fungi are a natural part of the mycobiota of dairy products. They are known to significantly influence flavour, aroma, colour, texture and sensorial properties of these products by their proteolytic and lipolytic activity, aroma and pigment formation, fermentation and/or assimilation of residual sugars, lactate and citrate [1]. On the other hand, the microscopic fungi including *G. candidum* contaminate processed foods and adversely affect the nutritional quality of contaminated food due to physical, chemical and sensorial changes [2–4].

G. candidum is a microscopic fungus that could be isolated from various nutritionally rich substrates such as silage, plant tissues, fruits, insects, digestive tract in humans and other mammals [5–9]. This species is commonly associated with food and is naturally found in milk and dairy products such as fermented milk, cream, fresh cheese, curd cheese, soft cheeses such as Camembert and semi-fresh goats' and ewes' milk cheese [10–12].

This microorganism from milk, similar to many other microscopic fungi, tolerates a wide range of environmental conditions, notably temperature and pH. It can grow at temperatures from 5 °C to 38 °C, with an optimum around 25 °C, and in a wide pH interval, from 3 to 11 [5, 13]. Generally, this microscopic fungus is considered to be sensitive to NaCl, however, the property is strain-dependent [14]. According to Marcellino and Benson [11], inhibition of the majority of strains can occur at 1.0–2.0 g·l^{-1} of NaCl in the growth medium and they are usually unable to grow on a medium containing more than 4 g·l^{-1} of salt.

G. candidum is generally associated with agri-food industry, but its presence has ambiguous effects. Due to the production of many volatile compounds important for the flavour, such as phenyl compounds, lactones, esters and volatile sulphur compounds, this fungus is frequently used as a ripening agent. Alone or in combination with *Penicillium camemberti*, *P. candidum* and/or *P. roqueforti*, it is associated with surface mycobiota in many mould-ripened cheeses (Camem-

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bert, Brie, St. Marcellin), blue-veined cheeses (Danablu, Roquefort, Stilton, Gorgonzola) and smear-ripened cheeses (Limburger, Münster, Livarot, Tilsit, Reblochon, Pont-l'Évęque cheese) [13, 15–17]. It stimulates the development of other microbiota in the next stages of cheese maturation and determines the texture, cohesiveness and thickness of the cheese rind [5, 17]. On the surface of mould-ripened cheeses, *G. candidum* is responsible for uniform white and velvety coat and contributes to the development of typical cheese flavours [18].

This microscopic fungus is also involved in beer making and industrial enzyme production [12]. Strains of *G. candidum* are utilized by a number of industries and recently have been employed in the bioremediation of olive mill and distillery wastewaters, where they were capable of reducing the phenolics, oxygen demand and antimicrobial compounds of these industrial by-products [9].

However, *G. candidum*, is also a potential spoilage agent responsible for deterioration of fresh cheeses, fermented milk, cream, butter, poultry meat, fruit juices and vegetables [9, 10, 19–21]. It is an example of a fungus capable of growing at refrigeration temperatures. During inappropriate growth on the surface of a raw soft ripened cheese (Camembert-type cheese), it metabolizes food components and causes defects resulting also in economic losses. This fungus with mouldy and yeasty tendencies produces yeasty, fruity or alcoholic off-flavours and odours, and is responsible for unequal covering of the cheese surface, thus ruining the integrity of the rind [2, 5, 17, 20, 22].

Geotrichum species are also indicative of food quality. The presence of *G. candidum* in pasteurized food products is generally an indication of unsatisfactory sanitary conditions during food preparation and storage, or the usage of inferior raw materials. This microscopic fungus is known to grow rapidly as a slimy layer submerged in liquid on concrete, metal or wood in an food-processing plants with a low hygiene level, with the result of non-viable mycelial fragments ending up within the processed products [7, 9].

Prediction of growth parameters related to the behaviour of food containing microorganisms is important for the design of dairy products, their quality and for maintaining food safety [23]. Growth of yeasts and moulds is a major concern because some of them are responsible for deterioration of food products and because some of them can potentially cause foodborne diseases [3]. Predictive models describe the microbial response to a number of preservation factors and provide detailed knowledge of the microbial behaviour in food products, condensed into mathematical models [24]. The predictive microbiological models facilitate an objective evaluation of microbial spoilage, safety and quality of food, and may be used to predict changes in quality and stability of foods, as well as to determine the deterioration rate [25].

The variability growth parameters that influences the microbial responses in foods should not be neglected in risk-based food quality/safety studies. Its major sources include variability in food characteristics, storage conditions (time-temperature) and individual cell variability. One of the most significant sources of variability is the inherent difference in microbial behaviour among strains of the same species. Considerable intra-species diversity is related to physiological requirements of microscopic fungi, e.g. growth rates and lag phase duration [26, 27].

Extensive research data indicate variability in characteristics of different *G. candidum* strains. Within *G. candidum* clade, two major morphotypes can be distinguished. The first corresponds to strains with cream-coloured, yeast-like colonies. From microscopic point of view, they produce generous arthrospores by breaking the hyphae. This morphotype exhibits only weak growth with optimum temperature between 22 °C and 25 °C. The second type is characterized by white felting colonies spreading out on agar plates, with microscopic structure composed mainly of vegetative hyphae and few arthrospores, with rapid growth at optimum temperatures of 25–30 °C. Between the two forms, strains form a continuum offering a wide diversity of morphological aspects [2, 5, 11, 13, 18, 28, 29].

Beyond the scientific interest in understanding the behaviour of *G. candidum* in the food context, the aim of this study was to describe the strain variability of *G. candidum* growth profiles in order to integrate it in exposure assessment. Data with the inherent variability can be used as effective tools for optimization of quality control systems in dairy production.

MATERIALS AND METHODS

G. candidum strains

Eighteen isolates covering a wide range of dairy origins, and six collection strains (*Galactomyces candidus* CBS 178.71, *G. candidum* CBS 180.33, *G. candidum* CBS 357.86, *G. candidum* CBS 557.83, *G. bryndzae* CBS 11176, *G. silvicola* CBS 9194; Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands) were evaluated in

the present study.

Based on the morphological, biochemical and molecular tests, all studied isolates were identified as *G. candidum* (data not shown). Identification was confirmed on the basis of internal transcribed spacer (ITS) sequencing (Dr. Domenico Pangallo, Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia). Phylogenetic analyses by GROENEWALD et al. [30] revealed affinity of novel species *Geotrichum bryndzae* [31] and *Geotrichum silvicola* [32] to *Geotrichum candidum*/*Galactomyces candidus* group.

Cultures of all isolates and collection strains were stored refrigerated (5 °C ± 1 °C) on Plate Count Skim Milk Agar (SMA; Merck, Darmstadt, Germany) slants and were subcultured monthly.

Growth experiments

Representatives of *G. candidum* were grown for 72 h on the top layer of a perpendicular SMA agar tube at 25 °C to reach heavy sporulation. Spores (conidia) were then suspended in 5 ml of sterile saline solution (8.5 $g \cdot l^{-1}$ NaCl, 0.1 $g \cdot l^{-1}$ of peptone) by scraping gently the surface of the medium with a sterile pipette tip. Immediately after preparation, suspension of each of the tested fungal strains was diluted in solution to yield an inoculum count of approximately 10^3 $CFU \cdot ml^{-1}$.

The standard growth SMA medium, which was acidified with 10 $ml \cdot l^{-1}$ lactic acid (Sigma-Aldrich, St. Louis, Missouri, USA) to pH 5.5 was used in the experiments. The medium was autoclaved and 30 ml of growth medium was poured into sterile Petri dishes (diameter 110 mm). After solidifying, 2 μl of appropriately diluted spore suspensions were used to inoculate the centre of the Petri dishes. The initial diameter of the inoculated spore suspension drop was established to 4.6 mm. For all experiments, zero time was defined as the time when the suspension was applied to the surface of agar plate.

After inoculation, the plates were sealed in polyethylene bags to prevent water loss and were stored under controlled storage conditions in programmable incubators (Pol-Eko Aparatura, Wodzisław Śląski, Poland) set at 15 °C. The experiments were performed in aerobic conditions and Petri dishes were incubated upside down. Growth experiments were carried out in triplicate.

The diameters of developing colonies were measured at appropriate time intervals, using a Vernier calliper (150 mm × 0.02 mm; Sinochem Jiangsu, Nanjing, China) in two orthogonal directions per plate, without opening the dishes. The final diameter of colonies was calculated as arithmetic mean. The measurements were taken from the early stages of growth in order to capture the lag phase.

Growth curve fitting

The growth was assessed as the change in diameter of growing circular colonies using the primary growth model of BARANYI and ROBERTS [33]. The growth response of each *G. candidum* isolate and strain was plotted against time and fitted to a model for the estimation of the growth rate (*Gr*) and the apparent lag phase duration (λ) using an in-house Excel Add-in package 'DMFit' version 3.5 (ComBase managed by United States Department of Agriculture-Agricultural Research Service, Washington D.C., USA and University of Tasmania Food Safety Centre, Hobart, Australia). The model is able to describe growth curves either with or without lag phase and with or without stationary phase. This curvature is controlled by two parameters, namely, upper asymptote (n) and lower asymptote (m). The m curvature parameter and n curvature parameter of the growth model were set to 10, by default. Growth curve quadruples (18 isolates and 6 collection strains), i.e. $(18 + 6) \times 4 = 96$ curves were fitted by the primary model.

Statistical analyses

Each experiment was performed in triplicates. Analysis of variance of medians was used to assess the significance of growth conditions, colony diameter and intraspecific differences in monitored isolates and collection strains. Results were presented as means of values with their standard deviations. Statistical analyses were carried out using Microsoft Excel 2016 (Microsoft, Redmond, Washington, USA) with addition of an analytical program Analyse-it (Analyse-it Software, Leeds, United Kingdom). Parametric data were treated by ANOVA test with 95% confidence interval.

The coefficient of variation (*CV*) shows the extent of variability in relation to the mean of the population. *CV* is calculated from the average and standard deviation as follows and reported as a percentage:

$$CV = \frac{SD}{\bar{x}} \times 100 \quad (1)$$

where *SD* is standard deviation and $\bar{x}$ is mean.

RESULTS AND DISCUSSION

In the present study, G. candidum was used as a target microorganism since it is an important dairy contaminant. It can be found in a wide

range of environments and has been isolated from different sources including dairy products. The *G. candidum* isolates and strains used in this study were not only strains with a long history of culturing in the laboratory, but also strains isolated from food products, which have a short laboratory history. We used also six collection strains of diverse geographical origins and isolation sources. We also included *Geotrichum silvicola* and *Geotrichum bryndzae* species in our study, because these species were recognized as synonyms of *Galactomyces candidus* (mitotic state *G. candidum* Link) by GROENEWALD et al. [30] based on phylogenetic analysis.

Several studies in quantitative microbiology showed that intraspecific variation in growth response is an important step in mathematical prediction models. Strain variability is defined as an inherent characteristic of microorganisms that cannot be reduced when strains are identically treated under the same set of conditions [34, 35]. Variability in growth kinetics of different bacterial species was reported in previous studies [34, 36–44]. However, only a limited number of studies was focused on intraspecific growth variation of yeasts and moulds [45–49].

In the present study, the impact of strain variability on maximum growth rate was quantified using twenty-four *G. candidum* representatives. Strain variability was investigated by fitting a primary growth model, a continuous sigmoidal function approach. Model of BARANYI and ROBERTS [33] can be used to obtain two kinetic parameters, namely, growth rate and lag phase duration. This model has been successfully adapted to fit colony diameter growth curves of *Aspergillus flavus* [50], *Penicillium roqueforti* [51], *Byssochlamys fulva*, *Neosartorya fischeri, Talaromyces avellaneus* [52],

Tab. 1. Growth parameters and origin of tested Geotrichum candidum isolates and collection strains.

Isolate/Strain	Gr [mm·h^{-1}]	λ [h]	α	d_{max} [mm]	R^2	Source
A	0.115 ± 0.001	27.3 ± 6.2	3.13 ± 0.71	76.05 ± 3.76	0.994	Ewes' lump cheese
B	0.168 ± 0.009	26.6 ± 3.4	4.47 ± 0.73	88.42 ± 5.59	0.990	Ewes' lump cheese
C	0.144 ± 0.002*	31.4 ± 4.2	4.53 ± 0.68	86.36 ± 0.66	0.999	Ewes' lump cheese
D	0.123 ± 0.002*	20.9 ± 4.2	2.57 ± 0.48*	74.54 ± 0.94	0.996	Bryndza cheese
E	0.156 ± 0.008	20.8 ± 3.1*	3.24 ± 0.57	76.69 ± 2.19	0.993	Bryndza cheese
F	0.154 ± 0.004	36.6 ± 4.7	5.65 ± 0.88	74.79 ± 0.54	0.998	Bryndza cheese
G	0.172 ± 0.002	27.4 ± 5.5	4.72 ± 0.96	90.32 ± 1.15	0.998	Cottage cheese
H	0.165 ± 0.007	48.8 ± 1.7	8.07 ± 0.55	80.65 ± 2.89	0.997	Bryndza cheese
I	0.135 ± 0.001	43.9 ± 4.6	5.91 ± 0.64	64.48 ± 2.53*	0.996	Ewes' lump cheese
J	0.164 ± 0.002	46.6 ± 0.6	7.65 ± 0.06*	86.30 ± 0.93	0.999	Bryndza cheese
K	0.175 ± 0.002	47.9 ± 2.3	8.38 ± 0.49	55.89 ± 0.99	0.999	Ewes' lump cheese
L	0.125 ± 0.007	48.3 ± 7.3	6.06 ± 1.25	69.70 ± 0.24*	0.998	Ewes' lump cheese
M	0.135 ± 0.003	30.3 ± 6.6	4.08 ± 0.86	76.84 ± 3.56	0.994	Ewes' lump cheese
N	0.137 ± 0.002	24.6 ± 4.5	3.37 ± 0.61	74.08 ± 1.04	0.998	Ewes' lump cheese
O	0.132 ± 0.004	26.6 ± 1.3	3.51 ± 0.14	76.57 ± 1.81	0.997	Ewes' lump cheese
P	0.144 ± 0.001	22.1 ± 0.8	3.19 ± 0.13	83.33 ± 0.96	0.999	Ewes' lump cheese
R	0.164 ± 0.002	30.3 ± 7.7	4.99 ± 1.30	80.50 ± 0.62	0.997	Ewes' lump cheese
S	0.159 ± 0.007	23.1 ± 2.9	3.67 ± 0.34	90.13 ± 2.33	0.995	Ewes' lump cheese
CBS 178.71	0.197 ± 0.004*	31.7 ± 2.4	6.25 ± 0.47	91.54 ± 0.53	0.999	Soil polluted with oil
CBS 180.33	0.124 ± 0.001	14.7 ± 1.8	1.83 ± 0.22	73.24 ± 1.79*	0.997	Unknown
CBS 357.86	0.171 ± 0.002	32.6 ± 2.1	5.56 ± 0.28	88.83 ± 0.68	0.999	Unknown
CBS 557.83	0.177 ± 0.002	30.6 ± 2.5	5.41 ± 0.50	94.31 ± 1.27*	0.999	Fruit
CBS 11176	0.170 ± 0.003	33.4 ± 0.6	5.70 ± 0.16	86.06 ± 0.76*	0.999	Bryndza cheese
CBS 9194	0.153 ± 0.006	23.1 ± 2.7	3.54 ± 0.53	78.97 ± 2.51	0.996	Insect

Values are expressed as mean ± standard deviation. Significantly different values are marked with an asterisk (*) ($p > 0.05$ in t-test and ANOVA test; $n = 4$).
Gr – growth rate; λ – lag phase duration; α – parameter expressing physiological state of cells; d_{max} – final diameter of colonies in stationary phase; R^2 – coefficient of determination of growth curve.

P. brevicompactum [53], *Monascus ruber* [54], *A. carbonarius* [55], *Rhizopus oryzae* [56], *A. flavus, A. parasiticus, Fusarium verticillioides* and *F. proliferatum* [57, 58] with satisfactory results. Radial growth rates described the colony diameter as a function of cultivation time.

The incubation temperature and pH value of solid growth medium were selected to reflect the ewes' cheese ripening process. The average growth parameters are summarized in Tab. 1. The strain-dependent growth variability was evaluated by one-way ANOVA using 'between groups' concept to study sources of variability. Tab. 2 also includes mean and median, minimum and maximum values of growth rates, lag phase duration, inoculum physiological state (α) and maximum diameter of colonies in stationary phase (d_{max}).

For all monitored strains and isolates under above mentioned conditions, growth kinetics followed a typical sigmoid curve with lower and upper asymptotes (Fig. 1). The growth curves based on colony diameters were typical for all isolates and collection strains, characterized by a lag phase, followed by linear growth and stationary phase in all cases. The experimental data were well-fitted to the used model with the mean coefficient of determination (R^2) for all fitting trials (n = 96) being 0.997 with standard deviation 0.002. Figs. 2–4 shows graphical illustration of the statistical analysis.

The average growth rates of *G. candidum* showed a low standard deviation represented by *CV* of 13.8 % (*Gr* = 0.152 mm·h^{-1} ± 0.021 mm·h^{-1}). Regarding *Gr*, few significant differences among some isolates (C and D) and collection strains (*Ga. candidus* CBS 178.71) were observed under the monitored growth conditions. The 5th and 95th percentiles of the growth rate distribution were 0.119 mm·h^{-1} and 0.180 mm·h^{-1}, respectively. The fitted quantile-quantile distributions of radial growth rates were strongly adjusted to data (Fig. 3A). An illustration of the estimated values of this kinetic parameter for the tested strains in the form of a cumulative probability distribution of the growth rates is presented in Fig. 4A.

Tab. 2. Descriptive average growth parameters of *G. candidum*.

	Gr [mm·h^{-1}]	λ [h]	d_{max} [mm]
Average value	0.152	31.2	80.08
SD	0.002	10.1	9.19
Median	0.154	30.0	80.36
Minimum	0.114	12.2	54.56
Maximum	0.201	58.5	95.79
n	96	96	96
CV	13.8 %	32.4 %	11.5 %

Radial growth at 15 °C based on 96 growth curves of 24 representatives (isolates and collection strains).
Gr – growth rate; λ – lag phase duration; d_{max} – final diameter of colonies in stationary phase; *SD* – standard deviation, *n* – number of growth data; *CV* – coefficient of variation.

Among all 24 strains, strain A, which was an isolate from ewes' lump cheese, belonged to strains with a lower radial growth rate (*Gr* = 0.115 mm·h^{-1} ± 0.001 mm·h^{-1}). In contrast, collection strain *Ga. candidus* CBS 178.71 turned out to be the fastest growing strain (*Gr* = 0.197 mm·h^{-1} ± 0.004 mm·h^{-1}). In general, fungal collection strains were growing faster compared to dairy isolates. These mentioned strains were accustomed to growing in a wide range of harsh conditions, which allowed them to adapt to less favourable conditions and, logically, these strains exhibited higher values of growth parameters in suitable conditions.

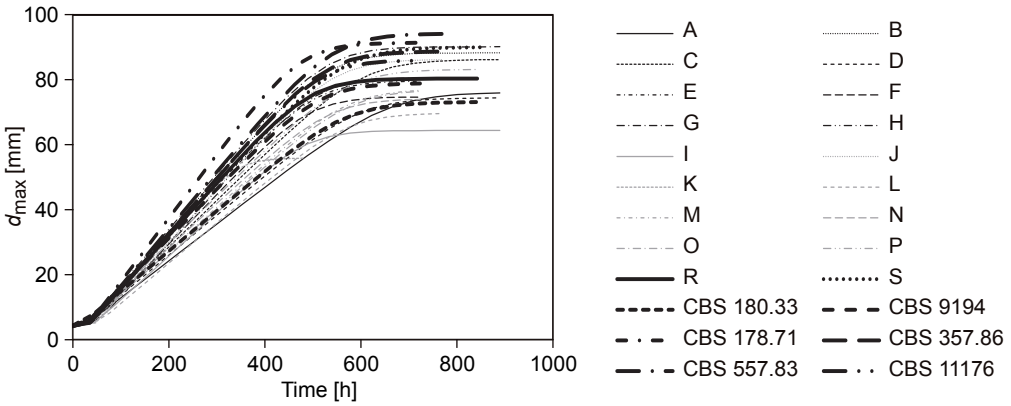

Fig. 1. Representative radial growth curves of 18 isolates and 6 collection strains of *G. candidum* colonies at 15 °C.

d_{max} – final diameter of colonies in stationary phase.

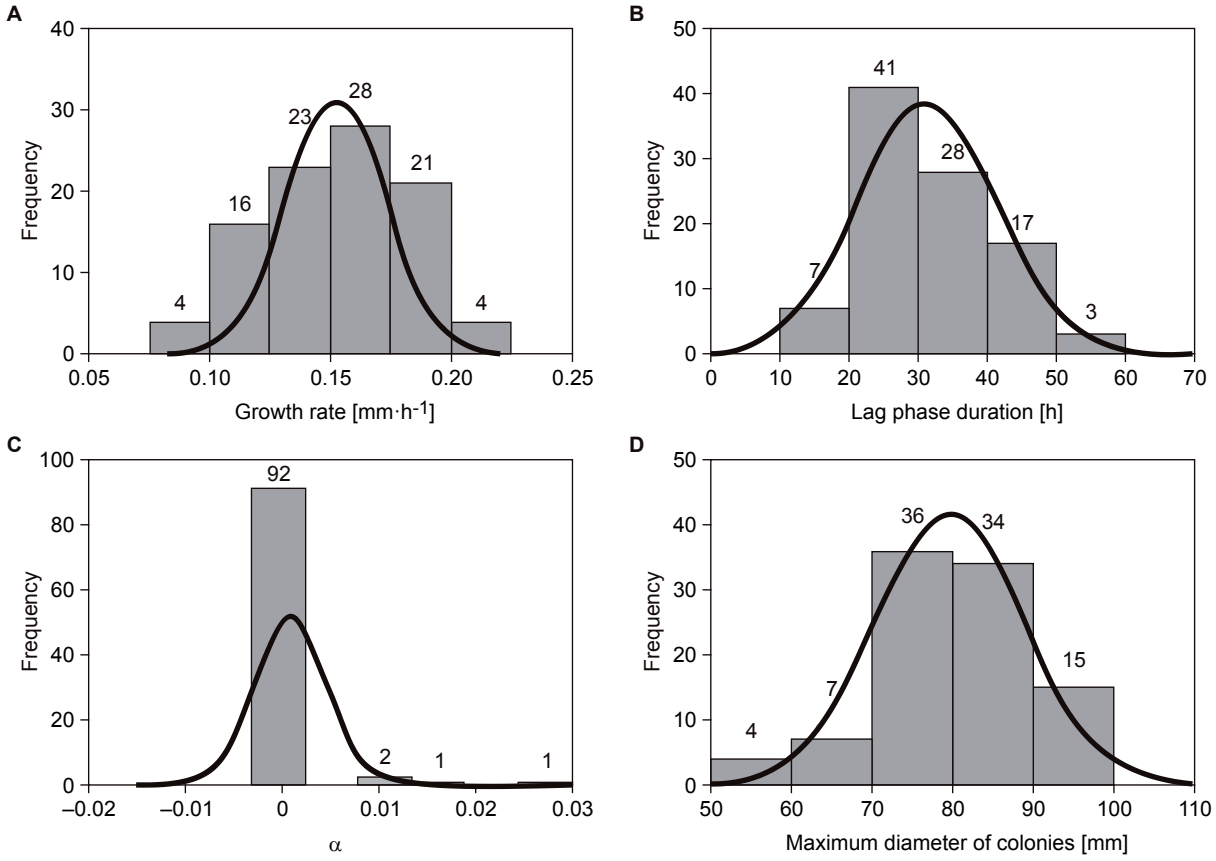

Fig. 2. Histograms of the growth values of *G. candidum* colonies (n = 96).

A – radial growth rate, B – lag phase duration, C – parameter α expressing physiological state of cells, D – maximum diameter of colonies in stationary phase.

The lag phase is a period of adjustment of the inoculated cell population to the medium. The lag times ranged from 12.2 h (strain *G. candidum* CBS 180.33) to 58.5 h (strain L) with the average value 31.2 h and standard deviation 10.1 h (n = 96; CV = 32.4 %). Statistical differences in lag phase duration followed the same trend as growth rate variation and only one significant difference was observed (between λ for *G. candidum* CBS 180.33 and strain E). The cumulative probability of estimated values is illustrated in Fig. 4B, with its mean, 5th and 95th percentiles being 31.2 h, 16.2 h and 48.5 h, respectively. There was no relationship between high growth rate and short lag phase or vice versa.

Repeatability of the lag phase duration among four replicate growth curves determined for each strain had the highest coefficient of variation among growth data (CV = 32.4 %). Strain variability in lag phase duration was previously presented in various studies [34, 43, 59–61]. This fact is in agreement with lag phase complexity depending on many factors, including potentially all physical, enzymatic or chemical conditions of the growth environment [24, 43, 62]. Strain variations in λ estimates were important and CV of growth parameters was up to three times higher among the monitored strains than within any individual strain.

The apparent growth was expressed as the difference between the initial and final diameter measurements of the colonies and its average value of 75.48 mm ± 9.19 mm was estimated. Final colony diameter in stationary phase ranged from 54.56 mm to 95.79 mm (CV = 11.48 %; n = 96). From histogram and normal distribution illustrated in Fig. 3D it is obvious, that the majority (70 from 96 values) of final diameters of monitored isolates and collection strains ranged from 70 mm to 90 mm. In general, five significant differences (p > 0.05) were found among estimated d_{max} levels, although the maximum diameters were generally observed at collection strains *Ga. candidus* CBS 178.71 and *G. candidum*. Primary growth model of Baranyi and Roberts [33] allowed us to determine the two dimensionless parameters, h_0 and α, which reflect the physiological state of

microorganisms and, accordingly, their preparedness to grow in a given environment. Parameter h_0 is a link from the history to the current growth environment. Normalization level of the procedures prior to inoculation is demonstrated by the distribution of h_0 parameter, which ranged from 3.52 to 20.65 with a standard deviation of 4.04, with similar average and median values of 11.13 and 10.71, respectively.

Higher prominent differences were observed among the tested strains with regard to the parameter α, considering its wide range (from 1.08×10^{-9} to 2.95×10^{-2}). The value of α, physiological state, is a parameter quantifying 'suitability' of the culture for the given environment. The physiological state of microbial population was represented by 'α right tail' and was estimated to be between 1.08×10^{-9} and 3.50×10^{-3} for the majority of the tested strains (Fig. 3C). More specifically, the mean and median of the α distribution were 9.67×10^{-4} and 2.24×10^{-5}, respectively. Cumulative function showed that four data points (all corresponding to *G. candidum* CBS 180.33) exceeded the range of this probability of α distribution.

Our study showed that strains from diverse habitats and isolation sources displayed closely related growth character, lag phase duration and final diameter (Fig. 1). Data presented in Tab. 1 confirmed that origin (wild or commercial) did not correlate with growth variability. However, in general, collection strains exhibited a little higher values of radial growth rates.

G. candidum presents unusual characteristics that complicate its taxonomic classification. For instance, this microscopic fungus reveals high morphological variability and wide phenotypic diversity, and has many features generally associated with filamentous fungi and yeasts [5, 13, 63]. Biochemical and genetic differences in *G. candidum* clade were observed in several studies assessing the behaviour of a small or a large number of strains of this microorganism [18, 28–30; 63–66].

Conclusions on the significance of intra-species variability for predictive models vary, possibly reflecting the conditions and strains used in the dif-

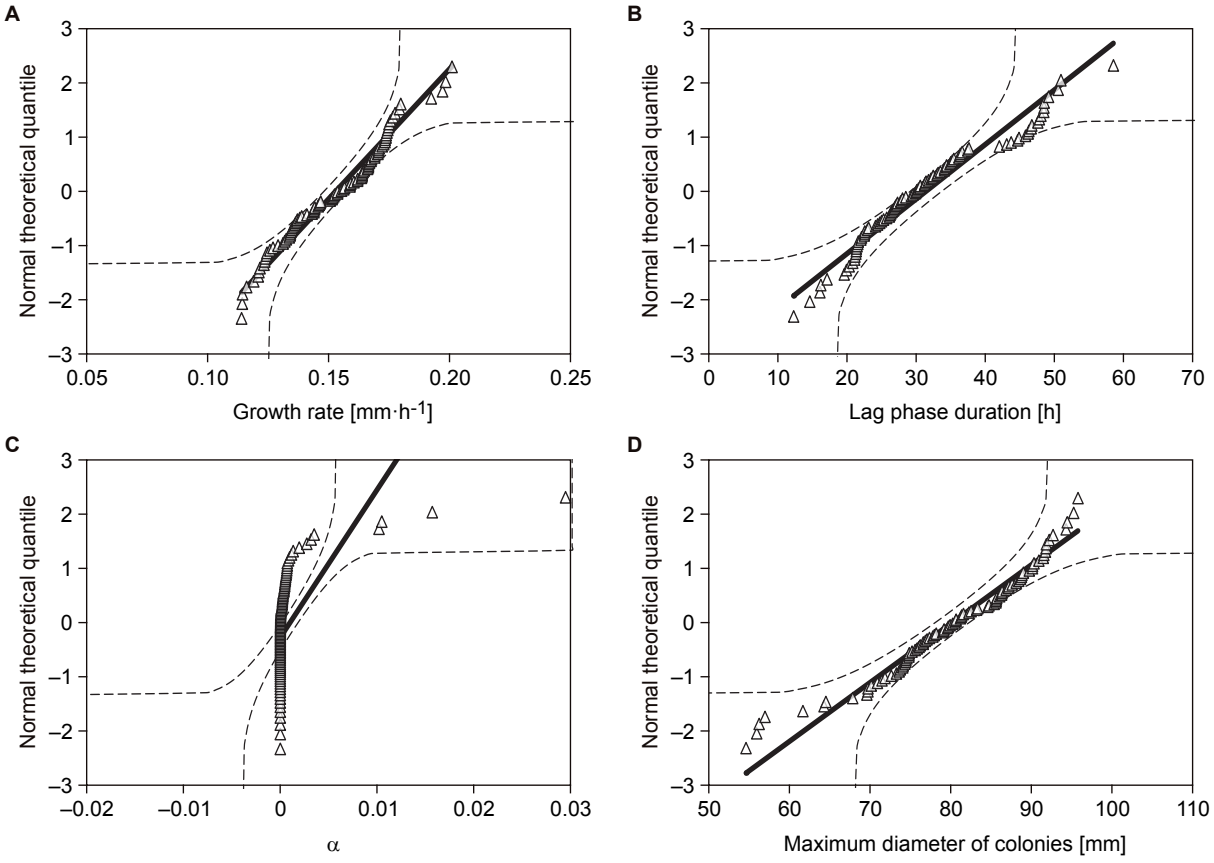

Fig. 3. Growth quantile-quantile distributions of estimated growth parameters.

A – radial growth rate, B – lag phase duration, C – parameter α expressing physiological state of cells, D – maximum diameter of colonies in stationary phase.

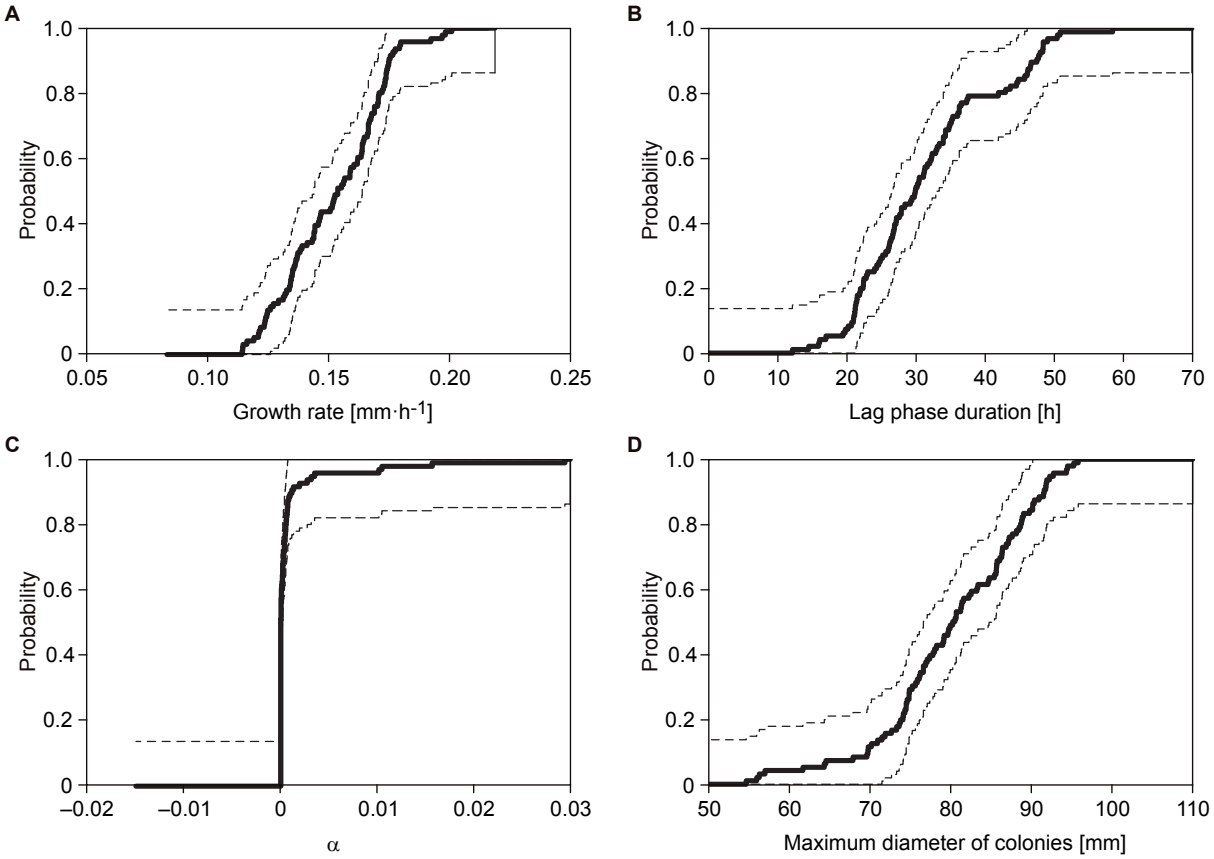

Fig. 4. Cumulative distribution function plots of estimated growth parameters of *G. candidum* at 15 °C.

A – radial growth rate, B – lag phase duration, C – parameter α expressing physiological state of cells, D – maximum diameter of colonies in stationary phase.

ferent studies. Alshareef and Robson [67] reported that 220 environmental and clinical isolates of *Aspergillus fumigatus* exhibited highly variable growth rates. In that study, statistical significance was determined by one tailed, paired *t*-test for means with a 95% confidence interval. Similarly, Ben-ami et al. [68] observed significant inter-strain differences in growth rates among *A. fumigatus* and *A. terreus* isolates. An extensive intra-species growth variation of 50 strains of *Sclerotinia sclerotiorum* demonstrated remarkable variability linked with geographic origin [69].

In contrast, Garcia et al. [46] reported that *CV* for growth rates of *Penicillium expansum* growing on apple concentrate agar at 20 °C was 13.5 % and growth of strains was similar. A strain-to-strain variation in growth among 30 strains of *Aspergillus carbonarius* was also assessed by Garcia et al. [47]. The authors revealed low variability (*CV* = 13.2 %) between strains cultivated at optimal conditions (water activity 0.98; temperature 25 °C) on malt extract agar (MEA) plates.

The level of strain-dependent growth character of *G. candidum* strains has not been clearly elucidated, yet. Strain-specific differences are of utmost importance for tracking of commercial strains, the selection and monitoring of commercial cultures selected for cheese ripening. In addition, data resulting from this study might be of interest to estimate microbiological risk and would be useful to quantify and model the effect of history via the actual physiological state of this microscopic fungus. This would allow food manufacturers to prevent the growth of *G. candidum* as a spoilage agent. Thus, the growth kinetics values obtained with these strains of *G. candidum* may be useful for predicting the growth of other strains of *G. candidum* for which data are currently unavailable.

The present study was conducted to evaluate the behaviour of 24 *G. candidum* strains aiming at (i) characterizing the variability of strain-specific growth differences, (ii) probability distribution of the growth parameters, and (iii) potential trends among the tested strains related to origin. Uniqueness of strain characteristics explains only one part of total variability stated in the literature, but the

inclusion of strain variability into mathematical models will contribute to more realistic prediction of *G. candidum* behaviour in dairy products.

CONCLUSIONS

Presence of fungi in dairy products may negatively affect not only the sensorial properties but, more importantly, their nutrional content. In order to preserve high quality of products where *G. candidum* presence is unacceptable, our study was aimed to analyse the intra-species variability of behaviour in terms of growth kinetics of this dairy microscopic fungus. The growth potential may have an important impact on accuracy of microbial risk assessment and, therefore, should be systematically assessed and accounted for. In the framework of such approaches, it is essential to have avaialable quantitative data on variability of growth parameters. Research data regarding variability and strain-specific differences of *G. candidum* were first provided by this study. They showed that the monitored isolates had similar radial growth rates (Gr = 0.152 mm·h^{-1} ± 0.021 mm·h^{-1}; CV = 13.8 %) and these were not associated with isolation source or geographical sources. The results are practically important as knowledge of probability distribution of growth parameters is a basis for estimation of growth probability of all *G. candidum* strains.

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