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Role of *Chrysonilia sitophila* in the quality of cork stoppers for sealing wine bottles

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The contribution of *Chrysonilia sitophila* in cork stopper manufacture was studied and a simulation of the industrial processing of cork stoppers was performed. Stoppers cut from slabs where mold development was inhibited were compared with others cut from slabs colonized by *C. sitophila* alone or with several molds, in terms of physical properties and chemical taints. *C. sitophila* does not produce 2,4,6-trichloroanisole, guaiacol, or 1-octene-3-ol on cork slabs incubated for 66 days. Since some chlorophenol-related compounds contaminate cork slabs during the production processes, metabolic tests were performed to investigate the capability of molds to produce 2,4,6-trichloroanisole by methylation of 2,4,6-trichlorophenol. Degradation of 2,4,6-trichlorophenol by *C. sitophila* resulted in a very high level of degradation without production of 2,4,6-trichloroanisole. *C. sitophila* restricted growth of other molds on maturing slabs for at least 30 days. These results show that *C. sitophila* can be exploited by industrial producers of cork stoppers since it is able to inhibit the development of other molds and it does not produce the compounds responsible for 'cork-taint', even in the presence of chlorophenols. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 256–261.

Keywords: *Chrysonilia sitophila*; cork stoppers; cork taint; 2,4,6-trichloroanisole

Introduction

The industrial manufacture of cork stoppers has a significant economic impact on the Portuguese economy since Portugal is the largest producer of cork worldwide. The cork stopper manufacturing process includes a maturing stage, after the cork slabs are steeped in boiling water, which allows physical stabilization of the slabs as well as a humidity level adequate to punch cork stoppers. During the maturing stage molds completely cover the slabs. This stage was studied as well as the empirical procedure which is used for the traditional production of cork stoppers [14,21].

The occurrence of tainting in bottled wine, causing unpleasant alterations in wine flavor or aroma, is responsible for large losses of stock in the wine industry. The most unpleasant of these 'off-flavors' is the so-called 'cork-taint', clearly distinguishable by the musty/moldy aroma of the wine. Several compounds such as 2,4,6-trichloroanisole (TCA), guaiacol, and 1-octene-3-ol have been associated in the past with 'cork-taint' [23]. Some microorganisms have the ability to perform chemical breakdown of lignin, producing guaiacol [25] which, when present in wine, can produce flavor modification by combining with other compounds [24]. 1-Octene-3-ol is a metabolite of molds and induces a mushroom-like flavor in wine [23]. TCA was identified as the major compound responsible for 'cork-taint' [9]. Some molds can methylate chlorophenols and related compounds as a detoxification process, producing chloroanisoles [12,18]. This led to the suggestion that the main cause of 'cork-taint' in wines could be attributed to molds growing on maturing cork slabs [23]. In addition, the high incidence of respiratory health problems in workers of the cork industry has been attributed to mold spores dispersed into the atmosphere during slab cutting [5,6]. These facts induced producers to realize that mold development should be suppressed during the manufacturing process. On the other hand, cork slabs were traditionally considered to be of good quality for stopper manufacturing when they were completely covered with white or salmon-colored molds. However, no evidence exists to adequately support either the traditional practice or the current belief of suppressing mold growth.

If *C. sitophila* contributes significantly to the reported wine alterations, these should be systematic, which is not the case [14]. Other authors described some *Penicillium* species as strong producers of off-flavors, and systematically referred to them as cork-dominant molds [22,23]. However, during the manufacturing process, the dominant mold normally associated with the maturing step was reported to be *Monilia sitophila (C. sitophila)* [14,21]. Some *Penicillium* species, as well as other TCA-producing mold species found in previous works, were associated with later colonization of the cork slabs and stoppers. This colonization may occur in the industry during packaging [32], transport [31,33], or in the winery environment [11,26]. The volatile metabolites are able to migrate into cork stoppers [31], even when the bottle of wine has been stoppered [11]. Finished stoppers (clarified and waterproof), with humidity levels of 6%, restrict the growth of molds [16]. If cork stoppers are stored under conditions

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of low humidity and in a package impermeable to volatile compounds, further contamination by molds and the associated production of TCA can be prevented.

The crucial phase in the manufacture of cork stoppers is the cork slabs maturing step. This work deals with the role of *C. sitophila* development on cork slabs during the maturing stage.

Materials and methods

Fungus

Chrysonilia sitophila (Montagne), obtained from the IBET/ITQB collection, was maintained in potato dextrose agar (PDA, Merck, Darmstadt, Germany) and stored at 4°C. To prepare *C. sitophila* inoculum, fungal spores were obtained from 72-h cultures (27°C) by suspension in 0.8% (w/v) NaCl. This suspension was filtered through glass wool. The optical density of the spore suspensions was standardized by adjusting the absorbance at 450 nm to 0.1.

Cork slabs

These were obtained from two industrial manufacturers of cork stoppers, in this work designated as Unit A and Unit B. Cork slabs (30 × 25 × 3 cm), obtained from Unit A, were boiled in water for 2 h and placed immediately inside a biological safety cabinet until they reached room temperature. Each 'inoculated slab' was inoculated with 2 ml of a spore suspension and then placed inside the sterile maturation chamber. 'Non-inoculated slabs' were placed directly in the sterile maturation chambers. The chambers were sealed and connected to tubes (Marprene tubing, 3.2 mm od, 1.6 mm id, Watson Marlow, Falmouth, UK) for gas admission.

Maturation chambers

Maturation chambers of acrylic material were designed to contain three cork slabs each. Gas flow was obtained by injection of filtered gas (Millex-FG 50, 0.2 μm pore size, Millipore, Bedford, MA, USA) through a false base with distribution in the three compartments via 2-mm diameter holes. The outlet was located on the front door of the maturation chamber, which was sealed by a rubber joint.

The experimental conditions are summarized in Table 1. The maturation chambers were exposed to a 'low flow period' (9 ml min^{-1}) and/or to a 'fast flow period' (35 ml min^{-1}) of air (Air K, Air Liquide, Estarreja, Portugal). Three chambers were saturated with carbon dioxide (CO_2, Air Liquide), with three exchanges of gas per day. Maturation chambers were maintained at 14°C in the dark.

Microbiological analysis of the cork slabs

This analysis was performed only on the slabs showing growth of molds other than *C. sitophila*. Enumeration of the different species was performed by sampling hyphae or spores from each mold observed on the slabs, followed by point inoculations on solid media. The cultures were incubated at 27°C. Species were isolated from mixed cultures after several subcultures, by taking advantage of differences in the rates of colony growth.

During the isolation procedure the following media were used: M1 base medium containing 15 g L^{-1} of agar and supplemented with 7.5 g L^{-1} carboxymethyl cellulose (CMC medium viscosity, Sigma, St Louis, MO, USA), potato dextrose agar, plate-count-agar (PCA, Merck), oxytetracycline glucose yeast agar (OGY, Merck), dichloranglycerol agar base (DG18, Oxoid, Basingstoke, UK). No oxytetracycline or chloramphenicol was added to OGY or DG18 medium, since no bacterial contamination was expected. Basal medium M1 (modified from Sternberg *et al* [30]) contained: $(NH_4)_2SO_4$ 1.4 g, KH_2PO_4 2.0 g, urea 0.3 g, $CaCl_2$ 0.3 g, $MgSO_4$ 0.3 g, $FeSO_4 \cdot 7H_2O$ 5 mg, $MnSO_4 \cdot H_2O$ 1.6 mg, $ZnSO_4 \cdot 7H_2O$ 1.4 mg, $CaCl_2$ 2 mg, and tryptone (Difco, Detroit, MI, USA) 0.75 g, per liter of distilled water. The pH was adjusted to 6.0 with 0.1 M NaOH. M1 containing CMC as the sole carbon source was used for increased selectivity. DG18 supplemented with 250 mg L^{-1} rose bengal (RB, Sigma) was also used to restrict colony spreading.

Purity of the isolates was observed microscopically by looking at the morphology of reproductive structures formed in slide cultures [22] using blocks of DG18 or PDA. Preparations for microscopy were stained with lactophenol for identification to the genus level.

The growth rates of the isolated molds were determined on M1 medium containing CMC, by measuring the change in colony diameter with incubation time. Linear equations for mold growth were obtained by plotting colony diameter as a function of time. Growth rates, μ, were defined as the slope of the linear curves obtained.

Processing of stoppers

From each cork slab, stoppers of 4.7 cm height and 2.5 cm diameter were prepared in the industrial installations of Unit A. The stoppers produced were packed separately and

Table 1 Maturation chamber conditions for inoculated and non-inoculated cork slabs

Maturation periods	Inoculated slabs Air		Non-inoculated slabs CO_2		Non-inoculated slabs Air
	E_1	E_2	E_1	E_2	E_2
Slow flow period 9 ml min^{-1}	20 days	60 days	–	–	60 days
Fast flow period 35 ml min^{-1}	6 days	6 days	26 days	66 days	6 days
Total duration	26 days	66 days	26 days	66 days	66 days

The terms E_1 and E_2 represent the total duration of the experiment. Two maturation chambers were used in experiment E_1 and one in E_2.

sent immediately for physical analysis and chemical quantification of 'off-flavor' compounds.

Chemical analysis: For quantification of TCA, guaiacol, and 1-octene-3-ol, the stoppers were ground down and the chemicals were extracted with ethanol for 48 h at room temperature. After filtration the alcoholic solution was extracted with pentane and concentrated under vacuum. The concentrated extracts were analyzed by gas chromatography using a Hewlett Packard 5890 II chromatograph (Hewlett Packard, Wilmington, DE, USA) with a HP 1 column (Hewlett Packard, 25 m, 0.20 mm id, 0.33 μm film) and a mass detector Hewlett Packard 5971A; SIM-TCA m/z 167, 195, 210; guaiacol m/z 81, 109, 124; 1-octene-3-ol m/z 27, 57, 72. The detection limit was 1 μg kg^{-1} for both guaiacol and 1-octene-3-ol, and 0.02 μg kg^{-1} for TCA (expressed by kg of cork).

Physical analysis: Physical analyses were carried out using the methodologies recommended by the Portuguese Authorities (Instituto Português da Qualidade) for current control of the industrial cork stopper quality. The resistance to compression (RC) was performed according to the NP 2803-03 [3]. This test calculates the transverse and radial forces that a stopper can support in the bottling process, at room conditions (19 ± 2°C and 52 ± 5% humidity). The dimensional recuperation (RD) was determined by mechanical compression of the stopper until it reached 33% of its initial diameter, followed by immediate relaxation. The value of RD is the percentage ratio between initial and final diameters (15 min after mechanical compression). Humidity was determined using a calibrated hygrometer according to NP-2803-2 [2].

Analysis of TCA synthesis by molds

These tests were performed using the basal medium M1 modified by adding 7.5 g L^{-1} D-glucose, replacing $CaCl_2$ by the same quantity of $CaCO_3$, and excluding $CoCl_2$. The liquid cultures were supplemented with 100 μl of a stock solution of 0.5 mg ml^{-1} TCP (final concentration 1 μg ml^{-1}). This medium was distributed into sealed 100-ml culture flasks. Each flask was inoculated using the following procedure: a small agar bloc (approximately 1 cm^3) was cut from the growing margin of a 72-h-old fungal colony (PDA, 27°C) and used as inoculum. All flasks inoculated, with or without TCP, as well as media controls, were incubated for 11 days at 27°C with agitation (70 rpm), in the dark. All experiments were performed in duplicate. After mold growth, the culture media were analyzed as follows.

Extraction of TCP and TCA: The flask containing the culture medium was cooled in ice and 10 ml of *n*-hexane (Merck) was added by injection through the teflon septum. A trap flask with ethanol was connected to prevent gas release to the atmosphere. The solution was then filtered through a nylon membrane with 0.22-μm pore size (Millipore) and the solution was transferred into a separatory funnel. A solution saturated with sodium chloride (1 ml) was added to achieve a better separation of the two phases. The organic phase was collected and kept at 4°C.

TCA quantification: TCA quantification in the hexane extracts was carried out using a Hewlett Packard 5890 II gas chromatograph, equipped with a DB-5 column (30 m, 0.32 mm id, 0.25 μm film, J&W, Folsom, CA, USA) and an electron capture detector. Samples (1 μl) were directly injected over 30 s splitless. The split ratio was 1:15. The operating temperatures were 285°C on the injector and 325°C on the detector; the column temperature was programmed starting at 60°C and increased to 130°C at a rate of 30°C min^{-1}, maintained at 130°C for 1 min, increased to 250°C at a rate of 5°C min^{-1} and maintained at 250°C for 3 min. The external standard method was used for TCA quantification.

TCP quantification: For TCP quantification, 5 ml of the hexane extract were mixed with 35 ml of 0.1 M K_2CO_3. In order to acetylate TCP, 0.5 ml of acetic anhydride was added to this mixture. This solution of TCP acetate in hexane was then analyzed using the operating conditions described above. The quantification limits in hexane solutions were 1 μg L^{-1} for TCA and 2 μg L^{-1} for TCP while the detection limits were 0.5 μg L^{-1} for TCA and 1 μg L^{-1} for TCP.

Results and discussion

Cork slabs

Characterisation of the isolated molds: Several molds were identified from cork slabs when the incubation time was increased from 26 days (experiment E_1) to 66 days (experiment E_2). The growth of *C. sitophila* was detected 48 h after inoculation of the slabs with a spore suspension of *C. sitophila*. After 30 days of incubation, different species of molds began to develop in small areas, though they never overgrew *C. sitophila*. Six species, other than *C. sitophila*, were isolated. The microscopic observation of their reproductive structures allowed the identification of four different *Penicillium* spp ($I^{(1)}$, $I^{(2)}$, $I^{(3)}$, $I^{(4)}$) and one *Cladosporium* sp. The sixth mould ($I^{(8)}$) was not identified as it was detected only in a small zone in one of the slabs inoculated.

In the non-inoculated slabs that were kept under a flow of air, *C. sitophila*, *Penicillium* spp $I^{(2)}$, $I^{(3)}$, $I^{(4)}$, *Cladosporium* sp, two different *Penicillium* species ($I^{(5)}$, $I^{(6)}$), and one related *Penicillium* sp ($I^{(7)}$) were observed after 2 days of incubation and subsequently isolated. One mold having the macroscopic characteristics of *C. sitophila* was predominantly observed after 30 days of incubation.

Since it has been reported that the cork boiling process destroys the naturally-occurring microbiota of cork and that slabs are normally re-infected with the natural surrounding inoculum [29], our experiments were performed under highly controlled aseptic conditions. The cork slabs were placed inside the maturation chambers sealed in a biological safety cabinet and the air applied to the reactors was filtered through a 0.22-μm pore size filter. Since several molds were observed on the slabs during experiment E_2, our results suggest that the boiling process does not destroy all cork microbiota. In fact, Jäger *et al* reported that during the boiling process the interior temperature of a box con-

structed of cork reached a maximum of only 62°C after 1 h [20].

In a previous study performed in Unit B [13], *C. sitophila* was isolated as the predominant mold throughout the industrial process, although *Penicillium* sp. *Mucor* sp, and *Trichoderma* sp were also observed. However, none of these species were detected in non-boiled cork. In a third industrial unit (Unit C), only *C. sitophila* development was detected on boiled slabs during the maturing stage (data not shown). From all these results and observations, it is evident that the source of mold species growing on cork needs careful reevaluation since one can not simply consider cork-borne microorganisms.

The molds isolated during this work were probably originated from the industrial environment. Therefore, it can be assumed that the resident microbial population of the industrial areas is the main factor for the new colonization of cork during processing, occurring mainly in the maturing compartments. This mechanism was also suggested in other traditional industries involving microbial activity, such as wine production [34]. The origin of such populations in the wine industry is explained by the presence of a favorable environment for the survival and growth of certain microorganisms [8].

During the initial period of the slab maturing stage, an appreciable content of soluble sugars is available in the moisture of the slab [28], which could support growth of moulds with low levels of cellulolytic activity. Although this is not the case for *C. sitophila* (data not shown), many substrates are available in boiled cork slabs and thus mold growth selectivity should not occur due to substrate. Since the moisture level after boiling is adequate for mold development, the decisive factor seems to be the abundance of inoculum. As stated above *C. sitophila* became the dominant mold only after 30 days of incubation. These results suggest that in the maturing step a competitive mechanism exists among *C. sitophila* and the other molds since on the inoculated slabs *C. sitophila* dominated from the beginning, it appears that the initial concentration of *C. sitophila* inoculum is the decisive factor controlling the order by which the molds will grow. It is also clear that the inoculum concentration of *C. sitophila* necessary for colonization as the dominant mold can be quite low. This conclusion is supported by the fast growth of *C. sitophila* on cellulose medium (Table 2). *C. sitophila* was the fastest growing mold, having an increase in colony diameter of almost 3 mm h^{-1} of growth. The *Penicillium* sp $I^{(2)}$, which exhibited the second highest growth rate, showed an increase in colony diameter almost ten times smaller than that of *C. sitophila*.

Table 2 Constant growth rates (μ) for the isolated molds

Fungus	μ (mm h^{-1})
C. sitophila	2.994
$I^{(1)}$ *Penicillium* sp	0.181
$I^{(2)}$ *Penicillium* sp	0.249
$I^{(3)}$ *Penicillium* sp	0.124
$I^{(4)}$ *Penicillium* sp	0.063
$I^{(5)}$ *Penicillium* sp	0.178
$I^{(6)}$ *Penicillium* sp	0.117
$I^{(7)}$ related *Penicillium* sp	0.181
Cladosporium sp	0.112

Cork stoppers

Physical tests: Cork stoppers were submitted to physical analyses to evaluate if mold growth over prolonged incubation times (especially in the case of *C. sitophila*) changed their physical characteristics. Given that finished stoppers usually contain a low level of humidity (about 6%) and that the humidity of the stoppers under study (unfinished stoppers) was between 11% and 15%, the quality of the stoppers could be negatively affected. However, the results of the physical tests for all the stoppers obtained from slabs matured under the described laboratory conditions (Table 3) are within the normal range of values accepted for finished stoppers prepared from slabs matured in the industrial environment (personal communication). Although the cellulase (data not shown), and L-peroxidase [17] activities of *C. sitophila* are good indicators of the capability for growth on cork materials, the physical quality of the stoppers made from slabs 'matured' during long periods with *C. sitophila* was well within the standards defined by the industry. These results were taken as a validation of the subsequent experiments.

Chemical tests: The quantification of TCA, guaiacol, and 1-octene-3-ol was made to evaluate if the molds observed in the 'maturing slabs' had the ability to produce these compounds without an external addition of chlorophenol or chloride.

In the case of experiment E_1, 2.05 μg kg^{-1} of TCA were detected in stoppers originated from the slabs 'matured under carbon dioxide' and 1.6 μg kg^{-1} were detected in the cork stoppers originated from slabs inoculated with *C.*

Table 3 Physical properties of cork stoppers obtained from inoculated and non-inoculated slabs under the conditions of experiments E_1 and E_2 (expressed in mean value)

Test	Inoculated slabs		Non-inoculated slabs		
	Air flow		CO_2 flow		Air flow
	E1[a]	E2[b]	E1[a]	E2[b]	E2[b]
RC	38	43	37	42	38
(transverse)	36		39		
RC	10	11	9	10	10
(radial)	9		9		
RD	98	98	nd	98	98
	98		97		
Humidity	11.6	nd	11	nd	nd
	10.9	nd	11		

RC, Radial compression (RC-transverse: SD 4.8–6, RC-radial SD 1.1–1.5); RD, Dimensional Relaxation (SD not available); Humidity (SD 0.37–1.1); nd, Not done.
[a]Results from two maturation chambers analysed separately; [b]result of one maturation chamber.
Air and CO_2 flow are reported in Materials and Methods. The results reported resulted from analysis performed by the Centro Tecnológico da Cortiça (CTCOR, Portugal) (Technological Cork Center).

sitophila and 'matured under air'. In slabs matured for 26 days, the only visible mold was *C. sitophila*. Since the detected concentration of TCA was similar to that of slabs 'matured' under carbon dioxide, it is unlikely that *C. sitophila* could contribute to the TCA present. This was confirmed by experiments E_2 that had a prolonged maturation period (66 days), where TCA was not detected in any of the stoppers originated from inoculated or non-inoculated slabs and 'matured' under either air or carbon dioxide.

From these results it seems that *C. sitophila* is not able to methylate contaminant chlorophenols occasionally present in cork. The presence of these compounds in cork can be associated with their use as wood preservatives and pesticides [1] or with the use of chlorinated derivatives, commonly used as disinfectants of water, equipment, and work areas [7,19], which may result in chlorination of lignin [9]. Furthermore, most of the known origins of chlorophenol contamination are now almost completely banned from the production process of cork stoppers. Thus, it can be assumed that the TCA detected in the stoppers analyzed (experiment E_1) was present in the cork slabs before *C. sitophila* growth, probably resulting from accidental aerial contamination during stopper cutting. The presence of guaiacol and 1-octene-3-ol was not detected in any of the stoppers produced. These results show that, in the absence of chlorophenol or chlorine, the growth of *C. sitophila*, alone or in association with other molds, can not be considered responsible for production of the off-flavor compounds under study.

TCA synthesis by the isolated molds

Several molds were reported to produce TCA by methylating TCP. Chlorophenol and related compounds can be used as disinfectants or preservatives, so their presence may occur in one or more steps of stopper production and distribution. Therefore, it is necessary to clarify if *C. sitophila*, or any of the other molds detected, can methylate TCP and thus induce 'cork-taint'.

The total percentage of TCP converted and the yield of TCA production are depicted in Table 4. Culture media from the negative control flasks (media not inoculated, and with no addition of TCP) had TCP and TCA concentrations below the detection level under the conditions of the experiment. Only 66% of the TCP available was recovered. This value was taken into account for the calculations, and assumed as the maximum concentration of TCP that it is possible to detect under the experimental conditions used. TCA was not detected in these media.

Table 4 TCA synthesis from TCP by cork molds

Fungus	% TCP transformed		Yield of TCA production (%)	
	A_1	A_2	A_1	A_2
C. sitophila	82	88	nc	0.03
$I^{(1)}$ *Penicillium* sp	94	97	0.94	0.53
$I^{(2)}$ *Penicillium* sp	24	21	nc	0.43
$I^{(3)}$ *Penicillium* sp	94	94	0.13	0.16
$I^{(4)}$ *Penicillium* sp	9	21	5.00	5.14
$I^{(5)}$ *Penicillium* sp	27	18	0.67	1.67
$I^{(6)}$ *Penicillium* sp	45	30	1.07	4.40
$I^{(7)}$ related *Penicillium* sp	6	6	8.50	9.50
Cladosporium sp	91	94	2.20	2.13

The yield of TCA production corresponds to the quantity of TCA produced from methylation of 100 μg of TCP. Experiments were performed in duplicate: A_1 and A_2; nc, not calculated (TCA concentration below the detection method).

C. sitophila, *Penicillium* spp ($I^{(1)}$ and $I^{(3)}$), and *Cladosporium* sp showed identical capability to consume high levels of TCP but significant differences in the yields of TCA production. *C. sitophila* converted more than 80% of the available TCP, but led to the lowest yield of TCA production (0.03%). *Cladosporium* sp used a similar amount of TCP, but led to a higher yield of TCA production (2%). The *Penicillium* related sp, $I^{(7)}$ showed the lowest TCP level of consumption, but was the mold that more efficiently methylated TCP into TCA. This comparative analysis for each of the molds shows that no direct relationship exists between the percentage of TCP consumed and the yield of TCA produced. Tindale *et al* reported the ability of several molds isolated from packaging materials to methylate TCP, producing TCA [32]. The molds can be regarded as strong methylators (yield of TCA $>$ 45%), moderate methylators (yield of TCA between 10 and 45%), or weak methylators (yield of TCA $<$ 10%) [32]; according to the results presented in Table 4, all nine molds under study are weak methylators. Although the enzymatic reactions that allowed *C. sitophila* to metabolize more than 80% of the TCP are not clearly understood, the results presented in this paper show that methylation of TCP is not the main detoxification process used by *C. sitophila*.

It is unlikely that the molds under study could grow well in the presence of wine under oxygen depletion [10,15]. It is also improbable that these species can show cellular activity inside a cork stopper [20]. Also the production of TCA is unlikely to occur under conditions of depleted nutrients [4]. Since under the experimental culture conditions used in this work *C. sitophila* achieved stationary phase after approximately 40 h (data not shown), and considering the duration of the experiment in the liquid media supplemented with TCP (11 days), it is improbable that additional TCA would be produced in longer incubation periods.

This is the first report clarifying the contribution of *C. sitophila* in the maturing step of the cork manufacturing process. It is clear that the cork industry needs to establish strict rules of process separation. Special attention should be given to the compartment where the maturing stage of slabs takes place; it must be separated from the area where the cork slabs are normally cut and chosen before stopper manufacture, and be provided with adequate sanitary conditions. Slab choice and cut shortly after boiling and before maturation may also reduce spore dispersion and thus reduce the diversity of species growing on boiled cork slabs during the 'maturing step'. The microbial population established in the industrial environment is highly influenced by plant layout and operating procedures. Visual control of the levels of mold development over the maturing slabs is also feasible since the macroscopic aspect of *C. sitophila* is easily recognized and distinguishable from contaminant molds.

C. sitophila has the ability to restrict, at least for a period

of 30 days, growth of other mold species that can compromise cork stopper quality, possibly reducing contamination of the maturing slabs with molds such as *P. glabrum* and *P. granulatum*, reported as being strong TCA producers [30]. *C. sitophila* can also be used as an indicator of humidity levels adequate to start the punch of the cork slabs for the production of the stoppers.

Finally, the results suggest that the controlled growth of *C. sitophila* can be exploited by the industry to establish a 'clean' process of stopper manufacturing. The dominance of *C. sitophila* inside the maturation rooms can be achieved by using a combination of technological changes aimed at favoring its development and inhibiting the growth of other molds. *C. sitophila*, though unable to produce the compounds more often associated with the cork taint in wine, is able to metabolize TCP and thus to prevent production of TCA by other microorganisms.

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