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International Journal of Food Microbiology 104 (2005) 51–60

INTERNATIONAL JOURNAL OF Food Microbiology

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The inhibitory effect of *Penicillium camemberti* and *Geotruchum candidum* on the associated funga of white mould cheese

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Received 24 July 2004; received in revised form 30 March 2005; accepted 5 April 2005

Abstract

The diameter of pinpoint inoculated cheese contaminates (*Cladosporium herbarum, Penicillium roqueforti, Penicillium caseifulvum* and *Penicillium commune*), isolated from either the dairy environment or directly from cheese, were inoculated 24 h after inoculation of the secondary starters to simulate contamination at the critical point of the salt brine. Pure *P. camemberti* had the largest inhibitory effect on the *C. herbarum* contaminant. Adding *G. candidum* in mixed cultures weakened the inhibitory effect of *P. camemberti* on *C. herbarum*. Low levels of *G. candidum* (10^3 spore/ml) promoted visible growth effects of *C. herbarum*, and this was most pronounced in the early stages of growth. The interaction mechanism of *C. herbarum* was not affected by the choice of the strain of *P. camemberti* whereas the *Penicillium* contaminants were very sensitive to the choice of the *P. camemberti* strain. The presence of *G. candidum* in the mixed cultures seems to decrease the suppressing effect of pour-plated *P. camemberti.* No correlation of any kind was found in the pour-plated spore concentration totals by the inhibition of the *C. herbarum* and *P. roqueforti* contaminants whereas *P. caseifulvum* and *P. commune* were sensitive to this.

Keywords: White mould cheese; Interactions; Secondary starter; Associated funga; NaCl; Multivariate data analysis; Analysis of effect of design factors by soft bi-linear modelling (PLSR)

1. Introduction

To investigate the competitive power of secondary starters to the associated funga in the production of white mould cheese, secondary starters including four *Penicillium camemberti* and one *Geotrichum candidum* strain, were used as both pure and mixed cultures in different concentration and ratios.

Penicillium camemberti, the dominant species of surface ripened white mould cheese, is suggested to be a domesticated form of *Penicillium commune* (Pitt et al., 1986). *P. commune* frequently appears as spoiler on cheese (Lund, 1996). *P. camemberti* and *P. commune* are closely related to *Penicillium caseifulvum*, which has been isolated from blue cheese, where it acts as a spoiler responsible for yellowish spots

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doi:10.1016/j.ijfoodmicro.2005.04.002

appearing on the surface of the cheese as well as during the production of Lund et al. (1998) and Suhr et al. (2002).

The secondary starter of white mould cheese often includes *Geotrichum candidum*. This fungus-like yeast specifically contributes to the cheese with aroma but it can also act as a contaminant. Nielsen et al. (1998a) found that *G. candidum* played a major role in interactions between cheese-associated contaminants. The viability of the secondary starters *P. camemberti* and *G. candidum* is essential for the competitive power against contaminants whilst at the same time the viability is strain specific. Molimard et al. (1997) investigated the interaction between *P. camemberti* and *G. candidum* in dual cultures with ratios of 100:1 and 20:1 in different strains. No effect of *G. candidum* on the growth (biomass) of *P. camemberti* was found but, in contrast, they found effects of *P. camemberti* strains on *G. candidum*. They measured differences in colony forming units between strains of the same species, which were valid for both species. Hardy (1985) found that the NaCl concentration at the rind of camemberti cheese just after brining was 8%, which decreased in the first 3 days of growth, afterwards it remained stable at 4%. In another study van den Tempel and Nielsen (2000) found that 4% NaCl inhibited the germination and sporulation of *Penicillium roqueforti*.

Previous work found that 1% NaCl promoted germination and sporulation of *P. roqueforti* whereas 3% NaCl showed an inhibiting effect (Godinho and Fox, 1981; Lopez-Diaz et al., 1996). Furthermore, Nielsen (1999) suggested that future study, with more focus on environmental effect, on the interactions of *G. candidum* together with other cheese related fungi, yeasts and bacteria should be undertaken. They also pointed out the importance of studying the fungi in their natural environment.

Strain characteristics such as salt tolerance, proteolytic and lipolytic activity, formation of aromatic compounds and temperature tolerance are normally described in the sales catalogue from the producer of starter cultures. This information seems to be sporadic, and no information about the interaction characteristics is given. The cheese maker must therefore depend very much on personally experience in the innovation of new cheeses and optimisation of production. The present works investigates the importance of strain specific inhibitory characteristics in relation to the cheese-associated fungi and suggest multivariate data analysis as a tool for interaction studies.

The terminology of interactions has been outlined by Boddy and Wimpenny (1992) and will be used throughout this study. The most widespread interaction mechanism known for mould cheese between commercial starters and contaminants are mutual inhibition by contact (competition) except for *G. candidum* that showed specific inhibition of fungal contaminants on cheese (Nielsen et al., 1998b). Dieuleveux et al. (1998) found that *G. candidum* secreted 2-hydroxy-3-phenylpropionic acids and that this compound is known to have a broad antimicrobial effect. Nielsen et al. (1998a) found an unknown compound in an interaction study of *P. roqueforti*, *P. camemberti* and *G. candidum* but could not relate this metabolite to a suppressing effect of the associated funga. Whereas, van den Tempel and Nielsen (2000) found that *G. candidum* suppressed the growth and conidiogenesis of *P. roqueforti* cultured on blue mould cheese medium without salt.

In this study experimental conditions close to real white mould cheese production conditions were chosen, since previous work by Nielsen et al. (1998a) and by Larsen and Knochel (1997) showed that interaction studies using standard medium or semi synthetic cheese medium could not be correlated to results found using camembert cheese and previous work by Choisy et al. (1987) has pointed out a critical level of *G. candidum* for interaction has a correlational effects on the sensory quality. Therefore, different levels and concentrations of pure and mixed secondary starter cultures were included in the study.

2. Objectives

The purpose of this study was to investigate strain specific differences of interaction between different technological and physiological strains and species of secondary fungal starters used in the production of white mould cheese as well as the effect of spore concentration, culture type (pure or mixed pour-plated cultures) and ratio of secondary starter and the NaCl concentration of the medium on the associated funga.

3. Materials and methods

3.1. Secondary starters

Cultures were maintained at the Culture Collection at the Centre for Microbial Biotechnology (CMB), Technical University of Denmark. The strains are commercial starters. Four different technological and physiological strains of *Penicillium camemberti* were selected out of 20 screened (results not shown), see Table 1.

In this study, the screening numbers will refer to the *P. camemberti* strains (2, 11, 14 and 17). One strain of *G. candidum* (IBT 9284=G2) was selected from another screening experiment of this species (results not shown).

3.2. Inoculums

Spore suspension was harvested from CYA (Samson et al., 2000) after 7 days at 25 °C in 1 ml spore suspension (0.5% Tween 80, Merck); 0.5% agar (Bie and Berntsen A/S) in doublet distilled water), draining the spore suspension gently from mycelia using a Drigalski spatula and then pipette the suspension using a Pasteur pipette. The spore count in the suspension was adjusted based on counts in a Thoma counting chamber followed by dilution.

3.3. Contaminants

Cladosporium herbarum (IBT 13732=32=Ch), isolated from salt brine in dairy. The following contaminants were all isolated from a dairy environment or directly from the cheese. *P. roqueforti* (IBT 12845=45=Pr); *P. caseifulvum* (IBT 15151=51=Pca) and *P. commune* (IBT 10253=53=Pco). Contaminants were point inoculated at three points by dropping 10 μl of spore suspensions 1×10^6 cfu/ml on top of the prepared plates 24 h after plating.

Table 2
Experimental setup: overview of cultures pour-plated in cheese media and the number of colony forming units per ml (spore/ml) in final media

Medium type	Secondary starter (Spore/ml)					Total spore/ml cheese medium
	G2	2 m	11 m	14 m	17 m[a]	
B	–	–	–	–	–	–
g3	1×10^3	–	–	–	–	1×10^3
g	1×10^5	–	–	–	–	1×10^5
p	–	1×10^5	–	–	–	1×10^5
p	–	–	1×10^5	–	–	1×10^5
p	–	–	–	1×10^5	–	1×10^5
p	–	–	–	–	1×10^5	1×10^5
pg3	1×10^3	1×10^5	–	–	–	1.01×10^5
pg3	1×10^3	–	1×10^5	–	–	1.01×10^5
pg3	1×10^3	–	–	1×10^5	–	1.01×10^5
pg3	1×10^3	–	–	–	1×10^5	1.01×10^5
pg4	5×10^4	5×10^4	–	–	–	1×10^5
pg4	5×10^4	–	5×10^4	–	–	1×10^5
pg4	5×10^4	–	–	5×10^4	–	1×10^5
pg4	5×10^4	–	–	–	5×10^4	1×10^5
pg	1×10^5	1×10^5	–	–	–	2×10^5
pg	1×10^5	–	1×10^5	–	–	2×10^5
pg	1×10^5	–	–	1×10^5	–	2×10^5
pg	1×10^5	–	–	–	1×10^5	2×10^5

[a] Experiment only at 1.5% NaCl.

3.4. Medium

A cheese medium with 1.5% and 3% (w/v) NaCl was prepared by combining three separately produced fractions. This was done to promote homogeneity and avoid a Maillard reaction between the protein and sugar component in the media:

(1) 15 g agar and 200 ml Milli-Q H_2O.
(2) 13.5 g NaCl (1.5W/V) or 27 g NaCl (3.0 W/V) and 200 ml Milli-Q H_2O, each adjusted to pH

Table 1
The strains characteristics based on the four selected *P. camemberti* strains

Analysis/strain	2 (IBT922372)	11 (IBT923813)	14 (IBT9222382)	17 (IBT922384)
Proteolytic activity	Low	High	High	Low
Lipolytic activity (1–2 growth days)[a]	Medium	High	Medium	Medium
Growth rate (Colony diameter)	High	Medium	Small	Medium–small
Conidiogenesis	Weak	Non	Medium/ high	High

Relative units based on variations between the screenings of 20 strains (results not shown).
[a] The measured differences in lipolytic activity between strains were decreasing after growth day 2.

Table 3
Contaminants added as 3-point inoculations onto pour-plated secondary starter media (B, g3, g, p, pg3, pg4 and pg, Table 2)

Contaminant	*Cladosporium herbarum*	*Penicillium roqueforti*	*Penicillium caseifulvum*	*Penicillium commune*
Code	Ch	Pr	Pca	Pco
B	x	x	x	x
g3	x	x	x	x
g	x	x	x	x
p	x	x	x	x
pg3	x	x	x	x
pg4	x	x	x	x
pg	x	x	x	x

6 using 1 M HCl and then autoclaved (121 °C, 20 min).

(3) 300 g unripe and unsalted cheese, 28% fat, 55% dry matter (sampled 24 h after coagulation just before salting and stored at −20 °C until use) was mixed with 200 ml boiling Milli-Q H_2O by blending for 3 min. Hereafter pH was adjusted to 6.0 using 1 M HCl and the media was boiled in a water bath for 30 min.

After cooling to 50 °C the three fractions were combined and poured into 9 cm Petri-dishes with 20 ml in each. The secondary starters (see Table 2) were added to the Petri dishes and mixed into the media.

The experiments were carried out at high relative humidity (RH%>90) and 14 °C in ventilated incubators (Forma). The contaminants were 3-point inoculated on cheese medium 24 h after pour plating of secondary starters. The four contaminants were point inoculated on seven different types of media containing different levels of pure and mixed pour-plated secondary starters (see Tables 2 and 3). The experiment was repeated using four different strains of *P. camemberti* at low NaCl concentration (1.5%) and one *G. candidum* strain (G2). Three of the *P. camemberti* strains were also used in repeating experiments at high NaCl concentration (3%).

4. Measurement

Radial growth of fungal colonies was determined by colony diameter with the use of digital image analysis using Videometer 2000 Accuracy of measurements 1 pixel corresponded to ±0.03 mm (Videometer, DK), Measurements were made after 3, 4, 5 and 7 days of growth. Minimum growth of all blinds was set to 6 mm, the size of the 10 μl spore suspension droplets, as the inoculation point from the 10 μl spore suspension drop on average forms a circle 6 mm in diameter.

4.1. Data analysis

Soft Bi-Linear Modelling (BLM) was used as data analysis and for validation, using the software package Unscramble Version 7.6 SR-1. *A*nalysis of effects in designed experiments by BLM (Partial Least Squares Regression=APLSR) described by Martens and Martens (2001) was performed on all isolates, using full cross-validation and Jack-Knifing. The effect of design variable (APLSR) represents the multivariate data analysis alternative to traditional statistical analysis of variance (ANOVA). The Jack-Knifing (s-JK) technique determines the reliability range defined as 2 standard uncertainties were the individual uncertainties are estimated by full cross validation calculating root mean square error of prediction (RMSEP) (Martens and Martens, 2000; Martens et al., 2001).

Partial Least Square Regression (PLS-2) correlation *X*- and *Y*-loadings of point-inoculated contaminants are shown in Fig. 3a–d. PLS-2 results are present as percent suppression/inhibition of references cultured on pure cheese medium and by that the inhibition was negatively correlated to the references (in the direction of the first principal component (PC 1). Fig. 3a–d presents the results as correlation loading bi-plots showing the relationship between *X* and *Y* in two dimensions (PC 1=the first principal component and PC 2=the second principal component). The two elliptical circles demarcate 50% and 100% of the total explained variance of the APLSR model. If a factor (*X* or *Y*) is placed in the area between circles then these strongly contribute to the APLSR model. On the other hand, a factor placed near the centre (0,0) is not contributing to the model. The percent (explained by *X*), depends on the number of factors included in the experimental design, e.g. seven factors, as in this case, will split up the model into approximately seven principal components. The corresponding percent (explained by *Y*) shows how fitted the measurement are to explain the variation in *X*.

5. Results

Examples of measured contaminant diameters are shown in Fig. 1. The types of pour-plated secondary starter are listed in order of increasing total spore concentration, (see Table 2). It can be seen that the growth of *C. herbarum* (Ch) was promoted by low concentrations of *G. candidum* (g3) at growth days 4, 5 and 7 and that pure *P. camemberti* (p, strain 11) had the largest inhibitory effect. *P. roqueforti* (Pr) was 100% inhibited by pure *P. camemberti* and the same pattern of inhibitory effects were seen for *P. caseifulvum* (Pca). The mixed secondary starters (ratio 1:1) had the largest inhibitory effect on *P. commune* (Pco), being 100% inhibited on all measured growth days for medium type pg4.

The inhibitory effects of secondary starters, in percent, were calculated based on the references grown the on cheese medium, without a secondary starter. These results were analysed using multivariate data analysis because this method makes it possible to compare the inhibitory effect of all the secondary starters at different ratios and levels. To simplify this analysis two different multivariate models were calculated. The first model type was based on a contaminant sample set (e.g. all samples inoculated with *C. herbarum* = 53 minus one out-lier→52 samples) choosing media type (B, g3, g, p, pg3, pg4 and pg, Table 2) and *P. camemberti* strain (2, 11, 14 and 17) as *X* variable and inhibitory effect, in percent, of references as y variable (growth days 3, 4, 5 and 7). These models do not include NaCl levels since a pre-study of the results showed only minor effects of this factor (see Fig. 2a–d) all the samples both at high and low NaCl level, were pooled into one sample set for each contaminant.

Growths of *C. herbarum* were best inhibited by pure *P. camemberti* cultures (p) or by mixed cultures

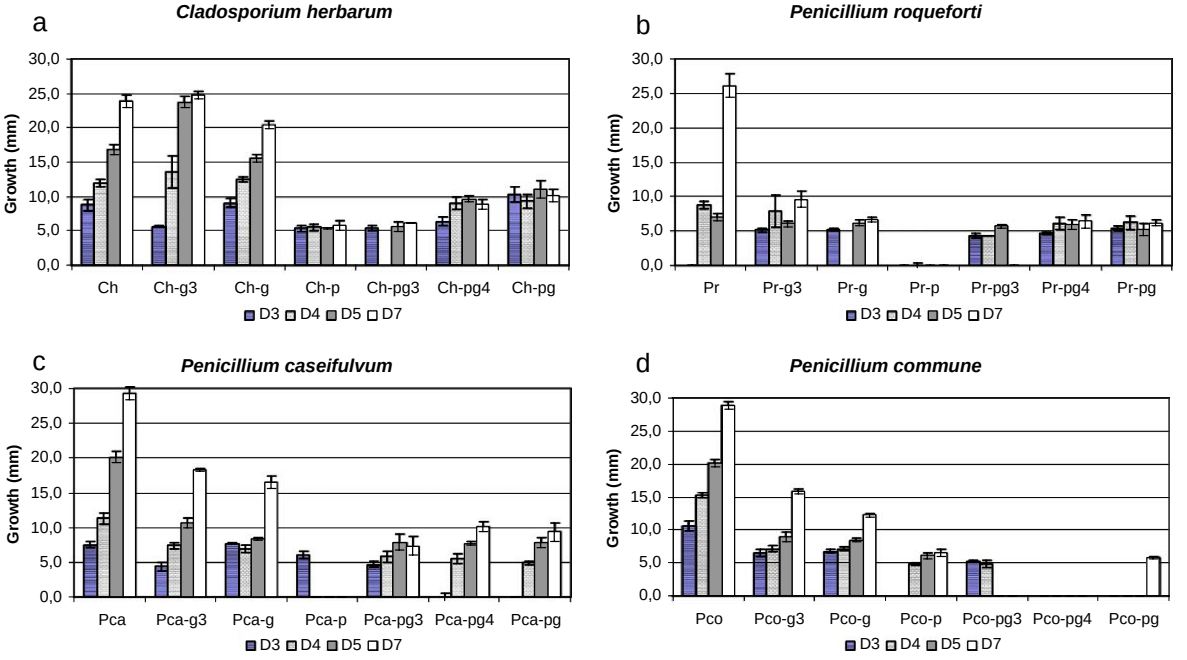

Fig. 1. (a–d) Colony diameter (mm) of the four contaminants three-points inoculated on different media types with secondary starter *P. camemberti* strain 11 and *G. candidum* at low salt level (1.5%). Standard deviation (mm) of six measurements on each sample (two measurements of each colony) is shown. Codes: Growth days 3, 4, 5 and 7 = D3, D4, D5 and D7. Ch = *Cladosporium herbarum* point inoculated on pure cheese medium, *Penicillium roqueforti* = Pr, *Penicillium caseifulvum* = Pca and *Penicillium commune* = Pco. -g3 = pour-plated *G. candidum* (10^3spore/ml), -g = pour-plated *G. candidum* (10^5spore/ml), -p = pour-plated *P. camemberti* (10^5spore/ml), -pg3 = pour-plated *P. camemberti* and *G. candidum* (10^5: 10^3spore/ml), -pg4 = pour-plated *P. camemberti* and *G. candidum* (5×10^4: 5×10^4spore/ml) and -pg = pour-plated *P. camemberti* and *G. candidum* (10^5: 10^5spore/ml).

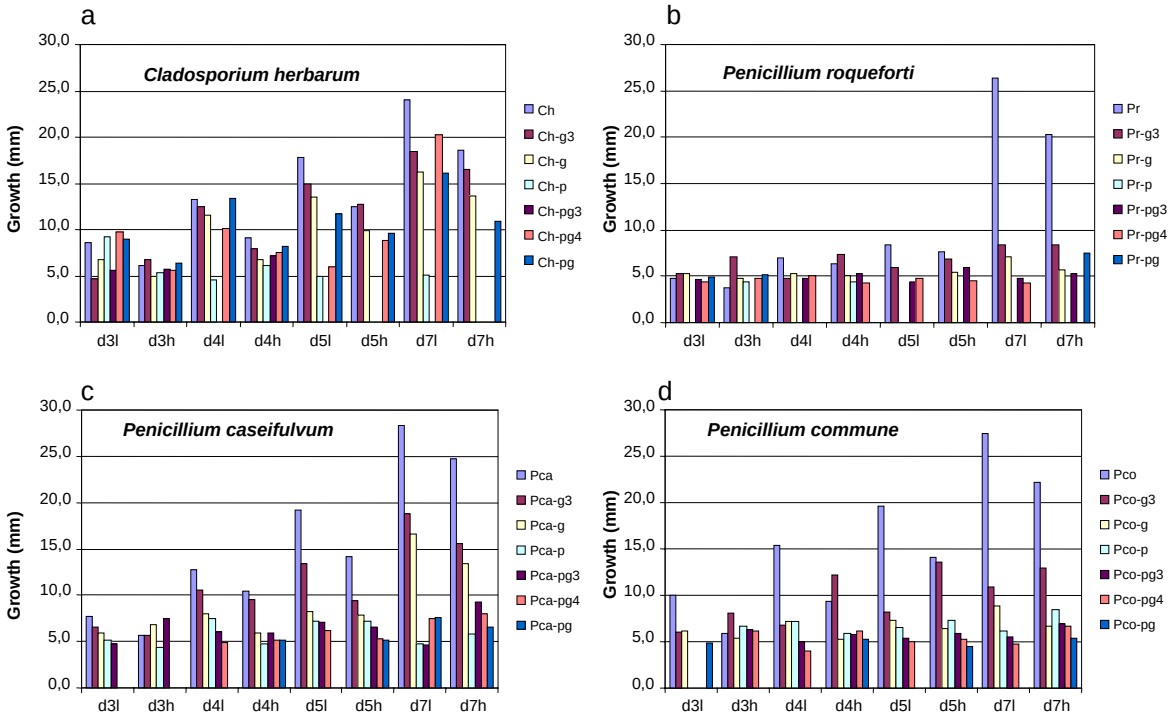

Fig. 2. (a–d) Colony diameter (mm) of the four contaminants three-points inoculated on different media types with secondary starter *P. camemberti* strain 11 and *G. candidum* at low and high salt level (1.5% and 3%). Standard deviation (mm) of six measurements on each sample (two measurements of each colony) is shown. Codes: Growth days 3, 4, 5 and 7 at 1.5% salt=d3l, d4l, d5l and d7l and growth days 3, 4, 5 and 7 at 3% salt=d3h, d4h, d5h and d7h . Ch=*Cladosporium herbarum* point inoculated on pure cheese medium, *Penicillium roqueforti*=Pr, *Penicillium caseifulvum*=Pca and *Penicillium commune*=Pco. -g3=pour-plated *G. candidum* (10^3spore/ml), -g=pour-plated *G. candidum* (10^5spore/ml), -p=pour-plated *P. camemberti* (10^5spore/ml), -pg3=pour-plated *P. camemberti* and *G. candidum* (10^5: 10^3spore/ml), -pg4=pour-plated *P. camemberti* and *G. candidum* (5×10^4: 5×10^4spore/ml) and -pg=pour-plated *P. camemberti* and *G. candidum* (10^5: 10^5spore/ml).

of *G. candidum* and *P. camemberti* at a ratio 1:100 (pg3) (see Fig. 3a). The medium composition of secondary starter spans out PC 1 and PC 2, 64% of the 12% total variation in PC 1 was explained by *Y* (percent inhibition) meaning that the composition of the secondary starter had a large influence on the inhibition of *C. herbarum*. The inhibiting effect became more pronounced as time went by as can be seen by growth days 4, 5 and 7 (d4l, d5l and d7l), which contribute more to the model than growth day 3 (they are further away from the centre and placed in the elliptical circles of 50–100% explained variance). *G. candidum*, as a pure culture, was significantly worse (significant by Jack-Knifing) when compared to the other secondary starters. The plate with the secondary starter culture is nearly placed at the same level as PC 1 of the control (B), indicating that *C. herbarum* (Ch) grew just as well on those. In Fig. 4a–d, the regression coefficients of each *X* variable for each growth day, marked with the respective uncertainty limits, for the APLSR model of *C. herbarum* are shown. The factors marked by stripes were significant. The spore concentration properly interferes with the explanation of *Y* (culture type Table 3) in the direction of PC 2 as g3 (10^3/ml), pg4 (1×10^5/ml) and pg (2×10^5/ml) span out PC 2 (6% explained *Y*). Antagonistic effects were seen for mixed cultures at a ratio of 1:1 for two different levels (pg=2×10^5/ml and pg4=1×10^5/ml) but these results were not significant.

Pure cultures of *P. camemberti*, showed the largest antagonistic effect on *P. roqueforti,* (see Fig. 3b). Ratios of 1:100 (pg3) of mixed pour-plated mixed cultures also had antagonistic effect but were not

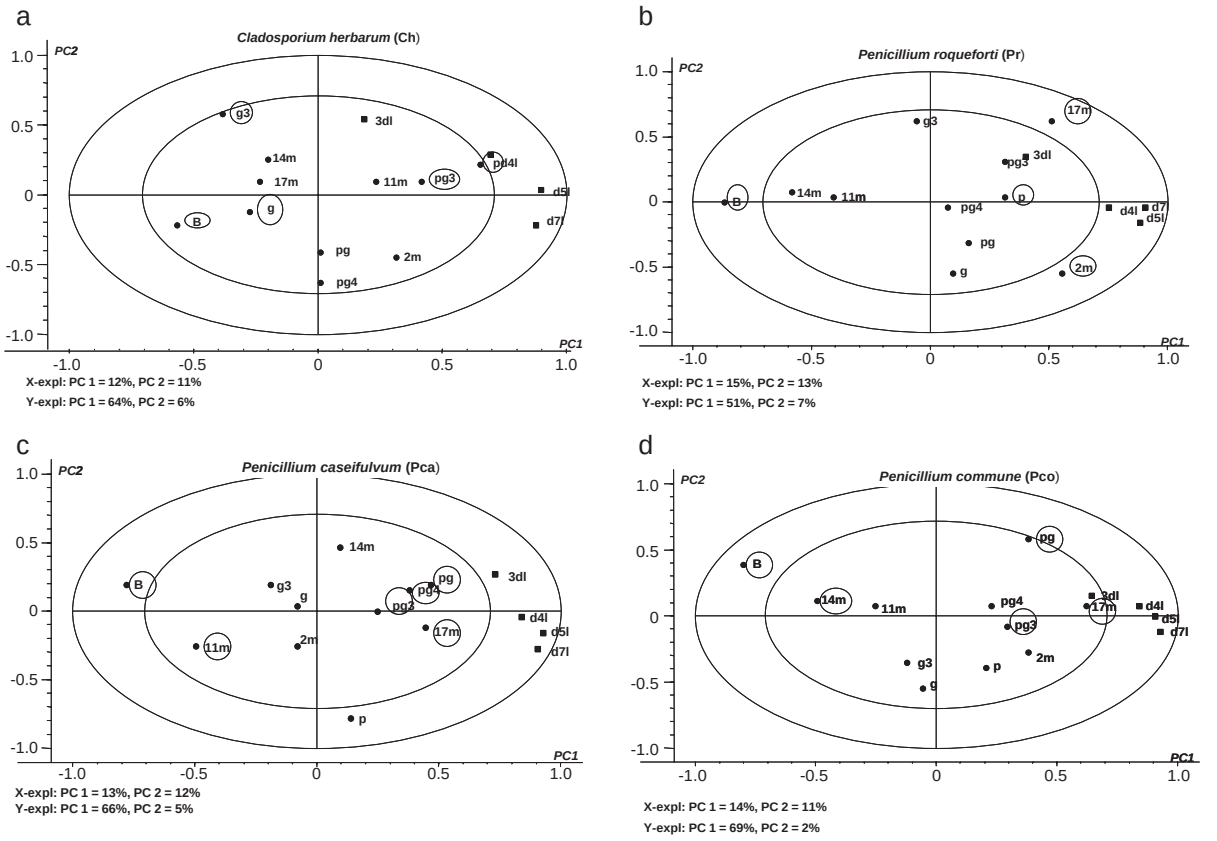

Fig. 3. (a–d) Correlation loadings bi-plots (*X* and *Y*). Inhibitory effect of secondary starters on point inoculated *C. herbarum, P. roqueforti, P. caseifulvum* and *P. commune* by multivariate design effect analysis (APLSR). Balanced experiments using four *P. camemberti* and one *G. candidum* strain pour-plated in pure and mixed cultures. Sample set=52 samples. *X*=●: Contaminant on pure cheese medium=B, *P. camemberti* strains=2m, 11m, 14m and 17m, medium type=g3=pour-plated *G. candidum* (10^3spore/ml), g=pour-plated *G. candidum* (10^5spore/ml), p=pour-plated *P. camemberti* (10^5spore/ml), pg3=pour-plated *P. camemberti* and *G. candidum* (10^5: 10^3spore/ml), pg4=pour-plated *P. camemberti* and *G. candidum* (5×10^4: 5×10^4spore/ml) and pg=pour-plated *P. camemberti* and *G. candidum* (10^5: 10^5spore/ml). *Y*=■=inhibitory effect on growth days 3, 4, 5 and 7=3dl, d4l, d5l and d7l. Circle: ○=marks the significant × variables. The two elliptical circles demarcate 50% and 100% of the total explained variance of the APLSR model.

significant. Low concentrations of pure cultures of *G. candidum* (10^3/ml) also inhibited the growth of *P. roqueforti* as compared to the control. The relatively strong inhibitory effects of *G. candidum* (g3 and g) were not seen towards the other contaminants (Fig. 3a, c and d). The choice of the *P. camemberti* strain had the largest influence on the inhibition of *P. roqueforti* whilst *P. camemberti* 2 and 17 had a significantly inhibiting effect, whereas the two *P. camemberti* strains, 11 and 14, had a low antagonistic effect.

Results for *P. caseifulvum* in Fig. 3c showed that all media containing mixed cultures had a significantly high antagonistic effect. For *P. caseifulvum*, both concentrations of *G. candidum* had a low inhibitory effect. *P. camemberti* 11 had a very low suppressing effect on the growth of *P. caseifulvum* whereas 17 had a significantly high effect on both.

In Fig. 3d it can be seen that the *P. camemberti* strains span out PC 1 (69% explained *Y* of 14% total variance) meaning that the suppressing effect on *P. commune* depended very much on the specific strain of secondary starter in action. This agrees with the results found for *P. caseifulvum* (see Fig. 3c). *P. camemberti* strain 14 had almost no effect and strain 17 had the most significant inhibitory effect. The

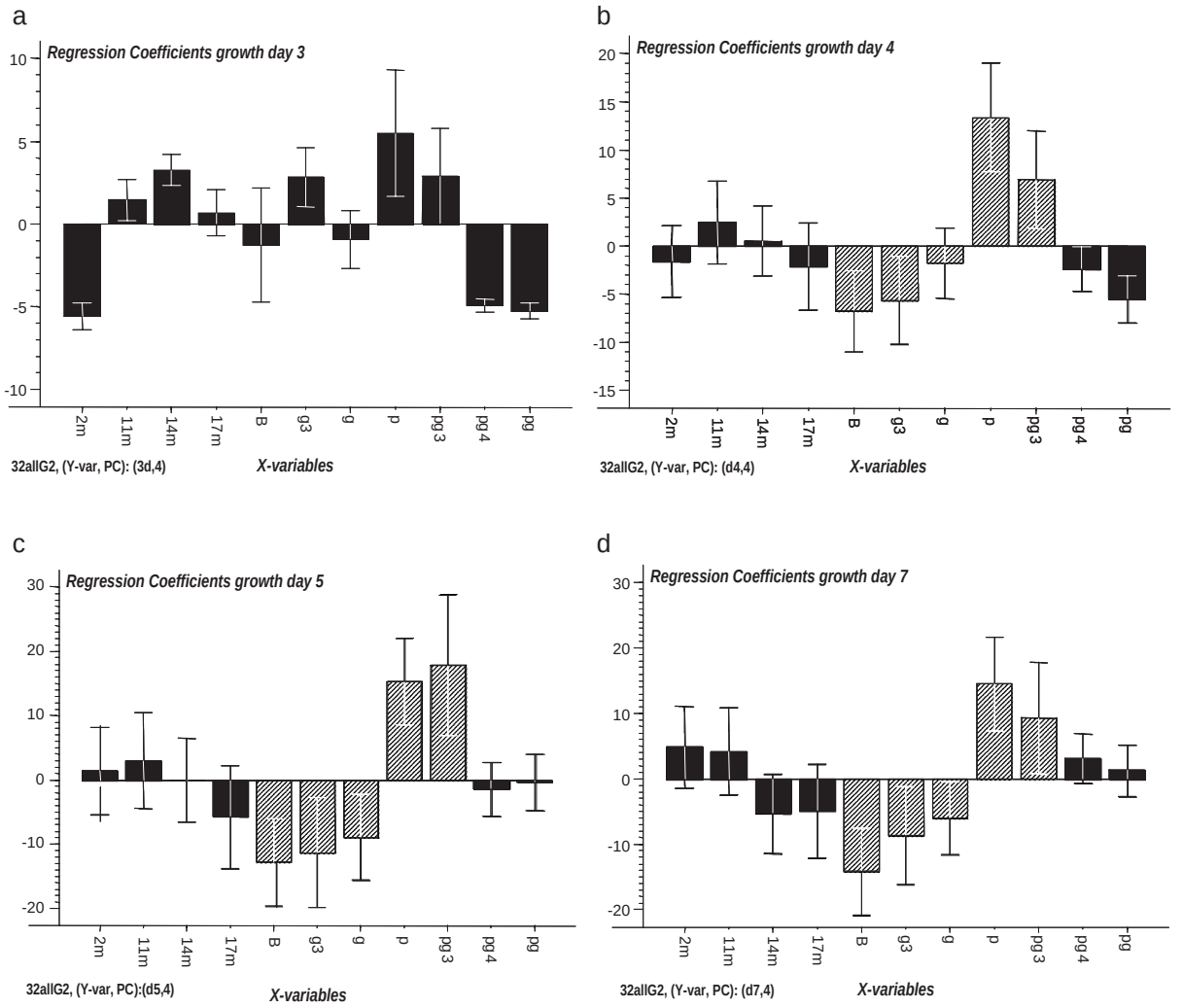

Fig. 4. (a–d) Regression coefficients of *X* variables in the APLSR model of *C. herbarum* growth days 3, 4, 5 and 7. Valuables significant by Jack-Knifing are marked by cross lines and standard uncertainties are shown.

mixed cultures, were the most inhibitory culture types where pg3 and pg, were significant.

6. Discussion

P. camemberti had the largest inhibitory effect towards *C. herbarum*. Low levels of *G. candidum* (10^3 spore/ml) showed commensalisms (one population benefits, but the other is unaffected) to the growth of *C. herbarum*, which is most pronounced in the early stages of growth. As this effect disappeared at high spore concentration, it can be surmised that competition or antagonistic effects overrule the commercialistic effect restrained by high spore concentration. The addition of *G. candidum* to the media weakened the inhibitory effect of *P. camemberti* and this can be due to competition between *P. camemberti* and *G. candidum*.

An explanation of the antagonistic effect seen for the mixed cultures at ratios of 1:1 could be that the secondary starters use more energy on mutual competition, which is more beneficial for *C. herbarum*.

Pure cultures of *P. camemberti* (p) and mixed cultures in the ratio of 100:1 (pg3) showed the largest

antagonistic effect on *P. roqueforti* (Pr) (see Fig. 3b). Cultures mixed 1:1 also had some antagonistic effect but were not significant. In contrast to *C. herbarum*, *P. roqueforti* was very sensitive to the choice of *P. camemberti* strains but no correlation to spore concentration was found, pointing to other interaction mechanisms other than competition. Therefore, it can be stated that the predominan interaction mechanism suppressing the growth of *P. roqueforti* is the antagonistic effect of both *P. camemberti* and *G. candidum*. The results shown for *P. roqueforti* agree with the results found by van den Tempel and Nielsen (2000). They found that *G. candidum* inhibited the growth and conidiogenesis of *P. roqueforti* cultured on blue mould cheese medium without salt. The inhibitory effect of secondary starters on contaminant *P. caseifulvum* showed concentration dependent inhibition (concentration of secondary starters) and this can be explained as competition. The level of inhibitory effect was also strains specific. High inhibitory effect was seen for *P. camemberti* 17 and low inhibitory effect was seen for *P. camemberti* 11. The results found for *P. commune* were consistent to the results found for *P. caseifulvum*, except that the inhibitory effect of the *P. camemberti* strain 14 was significantly lower for *P. commune*. The presence of *G. candidum* seems to decrease the suppressing effect of *P. camemberti* in mixed pour-plated cultures. No correlation was found between the total number of spores in the media and the inhibitory of *C. herbarum* (Ch) and *P. roqueforti* (Pr), whereas *P. caseifulvum* (Pca) and *P. commune* (Pco) were strongly inhibited at high spore density. As the spore concentration rose the effect of *P. camemberti* strains decreased.

7. Conclusion

The suppressing effect of *P. camemberti* on penicillia contaminants (*P. roqueforti*, *P. caseifulvum* and *P. commune*) were strain specific whereas the suppression of *C. herbarum* was more sensitive to the type of cultures mixtures in the medium. The results unambiguously point to the fact that the *P. camemberti* strain 17 is the most effective suppressor of the investigated strains. The optimal culture type for suppressing *C. herbarum* was found to be pure *P. camemberti* strain 2 or 11. Commensalisms beneficial for *C. herbarum* at low spore concentration of *G. candidum*, were observed. This effect was not observed for high spore concentration of *G. candidum.*

The results pointed out strain specific interaction characteristics, both in pure and mixed pour-plated cultures of secondary starters, which can influence the shelf life of white moulded cheese. Analyse of the effect of design factors by soft bi-linear modelling (APLSR) was found to be an excellent tool in the study of interaction between more than two species.

Acknowledgements

The authors wish to acknowledge the technical assistance of Mrs. M. Simkus, the material supported by Arla Foods and the financial support provided by the Danish Dairy Scientific Foundation.

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