Inhibition of *Listeria monocytogenes* by Food-Borne Yeasts†

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Many bacteria are known to inhibit food pathogens, such as *Listeria monocytogenes*, by secreting a variety of bactericidal and bacteriostatic substances. In sharp contrast, it is unknown whether yeast has an inhibitory potential for the growth of pathogenic bacteria in food. A total of 404 yeasts were screened for inhibitory activity against five *Listeria monocytogenes* strains. Three hundred and four of these yeasts were isolated from smear-ripened cheeses. Most of the yeasts were identified by Fourier transform infrared spectroscopy. Using an agar-membrane screening assay, a fraction of approximately 4% of the 304 red smear cheese isolates clearly inhibited growth of *L. monocytogenes*. Furthermore, 14 out of these 304 cheese yeasts were cocultivated with *L. monocytogenes* WSLC 1364 on solid medium to test the antilisterial activity of yeast in direct cell contact with *Listeria*. All yeasts inhibited *L. monocytogenes* to a low degree, which is most probably due to competition for nutrients. However, one *Candida intermedia* strain was able to reduce the listerial cell count by 4 log units. Another four yeasts, assigned to *C. intermedia* (three strains) and *Kluyveromyces marxianus* (one strain), repressed growth of *L. monocytogenes* by 3 log units. Inhibition of *L. monocytogenes* was clearly pronounced in the cocultivation assay, which simulates the conditions and contamination rates present on smear cheese surfaces. We found no evidence that the unknown inhibitory molecule is able to diffuse through soft agar.

Many foods, such as meat, egg products, vegetables, seafood, and dairy products, have a risk of contamination by *Listeria monocytogenes* (13). Within dairy products, a high incidence of *Listeria monocytogenes* was observed in red smear cheeses (27), a cheese type extensively produced mainly in Austria, Belgium, France, and Germany (6) that is characterized by a red-brown to red-orange colored, smear-like microbial consortium on its surface (4, 25). Biopreservation by protective cultures is considered to be a promising concept to improve food safety without changing the sensory quality of a product. The antimicrobial effect of protective cultures is caused by pH reduction due to the production of organic acids, such as lactic acid, by hydrogen peroxide; enzymes, such as lysozyme; low-molecular-weight metabolites, such as fatty acids; or bacteriocins, such as Nisin, or by competition for nutrients (for a review, see reference 15).

In order to develop potential countermeasures against *Listeria* in red smear cheese, antilisterial actions exerted by red smear cheese-ripening bacteria have been studied in some detail (8, 9, 12, 19, 28, 32, 33). In some cases, the antilisterial effect was assigned to bacteriocins (20, 33) or bacteriocin-like substances (5, 28). So far, linocin M18 is the only bacteriocin produced by a *Brevibacterium linens* strain, a typical red smear cheese-ripening bacterium that has been characterized at the molecular level (33). Micrococccin P₁, an antilisterial macrocyclic peptide antibiotic, was found to be secreted by a *Staphylococcus equorum* strain isolated from Raclette, a French red smear cheese (9). In other cases, the characters of the antilisterial

substances produced by different red smear cheese-ripening bacteria, or even details about the molecular basis of the inhibitory action, remain unresolved (8, 32). Unknown factors inhibiting *Listeria*, such as competitive or symbiotic interactions within the complex cheese smear ecosystem, may well exist (12).

Growth and therefore bacteriocin production of cheese-ripening bacteria depend on a pH increase on the cheese surface from around 5.0 to around 6.0, which is the result of aerobic lactic acid assimilation by yeasts, which form an important part of the surface microbial consortia of red smear cheeses (11, 25). Thus, antilisterial substances produced by ripening bacteria become effective only after the bacterial members of the consortia develop. This is a potential problem, since *Listeria monocytogenes* is more pH tolerant than the ripening bacteria and may grow even at pH values of around 5.0 (13). During the early stages of ripening, yeasts dominate the microbial flora of red smear cheeses due to their pH tolerance (11). Therefore, it would be reasonable to search for yeast expressing an antilisterial activity to combat *Listeria* in its initial stages of multiplication on cheese.

Generally, antagonistic effects of yeasts against other yeasts are well known. The production of killer toxins was already recognized in the 1960s (18). Killer toxins were described as exotoxins lethal against susceptible strains of the same or congeneric species, whereas the toxin-producing yeast itself is immune to its own killer toxin (18, 23). Polonelli and Morace (23) also reported on a killer phenomenon directed against unrelated microorganisms, among others, bacteria. However, in their study, a screening medium supplemented with methylene blue was used, and a correlation between the antibacterial activity in yeast and the use of methylene blue in the medium was demonstrated by Bilinski et al. (2). There exist some older clinical studies which describe antibacterial activities of yeasts (7, 14, 24, 35). However, little effort has been devoted to

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TABLE 1. *Listeria monocytogenes* indicator strains

Strain (WSLC) ^a	ATCC ^b no.	Serovar	Source
1001	ATCC 19112	1/2c	Spinal fluid
1039	ATCC 13932	4b	Spinal fluid
1211		3a	Cheese
1364		4b	Cheese
1416		1/2a	Cheese

^a WSLC, Weihenstephan *Listeria* Collection, Abteilung Mikrobiologie, ZIEL Weihenstephan, Freising, Germany.

^b ATCC, American Type Culture Collection, Manassas, Va.

investigating yeasts isolated from food or food environments for inhibitory potential against pathogenic bacteria, such as *L. monocytogenes*. To our knowledge there are only two studies reporting on this topic. One deals with biofilms from floor drains at different food-processing plants (37). Three out of 156 unidentified yeast isolates showed very low antilisterial activity. In liquid medium, listerial growth was repressed by 0.7 log₁₀ CFU/ml, and in a biofilm on stainless steel by 0.2 and 0.5 log₁₀ CFU/cm², but no further details were reported. Dieuleveux et al. (10) described an antilisterial *Geotrichum candidum* strain that was isolated from a French red smear cheese. Antilisterial properties were tested in solid and liquid media using ultrafiltrates of a *G. candidum* culture instead of living cells, as in the present study.

The aim of the present study was to search for yeasts isolated from food products, especially from red smear cheeses, expressing antilisterial activity. We used two different screening methods, an agar-membrane-based assay (no direct cell contact between *Listeria* and yeast) and a cocultivation assay (with direct cell-cell contact possible). We found several strains that display very significant antilisterial potentials. These are promising candidates for further characterization of the inhibitory mechanism and potential use as protective cultures.

MATERIALS AND METHODS

***Listeria monocytogenes* indicator strains.** *Listeria monocytogenes* strains WSLC 1001, 1039, 1211, 1364, and 1416 were selected from the Weihenstephan strain collection to serve as indicator strains in the agar-membrane screening assay (Table 1). For the cocultivation experiment, *L. monocytogenes* WSLC 1364 was used.

Cultivation of *L. monocytogenes* indicator strains. Five milliliters of brain heart infusion (Merck) was inoculated with *L. monocytogenes* cells from a fresh brain heart infusion agar plate and incubated at 30°C for 17 to 18 h using a CERTOMAT S (B. Braun Biotech International, Sartorius group) at 180 rpm.

Yeast strains. A total of 404 yeasts were screened for antilisterial potential using an agar-membrane-based screening assay (Table 2). One hundred of these, isolated from different sources but mainly from dairy products, were selected from the Weihenstephan yeast collection, and 304 yeasts were collected from various European smear-ripened cheeses (for details, see Table S1 in the supplemental material). The yeast isolates were identified using Fourier transform infrared spectroscopy (16), and where necessary, by physiological tests according to the methods of Barnett et al. (1), modified by Seiler and Busse (31), as well as by sequence analysis of the 26S rRNA gene D1/D2 domain (17), using a PCR protocol described by Oberreuter et al. (21). Fourteen yeasts out of the set of 304 were cocultivated with *Listeria monocytogenes* WSLC 1364 and, additionally, were tested for inhibitory activity against the same *L. monocytogenes* strain using an assay which was developed by Provost et al. (24) to type species of the *Nocardia asteroides* complex by a yeast killer system.

Cultivation of yeast strains. For preculture, one loop (2 mm in diameter) of cells of a fresh culture grown on yeast extract glucose chloramphenicol agar (Merck) supplemented with 10 mg/liter bromophenol blue (YGCBA) (29) was inoculated in 5 ml of yeast extract glucose broth (5.0 g yeast extract [Oxoid],

TABLE 2. Yeast species investigated for antilisterial activity by the agar-membrane screening assay

Species	No. of isolates
<i>Candida anglica</i>	3
<i>Candida catenulata</i>	3
<i>Candida ethanolica</i>	1
<i>Candida glabrata</i>	1
<i>Candida intermedia</i>	21
<i>Candida magnoliae</i>	1
<i>Candida maltosa</i>	3
<i>Candida parapsilosis</i>	4
<i>Candida tropicalis</i>	1
<i>Candida zeylanoides</i>	6
<i>Clavispora lusitanae</i>	43
<i>Debaryomyces hansenii</i>	206
<i>Dekkera anomala</i>	1
<i>Galactomyces geotrichum</i>	23
<i>Hanseniaspora uvarum</i>	1
<i>Issatchenkia occidentalis</i>	2
<i>Issatchenkia orientalis</i>	3
<i>Kluyveromyces lactis</i>	8
<i>Kluyveromyces marxianus</i>	8
<i>Pichia anomala</i>	5
<i>Pichia cactophila</i>	1
<i>Pichia guilliermondii</i>	1
<i>Pichia jadinii</i>	5
<i>Pichia membranifaciens</i>	5
<i>Pichia triangularis</i>	5
<i>Saccharomyces castellii</i>	1
<i>Saccharomyces cerevisiae</i>	1
<i>Saccharomyces dairenensis</i>	1
<i>Saccharomyces unisporus</i>	2
<i>Torulaspora delbrueckii</i>	5
<i>Trichosporon beigeli</i>	4
<i>Trichosporon ovoides</i>	6
<i>Williopsis californica</i>	1
<i>Yarrowia lipolytica</i>	12
Unidentified cheese yeasts	10
Total	404

20.0 g glucose [Fluka], 1.0 liter distilled water, adjusted to pH 6.6 with HCl) and incubated at 27°C for 24 h using a CERTOMAT S (B. Braun Biotech International, Sartorius group) at 180 rpm. To prepare the main culture, 50 µl of the incubated preculture was transferred to 5 ml yeast extract glucose broth prewarmed to 27°C. The incubation conditions used were as described for the preculture. The main culture, in which cell numbers of 10⁷ to 10⁸ CFU/ml were commonly reached, was either spotted onto the membrane in the agar-membrane screening assay or mixed with a *L. monocytogenes* culture for the cocultivation assay.

Agar-membrane screening assay. An overnight liquid culture (100 µl) of a *Listeria* indicator strain was mixed with 10 ml of melted yeast extract glucose soft agar containing 1% tryptone (YGSA) [5.0 g yeast extract (Oxoid), 20.0 g b(+) glucose (Fluka), 10.0 g tryptone (Oxoid), 8.0 g agar (Oxoid), 1.0 liter distilled water, adjusted to pH 6.6 with HCl], which was kept warm at 50°C until use. The inoculated soft agar was immediately poured into a petri dish (9 cm in diameter). After 15 min of solidification, a 4- by 5-cm piece of nonautoclaved nitrocellulose membrane (S&S PROTRAN; type BA 83; pore size, 0.2 µm) was placed onto the YGSA. Five microliters of a 24-h main liquid yeast culture was applied to the membrane. The antilisterial activities of four yeasts were studied using one membrane. As a control, the supernatant of *Lactobacillus plantarum* ALC01 containing pediocin was pipetted in the middle position of the membrane. After incubation at 27°C for 24 h, the assay was evaluated for inhibition zones using Henry's illumination, where light is transmitted at a 45° angle to the bottom of the agar plate. The inhibitory potential was classified according to a score-based system (Fig. 1).

Cocultivation assay. In the cocultivation experiments, 10⁵ to 10⁶ yeast cells per cm² were cocultivated with ~6 *Listeria* cells per cm², which is a realistic contamination rate on red smear cheese (27). Cell counts of the liquid *Listeria*

FIG. 1. Score-based evaluation system for antilisterial activities of yeasts in an agar-membrane assay. Score 0, no inhibition; score 1, very weak inhibition; score 2, weak inhibition; score 3, moderate inhibition; score 4, clear inhibition; score 5, very clear inhibition; n.e., not evaluable—white staining in the soft agar under the yeast spot grown on the nitrocellulose membrane; P, supernatant of *Lactobacillus plantarum* containing pediocin applied to the soft agar.

culture were determined by measurements of optical density at 600 nm. Due to the standardized cultivation described above, the desired cell numbers of the *Listeria* indicator strain WSLC 1364 could be adjusted with low variability. An optical density at 600 nm of 0.5 corresponded to 5.8×10^8 CFU/ml. The culture was diluted serially according to the cell density desired on the agar plate used in the cocultivation experiment. A 100- μ l aliquot was mixed with a yeast suspension prepared by centrifuging 300 μ l of the yeast main culture at 5,000 rpm for 5 min, discarding the supernatant, and resuspending the pellet in 100 μ l of 1/4 Ringer solution. The *Listeria*-yeast mixture was then spread onto yeast extract glucose agar containing 1% tryptone [5.0 g yeast extract (Oxoid), 20.0 g D(+)-glucose (Fluka), 10.0 g tryptone (Oxoid), 15.0 g agar (Oxoid), 1.0 liter distilled water, adjusted to pH 6.6 with HCl] and incubated for 24 h at 27°C. Cell counts of *Listeria* and yeast used in the cocultivation experiment were checked separately on yeast extract glucose agar plates containing 1% tryptone incubated at 27°C for 48 h in duplicate. After cocultivation, 3.5-cm² agar pieces were transferred upside down to a 100-ml Erlenmeyer flask containing 2 ml of 1/4 Ringer solution. Cell lawns were removed by shaking the flasks for 30 min at 200 rpm. The resulting *Listeria*-yeast suspensions were transferred to sterile tubes. Residues in the flasks were washed with another 1 ml of 1/4 Ringer solution and added to the respective tubes. The *Listeria*-yeast suspensions were serially diluted, and appropriate dilutions were plated in duplicate on YGCBA or Oxford agar (Oxoid) for determining yeast or *Listeria* counts. Two to four independent tests were performed on each yeast strain. As a control, an equivalent amount of 1/4 Ringer solution was mixed with the *Listeria* cells instead of the yeast culture. Afterward, the same procedures described above were followed. Residual yeast and *Listeria* cell counts on the agar pieces were assayed by homogenization and plating of appropriate dilutions in duplicate on YGCBA or Oxford agar (Oxoid), respectively, but these were low and did not significantly influence the yeast and *Listeria* cell counts obtained by washing off the cell lawns from the agar pieces.

Assay for assessing "killer toxin activity." The "killer toxin activity" assay was performed according to the method of Provost et al. (24) with some modifications. YG⁺SA was inoculated with an aliquot of a liquid culture of *L. monocytogenes* WSLC 1364 as described above for the agar-membrane screening assay. After solidification, one loopful (2 mm in diameter) of yeast cells, taken from a YGCBA plate incubated for 48 h at 27°C, was streaked in a line (2 to 3 cm) onto the soft-agar surface. The plates were incubated at 27°C for 24 h and evaluated for inhibition zones around the yeast cells using Henry's illumination.

RESULTS AND DISCUSSION

Agar-membrane screening assay of yeasts from various sources. To test whether yeasts in general are able to produce substances inhibitory to *Listeria*, 100 yeasts selected from the Weiherstephan Yeast Collection were screened against two *Listeria monocytogenes* indicator strains, WSLC 1001 and 1364, from the Weiherstephan *Listeria* Collection using an agar-membrane-based screening assay which was easy and fast to perform, allowing us to screen a high number of strains. According to the different inhibition zones obtained, a score-based evaluation system was developed ranging from 0 (no inhibition) to 5 (very clear inhibition) (Fig. 1). The experiments showed that around 10% of the yeasts had a clear or very clear antilisterial activity, whereas no effect to moderate inhibitory effect was ascribed to the majority of the strains.

However, within the inhibition zone, growth of *L. monocytogenes* was still observed (data not shown). A higher inhibition rate would have resulted in a transparent zone, as is observed when the supernatant of *Lactobacillus plantarum* including the antilisterial pediocin is applied to the soft agar surface (Fig. 1). In some cases, a white staining of the soft agar below the yeast spots grown on the membrane occurred due to unknown causes and was therefore considered not to be evaluable. It is also known that only a minority of isolates of cheese-ripening bacteria inhibit *Listeria* (8, 28, 32).

Due to yeast growth, the liquid cultures reached pH values of around 4.5. To exclude the possibility that acidification of the yeast liquid culture used for the agar-membrane screening assay was the cause of inhibition, the liquid medium for the cultivation of a selection of yeasts was buffered with bis-Tris adjusted to pH 6.5. There were no significant differences between buffered and unbuffered yeast cultures. Also, the pure buffer solutions did not influence listerial growth. Furthermore, liquid medium for yeast cultivation acidified to pH 4.3 did not show any inhibitory effect when spotted on the membrane.

Ten yeasts with different inhibitory potentials were then tested for their inhibitory potentials against four *L. monocytogenes* strains. The five inhibitory yeasts (Table 3) demonstrated their activities in four independently performed experiments. Depending on the *Listeria* indicator strain used, slight differences in inhibition were noticed, which is well known for bacteria exhibiting antilisterial activity (8, 32). The inhibitory potentials of the yeasts were clearly strain dependent (compare the two *Kluyveromyces marxianus* strains in Table 3).

Agar-membrane screening assay of yeasts from various European red smear cheeses. Since the focus of the present study was on yeasts originating from smear-ripened cheeses, a total of 304 yeasts from various European red smear cheeses were screened against the *L. monocytogenes* strains WSLC 1039, 1211, 1364, and 1416. Eleven yeasts out of the 304 were able to inhibit two or more *L. monocytogenes* indicator strains significantly in at least three independently performed experiments. Ten of these isolates belonged to *Candida intermedia* and were isolated from three different cheeses, two of which were produced in the same dairy. The other yeast was identified as *Yarrowia lipolytica*. The isolates from most cheeses did not show significant antilisterial activity. Ryser et al. (28) investigated bacteria for their antilisterial activities and found inhibiting isolates on only 15 out of 105 red smear cheeses.

Compared to the previously performed analyses of yeasts from various sources, the fraction of inhibitory yeasts from

TABLE 3. Antilisterial activities against four different *L. monocytogenes* indicator strains

Species	Strain ^c	Score for <i>L. monocytogenes</i> indicator strain WSLC ^a :				Score of total antilisterial activity ^d
		1039	1211	1364	1416	
<i>Issatchenkia occidentalis</i> ^b	WSYC 312	17	15	16	15	63
<i>Pichia membranifaciens</i> ^b	WSYC 223	17	15	17	14	63
<i>Issatchenkia orientalis</i> ^b	WSYC 263	17	13	17	14	61
<i>Candida parapsilosis</i> ^b	WSYC 51	12	13	15	17	57
<i>Kluyveromyces marxianus</i> ^b	WSYC 1	9	12	13	14	48
<i>Galactomyces geotrichum</i>	WSYC 122	5	7	10	7	29
<i>Debaryomyces hansenii</i>	WSYC 184	3	2	4	3	12
<i>Kluyveromyces marxianus</i>	WSYC 215	2	2	1	2	7
<i>Pichia triangularis</i>	G 1077	1	3	1	1	6
<i>Candida zeylanoides</i>	WSYC 22	0	0	1	1	2

^a Semiquantification is given as a sum of scores obtained in four independent experiments in the agar-membrane screening assay (Fig. 1). Maximum value, 20 (highest level of inhibition); minimum value, 0 (lowest level of inhibition). WSLC, Weihenstephan *Listeria* Collection, Abteilung Mikrobiologie, ZIEL Weihenstephan, Freising, Germany.

^b Strain with a clear inhibitory action against *L. monocytogenes*.

^c WSYC, Weihenstephan Yeast Collection, Abteilung Mikrobiologie, ZIEL Weihenstephan, Freising, Germany. G, glycerol number, Abteilung Mikrobiologie, ZIEL Weihenstephan, Freising, Germany.

^d Sum of inhibition values obtained for the different indicator strains.

smear-ripened cheeses was quite low. In total, 65% of 304 red smear cheese yeasts examined in this study were assigned to *Debaryomyces hansenii*, and only one strain showed noteworthy inhibition of two of the tested *L. monocytogenes* strains in two independent experiments. The high frequency at which *D. hansenii* occurs on red smear cheeses (3, 4, 11, 26, 34), on cheeses in general (22, 36), or in different dairy products (26, 30) has often been described.

Inhibition of *L. monocytogenes* in a yeast cocultivation assay.

It became clear from the agar-membrane screening assay that some yeasts exhibit antilisterial properties. In the next step, a cocultivation assay was developed in order to simulate the conditions (direct contact of yeast and *Listeria* cells) and con-

tamination rates found on the surfaces of smear-ripened cheeses. A total of 14 yeasts from various species and with different inhibitory potentials according to the agar-membrane screening assay were selected from the 304 European red smear cheese isolates for cocultivation with *L. monocytogenes* strain WSLC 1364. This indicator strain proved to be very sensitive in the agar-membrane screening assay.

As shown in Fig. 2, all yeasts tested in a 24-h cocultivation with *L. monocytogenes* showed some inhibition compared to the controls, in which *Listeria* cells always grew up to around 5×10^7 CFU/cm². In the presence of up to 10^8 yeast cells per cm², *Listeria* cell counts 1 to 5 log units lower than in the controls were found (Fig. 2). Differences in the antilisterial

FIG. 2. Inhibition of *Listeria monocytogenes* WSLC 1364 when cocultured with yeast strains isolated from different European smear-ripened cheeses. Cell counts are given as mean values of two to four independent experiments, with error bars representing standard deviations. *, yeasts in which a marginal inhibition zone was obtained in the "killer toxin assay"; WSLC, Weihenstephan *Listeria* Collection; WSYC, Weihenstephan Yeast Collection.

TABLE 4. Inhibitory potentials of 14 yeasts isolated from European red smear cheeses tested in the agar-membrane screening assay, cocultivation assay, and "killer toxin" assay

Strain	Species	Agar-membrane assay ^a score	Cocultivation assay ^b (log units/cm ²)	"Killer toxin" assay ^c (mm)
WSYC 512	<i>D. hansenii</i>	1	1	
WSYC 513	<i>D. hansenii</i>	3–4	1	
WSYC 514	<i>C. intermedia</i>	1	5	
WSYC 515	<i>Cl. lusitanae</i>	2	1	
WSYC 516	<i>C. intermedia</i>	2–3	4	
WSYC 517	<i>C. intermedia</i>	4	4	
WSYC 518	<i>C. intermedia</i>	3–4	1	
WSYC 519	<i>C. intermedia</i>	4	4	
WSYC 520	<i>C. anglica</i>	3	2	1
WSYC 521	<i>S. unisporus</i>	2–3	1	1
WSYC 522	<i>D. hansenii</i>	1	1	
WSYC 523	<i>C. intermedia</i>	5	3	
WSYC 524	<i>Y. lipolytica</i>	2	1	
WSYC 525	<i>K. marxianus</i>	2/NE	4	1

^a Average scores obtained in four independent experiments according to the score-based system (Fig. 1). NE, not evaluated.

^b Average reduction of *Listeria* cell counts obtained in two to four independent experiments compared to the controls. A reduction of 1 log unit/cm² is considered to be nonspecific.

^c Inhibition zone around the yeast cells grown on the soft agar surface.

potentials were not due to different yeast cell numbers (data not shown). In 7 out of 14 cases, variations in the *Listeria* cell counts per cm² of only around 1 log unit were observed. This weak reduction of listerial growth might be due to competition for nutrients. Taking this into account, four yeasts, belonging to *C. intermedia* and *Kluyveromyces marxianus*, inhibited *Listeria* by 3 log units (CFU/cm²). The strongest inhibitory effect was observed for a yeast identified as *C. intermedia*. *Listeria* cell counts showed a difference of 4 log units (CFU/cm²).

Assay for assessing a "killer toxin activity." The same 14 yeasts that were cocultivated with *L. monocytogenes* WSLC 1364 were tested in an assay used for assessing killer toxin activity (24). In three of these yeasts, identified as *Candida anglica* WSYC 520, *Kluyveromyces marxianus* WSYC 525, and *Saccharomyces unisporus* WSYC 521 (Fig. 2), a very small inhibition zone of 1 mm around the yeast cells grown on the soft-agar surface was reproducibly observed. Provost et al. (24), however, reported very large, clearly visible inhibition zones surrounding the killer toxin-producing yeast. The marginal inhibition zones obtained in the "killer toxin assay" did not correlate with the inhibition found in the agar-membrane assay or with the cocultivation assay (Table 4).

Conclusion. Our results show that a minority of yeasts tested have significant inhibitory potential. Antilisterial activity is not restricted to a single yeast species, but only a few strains within a species exhibit inhibitory properties. These results are comparable to those reported for antilisterial bacteria (8, 32). The inhibitory potential of yeast against *Listeria* was clearly pronounced in the cocultivation experiment, in which conditions (direct contact of yeast and *Listeria* cells) and contamination rates were chosen to simulate those found on smear cheese surfaces. This is important for a potential application of antilisterial yeast in red smear cheese microbial consortia. Furthermore, the active substance did not show a visible capacity for diffusion, as demonstrated in the "killer toxin assay."

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