

The effect of disinfection substances on the propagules  
of heat-resistant fungi in vitro

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Inoculum from the strains of thermoresistant strains of the fungi *Botryotrichum* (Bo.) *piluliferum*, *Byssoschlamys* (B.) *fulva*, *B. nivea*, *Neosartorya* (N.) *fischeri*, *Talaromyces* (T.) *avellaneus*, *T. bacillisporus*, *T. flavus* and *T. trachyspermus* consisting from the mixture of mycelium, spores, asci, ascospores, kleistothecia or aleuriospores was exposed in vitro to the action of 7 various types of disinfection solutions, the exposure time being 15 and 60 minutes. Under the experimental conditions, the most effective solutions proved to be the 0.2% Persteril and 1% Septonex solutions, the least effective was 1% Chloramine B solution. Among the tested strains, strain Bo. *piluliferum* was the most sensitive; *B. nivea*, *B. fulva* and *N. fischeri* were the most resistant strains.

**Key words:** Heat-resistant fungi, disinfection substances

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Inokulum z kmeňov termorezistentných mikromycét *Botryotrichum* (Bo.) *piluliferum*, *Byssoschlamys* (B.) *fulva*, *B. nivea*, *Neosartorya* (N.) *fischeri*, *Talaromyces* (T.) *avellaneus*, *T. bacillisporus*, *T. flavus* a *T. trachyspermus* pozostávajúce zo zmesi mycélia, spór, askov, askospór, kleistotécií, resp. aleuriospór bolo exponované in vitro účinku 7 dezinfekčných látok počas 15 a 60 min. V experimentálnych podmienkach sa najúčinnším javil 0,2 %-ný roztok Persterilu a 1 %-ný roztok Septonexu, najmenej účinný bol 1 %-ný roztok Chlóraminu B. Najcitlivejší bol kmeň Bo. *piluliferum*, najodolnejšie kmene *B. nivea*, *B. fulva* a *N. fischeri*.

Heat-resistant fungi cause serious problems in the canning industry at the production of fruit preserves. The germs of these fungi which survived the effect of higher temperatures begin to grow - after some time of latency - and the products become mouldy (Beuchat et Rice 1979, Beuchat et Toledo 1977, Hocking et Pitt 1984, King 1986, Scott et Bernard 1987, Splittstoesser 1978, Splittstoesser et al. 1970).

The not at all negligible source of fruit contamination and thus of production rooms and equipment in the canning industry are the propagules of the heat-resistant fungi in the soil. In Slovakia the heat-resistant germs of the *Neosartorya fischeri* strains occurred in the investigated samples of the soil in the amounts up to 149 colonies forming units (CFU) and the germs of the strains *Talaromyces flavus* up to 39 CFU/10 grams of the soil (Jesenská et Piecková 1991, Jesenská et al. 1991).

Colonies of other surviving species of fungi were also isolated from the soil after the effect of higher temperatures, e.g. *Botryotrichum piluliferum*, *Byssosclamyces (B.) fulva*, *B. nivea*, *Talaromyces (T.) avellaneus*, *T. bacillisporus*, *T. trachyspermus*, and others (Jesenská et al. 1992).

The question of the effectiveness of the disinfection substances on the heat-resistant fungi arose into foreground. This question was not studied until now, nevertheless, the elucidation of this would enable to perform directed sanitation of the production facilities in the canning industry.

The aim of our work was to obtain certain basic information about the *in vitro* effect of selected disinfection substances on the germs of such an important fungi strains as are the fungi able to resist to the temperatures used in the fruit canning industry.

## MATERIAL AND METHODS

### Fungi strains

There are introduced the tested strains in the Table 1; the cultures of these strains were isolated from the soil samples which had been exposed to the temperature of 70 °C in the environment of Sabouraud agar with Bengal Rose (Jesenská et al. 1992). The strains were isolated, then inoculated on the oblique Sabouraud agar (IMUNA, Šarišské Michaľany, Slovakia) in the test tubes and incubated at 25 °C for the time necessary for the formation and maturation of the reproductive structures, namely ascospores. The duration of incubation was from 4 to 6 weeks. Formation and maturation of kleistothecia, resp. asci and ascospores was examined microscopically in the native preparations.

### Inoculum

The grown-up strain was transported by inoculation needle from one test tube into Erlenmayer flask containing 10 ml of sterile saline and was homogenized by sterile balls during 1 hour in the laboratory quiver-machine. The number of the colony-forming units (CFU)/ ml during 72 hours of the incubation on the surface of Sabouraud agar was determined by the dilution method. The inoculum consisted – as verified microscopically in the native preparation – from the mixture of mycelium, spores, asci, ascospores and rest of kleistothecia (as far as the strains of *Neosartorya fischeri* or *Talaromyces* strains were present) and aleuriospores of *Botryotrichum piluliferum*.

### Disinfection substances

Disinfection substances usual in the common practice in Slovakia were used in the experiment:

Table 1: The effect of disinfection substances on the propagules of heat-resistant micromycetes in vitro.

Micromycetes	BP		BF		BN		NF		TA		TB		TF		TT	
	Time of effect of disinfection substances in minutes															
	15	60	15	60	15	60	15	60	15	60	15	60	15	60	15	60
Disinfection substances	Number of isolated colonies after effect of disinfection substances															
Ajatin (1%)	S	S	180	100	110	40	30	20	20	S	S	S	S	S	S	S
Septonex (1%)	S	S	30	S	16	1	S	S	S	S	S	S	C	S	S	S
Chloramin (1%)	32	S	C	C	C	100	43	6	C	1	S	S	C	77	C	100
Jodonal (2%)	S	S	S	S	5	S	S	S	C	6	C	C	100	4	C	C
Desigalin (2%)	S	S	10	6	16	10	3	S	5	S	S	S	S	S	S	S
Chlorhexidin (2%)	S	S	25	40	10	2	20	20	80	15	2	S	S	S	S	S
Persteril (0,2%)	S	S	30	S	S	S	60	S	15	S	S	S	4	S	S	S
Inoculum number of CFU.10 <sup>7</sup> /3 ml	3		0,3		0,3		30		3		40		50		10	

Notes a: BP...*Botryitrichum piluliferum*, BN...*Byssochlamys nivea*, BF...*Byssochlamys fulva*, NF...*Neosartorya fischeri*, TA...*Talaromyces avellaneus*, TB...*Talaromyces bacillisporus*, TF...*Talaromyces flavus*, TT...*Talaromyces trachyspermus*  
 S...Inoculated media stayed sterile  
 C...Uncountable number of colonies

**AJATIN:** 10% of active substance: dimethylbenzyl-dodecylammonium bromide. Manufacturer: SLOVAKOFARMA, Hlohovec (Slovakia).

**SEPTONEX:** 1-(etoxy-carbonyl)-pentadecyl-trimethylammonium bromide. Manufacturer: SLOVAKOFARMA, Hlohovec.

(Note: The quarterly ammonium compounds used in Slovakia are comparable to foreign ones. Ajatin is a substance similar to Benzalkonium chloride, Septonex has antimicrobial spectrum corresponding with Cetrimide).

**CHLORAMIN B:** Trihydrate of sodium salt of benzensulfochloramine. The preparation contains from 25 to 28% of active chloramine. Manufacturer: LACHEMA, Brno (Czech Republic).

**JODONAL B:** Active component: 1,7% of active iodine. Manufacturer: LACHEMA, Brno.

(Note: Substance with a wide antimicrobial spectrum which is comparable with iodinephores used abroad. The activity of this substance is significantly reduced in the protein-containing environment).

**CHLORHEXIDIN:** Chlorhexidin-gluconate 20% water solution. Active substance: 1,6-di-(4-chlorfenyl-diguanido)-hexan. Manufacturer: POLFA, Lodž (Poland).

**DESIGALIN:** Chlorhexidin-gluconate 7,5%. Manufacturer: POLFA, Lodž (Poland).  
**SEPTONEX 15 %;** additives. Manufacturer: GALENA, Komárov (Czech Republic).

**PERSTERIL:** Content of the peroxyacetic acid 28-32%, hydrogen peroxide 8-12%, sulfuric acid 1%. Manufacturer: CHEMICAL INDUSTRIES, Sokolov (Czech Republic).

Concentrations of above mentioned disinfection preparations were applied in the experiment and are quoted in the Table 1. These concentrations were chosen with respect to the concentrations used in the practice.

#### Experimental devitalization of germs of the tested strains

0.1 ml of the inoculum suspension was pipetted by a sterile pipette into 0.9 ml of disinfection solution. After the exposition time (15 to 60 minutes), 0.1 ml of the sample was inoculated and spread on the surface of Sabouraud agar (IMUNA, Šarišské Michaľany, Slovakia) in a Petri dish. Dishes were incubated for 5 to 7 days at 25 °C and the number of the colonies on the agar surface was determined.

#### RESULTS

On the basis of our results (Table 1) which were obtained by the above mentioned method, we can conclude that the germs of the *Botryotrichum piluliferum* strains were the most sensitive to the tested solutions. All the propagules in the inoculum

were devitalized after the exposure to disinfection solutions with the exception when after 15 minutes exposure to 1% Chloramin B solution only 32 CFU survived from the original number of the germs. The germs of the strains *T. bacillisporus* and *T. trachyspermus* were sufficiently sensitive to 15 minutes exposure to disinfection solutions with one exception, when these germs proved to be resistant to 1% solution of Jodonal. Among the tested strains, the germs of the *B. fulva*, *B. nivea* and *N. fischeri* showed to be the most resistant to the tested disinfection solutions. The 1% solution of Chloramin B showed to be the least effective in our experiment: after 15 minutes exposure we had observed growth of germs of the strains *B. fulva*, *B. nivea*, *T. avellaneus*, *T. flavus* and *T. trachyspermus* in the dishes with Sabouraud agar; after 60 minutes exposure there was growth of *B. fulva* with countless number of the colonies of this strain. The 2 % solution of Chlorhexidin and 1 % solution of Ajatin also proved to be less effective. 1% solution of Septonex showed to be the most effective; the 15 minutes exposure had devitalized the germs in four and the 60 minutes exposure in seven out of the eight tested strains. Good effect was observed also in 0.2% solution of Persteril, which after 15 minutes exposure devitalized the germs in four and after 60 minutes in all of the tested strains.

#### DISCUSSION

Fungi, whose germs are able to survive certain grade of the thermal processing of fruits and fruit juices, cause considerable problems in the canning industry by the moulding of the ready products. *B. fulva*, *B. nivea*, *N. fischeri*, and *T. flavus* are the most important thermoresistant fungi. These fungi occur in the soil in various amounts and with variable share of individual species (Bettucci et Rodriguez 1989, Beuchat et Rice 1979, Fravel et Adams 1986, Gochenaur 1975, Jesenská et Piecková 1991, Jesenská et al. 1991, 1992, Moubasher et Abdel-Hafez 1979, Mouchacca et Joly 1976, Okagbue 1989).

Other investigations of the ecology of heat-resistant fungi showed that – besides above mentioned species – there are germs of other fungi which are able to resist to the limited thermal processing (Jesenská et al., 1992, Lacey 1989, Lambert 1990, Pitt et Hocking 1985, Raper et Thom 1949, Samson 1989, Samson et Liuten 1975, Samson et Tansey 1975, Splittstoesser et al. 1989, van der Spuy et al. 1975). Thus, it is nearly certain, that these other species of fungi can take part – besides the known species – in the moulding of canned fruit.

The germs of these heat-resistant fungi get into the environment of food processing factories together with the soil rests which are stuck on fruit. For this, it is necessary to consider – among others – also the sanitation of the environment and equipment in the factories by the use of disinfection solutions.

In the literature we did not find information on the efficacy of disinfection solutions on the germs of heat-resistant fungi. For this reason, in our experiment 8

selected strains of fungi were investigated; these strains were isolated from the soil samples. We have examined the possibilities of devitalization of their propagules 7 various commonly used disinfection substances.

Certain differences in the sensitivity of individual strains and in the efficacy of individual disinfection solutions were shown. The strain *Botryotrichum piluliferum* – whose typical trait is to produce aleuriospores – showed to be the relatively most sensitive. The strains *B. nivea* and *B. fulva* – whose teleomorphic stadium is expressed by the formation of asci and ascospores – and *N. fischeri* strain with formation of kleistothecia, asci and ascospores showed to be the most resistant. Among the disinfection preparations, 1% Chloramin B and 2% Jodonal solutions were less effective, whereas the 0.2% Persteril and 1% Septonex solutions showed to be the most effective. The results were obtained *in vitro*; nevertheless, the effective sanitation of the environment under the operation conditions in food industry can be influenced by many other factors, i.e. not only from the aspect of the interaction between disinfection substances and thermoresistant fungi. In spite of this, the importance of performing disinfection is indisputable.

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