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Tolerance of intersterility group isolates of *Heterobasidion annosum* to low pH and aluminium on solid medium

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Abstract: Tolerance of several strains of the P-, S-, and F- intersterility groups of *Heterobasidion annosum* to low pH and high concentration of aluminium ions was studied on malt extract agar. There were marked intraspecific, but not intergroup, variations in tolerance to both factors. Some strains were relatively tolerant or sensitive to low pH and aluminium, while the others were tolerant to low pH but sensitive to Al. There was no relationship between tolerance of the strains to low pH and their tolerance to aluminium stress.

Additional key words: *in vitro*, plant pathogenic fungi, root rot, variation in tolerance to aluminium and low pH

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Introduction

Heterobasidion annosum (Fr.) Bref. is one of the severe root and butt-rotting pathogens of conifers in the boreal and temperate zones of the Northern Hemisphere (Hodges 1969). In Europe, Heterobasidion annosum consists of three intersterility (IS) groups showing preferential specialization to host trees (Korhonen 1978; Capretti et al. 1999). It is a white-rot fungus causing degradation of lignin, cellulose and other carbohydrates of the wood (Korhonen and Stenlid 1998). Infection starts when germinating air-borne spores or vegetative mycelia enter freshly exposed wood. *H. annosum* infects wounds on roots and stems. In managed forests the fungus spreads from tree to tree via root contacts and grafts (Rishbeth 1950). In Poland, the most severe damage caused by annosum root rot occurs in forest growing on former agricultural soils (Sierota 1995). In vegetative stage *H. annosum* lives almost exclusively in wood. It is a necrotroph in living trees or saprotroph on dead wood. The mycelium of *H. annosum* is rarely exposed to the soil environment. However, its spores may germinate in soil and infect roots of stressed trees (Korhonen and Stenlid 1998). Hyphae of *H. annosum* can also externally colonize root surfaces and grow between bark scales (Werner 1993; Korhonen and Stenlid 1998). This causes the exposure of the fungus to potentially toxic metals in polluted areas.

Aluminium, especially Al^{3+} ions, are toxic to plant roots (Kinraide 1991), and the stress caused by Al increases with high rates of atmospheric acidic depositions (Smith 1987). Risk of infection by *H. annosum* increases, generally, with soil fertility and lime content, specifically in the major rooting zone (Wallis 1960; Schönhar 1969). However, the fungus attacks also trees growing on polluted soils (Grzywacz and Ważny 1973; Domański 1978), where in consequence of low soil pH the availability of of Al^{3+} ions increases. To date, there is no available information

concerning the influence of the elevated aluminium concentration in forest soils on the incidence of the disease.

Although numerous laboratory data exist on the effect of aluminium on higher fungi, its importance under field conditions is still unclear. This raises the question of how, if at all, the exchangeable Al ions influence the fungal community in the field. Thus, there is a need to confront the response of fungi on aluminium stress observed *in vitro* and *in vivo*. Reductionist approaches, however, are useful in qualitative studies on tolerance of fungi to toxic metals.

The objectives of the study were (i) to assess the tolerance of nine strains of *H. annosum* to low pH and high concentration of Al on agar medium; (ii) to find out whether a relation exists between the aluminium and low pH tolerance; (iii) to assess intraspecific variation of this fungus in the tolerance to the both factors.

Material and methods

Nine strains of the three intersterility groups of *Heterobasidion annosum* (Fr.) Bref. were selected for the study. The strains were isolated from dead trees, stumps and logs of *Pinus sylvestris* L., *Picea abies* (L.) Karst. and *Abies alba* Mill., originating from forest stands in Poland. Details of origin of the strains are listed in Table 1.

Isolates were assigned to the P-, S-, and F-IS groups based on their ability to heterokaryotize homokariotic known tester mycelia (Korhonen 1978). The standard sources of the fungi were mycelia growing on malt extract agar MEA (Difco), 30 g l^{-1} malt extract, 15 g l^{-1} agar, at +5°C with transfer about four times a year.

The effect of low pH on the growth of P strains was investigated on MEA at pH 5.0 and 3.5. The S and F isolates were grown at pH 5.0 and 4.0. Medium was acidified after sterilization at 121°C for 20 minutes with sterile 0.1N HCl. Then the media were aseptically poured into Petri dishes (9 cm in diameter) and inoculated in the centre of the dishes with discs (5 mm in diameter) of young mycelial mats. Except for P strains, which were incubated for ten days, the fungi grew for seven days at 25°C in the dark and after this period the radial growth of the fungi was measured according to Eckstein and Liese (1970).

The effect of aluminium chloride ($AlCl_3 \cdot 6H_2O$), pure pro analysi, on fungal growth was investigated under the same conditions. Strains were grown for two weeks on MEA at pH 4.0, with the exception of P strains which grew at pH 3.5, with $AlCl_3$ in the concentrations of 6.0, 7.0 and 8.0 mM. The fungi growing without $AlCl_3$ served as control. The experiments were replicated two times. Each replication consisted of three plates.

Analysis of variance (Anova), Tukey's HSD test, and t-test were conducted using statistical analysis software Statistica PL 1997 (StatSoft Polska Inc., USA).

Results

The radial growth of all the *H. annosum* strains was inhibited at the lower pH, and with the exception of one strain (S-96076) the growth of all the remaining strains was also inhibited in the presence of aluminium (Figs. 1 and 2). The fungi varied significantly in the reaction to the low pH ($p<0.000$) and Al stress ($p<0.000$), but there was no statistically significant intergroup-variation in the tolerance to low pH and Al stress. Strains within the P, S, and F groups varied in their response to low pH ($p<0.000$, $p<0.05$, $p<0.000$, respectively). Similarly, the differences between the strains within each of the group in the response to aluminium stress were also highly statistically significant ($p<0.000$, $p<0.001$, $p<0.001$, respectively). In the case of the effect of low pH, three strains of the P group did not vary at pH 5.0, but varied significantly ($p<0.000$) at pH 3.5. Strain P-95092 was much more sensitive to low pH than the two others (Fig. 1A). Strains within the S group varied in their growth significantly at pH 5.0 ($p<0.01$) and at pH 4.0 ($p<0.000$). Strain S-96076 exhibited a greater tolerance to low pH than the other S strains (Fig. 1B). Radial growth of F strains varied significantly at pH

Table 1. Origin of *Heterobasidion annosum* strains

Isolate No.	IS group	Year of collection	Locality (Forest stand)	Host
P-97067	P	1997	Niepołomice	*P. sylvestris* (stump)
P-95107	P	1995	Podanin, Klotyldzin	*P. sylvestris* (dead young tree)
P-95092	P	1995	Podanin, Klotyldzin	*P. sylvestris* (dead young tree)
S-96076	S	1996	Węgierska Górka, Morońka	*P. abies* (log)
S-96049	S	1996	Nowy Targ	*P. abies* (stump)
S-96043	S	1996	Białowieża	*P. abies* (stump)
F-96067	F	1996	Węgierska Górka, Morońka	*A. alba* (dead tree)
F-96071	F	1996	Węgierska Górka, Morońka	*P. abies* (log)
F-96084	F	1996	Węgierska Górka, Morońka	*A. alba* (log)

5.0 ($p<0.001$), but not at pH 4.0. Growth of strain F-96084 was very slow even in the control (Fig. 1C). Concentration of 10 mM HCl in the medium decreased growth of four sensitive strains below 50% in comparison with the control (Fig.1).

Aluminium chloride added to the medium in similar molar concentration as HCl showed more toxic effect on the fungus (Fig. 2). The lowest level of aluminium (6 mM) reduced the radial growth of six fungi (P-95107, P-95092, S-96049, F-96067, F-96084, F-96071) below 50% in comparison to the control. Strain S-96076 appeared to be the most tolerant to aluminium stress. Relatively tolerant was also strain P-97067. Growth of all the F strains was strongly inhibited by $AlCl_3$ (Fig. 2C).

From the present data we can state that the tolerance to low pH and aluminium stress was not correlated ($r^2=0.006$, $p=0.84$). Some strains were tolerant or sensitive to both aluminium and low pH, while the

Table 2. Various tolerance of *Heterobasidion annosum* strains to low pH and aluminium

Fungi tolerant to low pH and Al	Fungi tolerant to low pH and sensitive to Al	Fungi sensitive to low pH and Al
S-96076	P-95107	S-96043
P-97067	S-96049	P-95092
	F-96084	F-96067
		F-96071

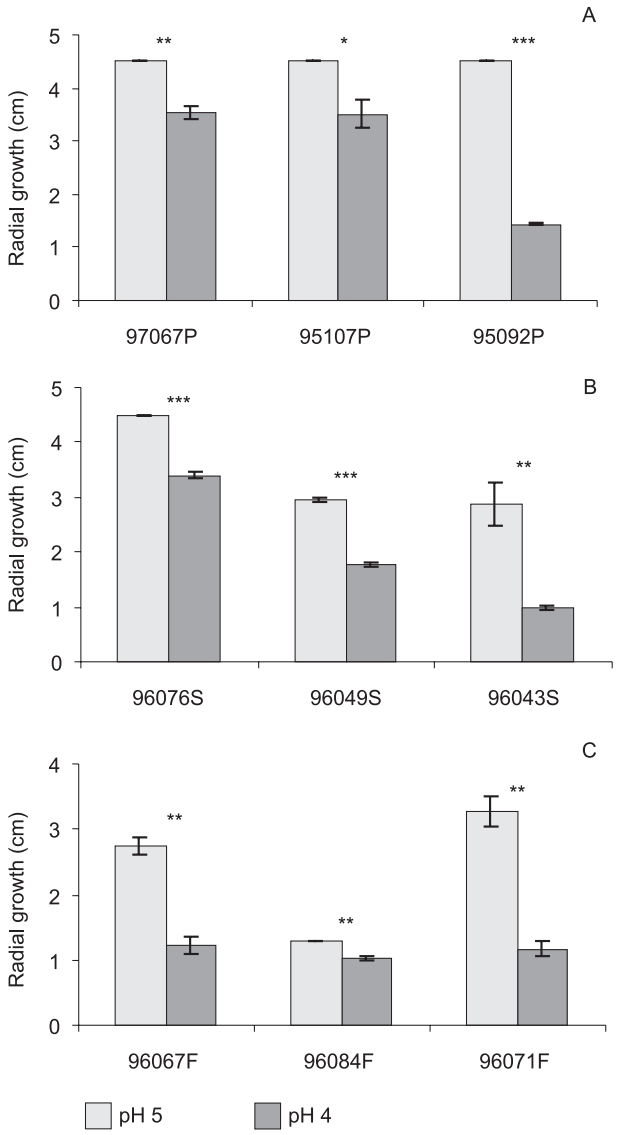

Fig. 1. Radial growth of *Heterobasidion annosum* P-strains (A), S-strains (B) and F-strains (C) on malt agar at two initial pH levels. Means (±SE)
* – $p<0.05$, ** – $p<0.01$, *** – $p<0.01$
according to test t

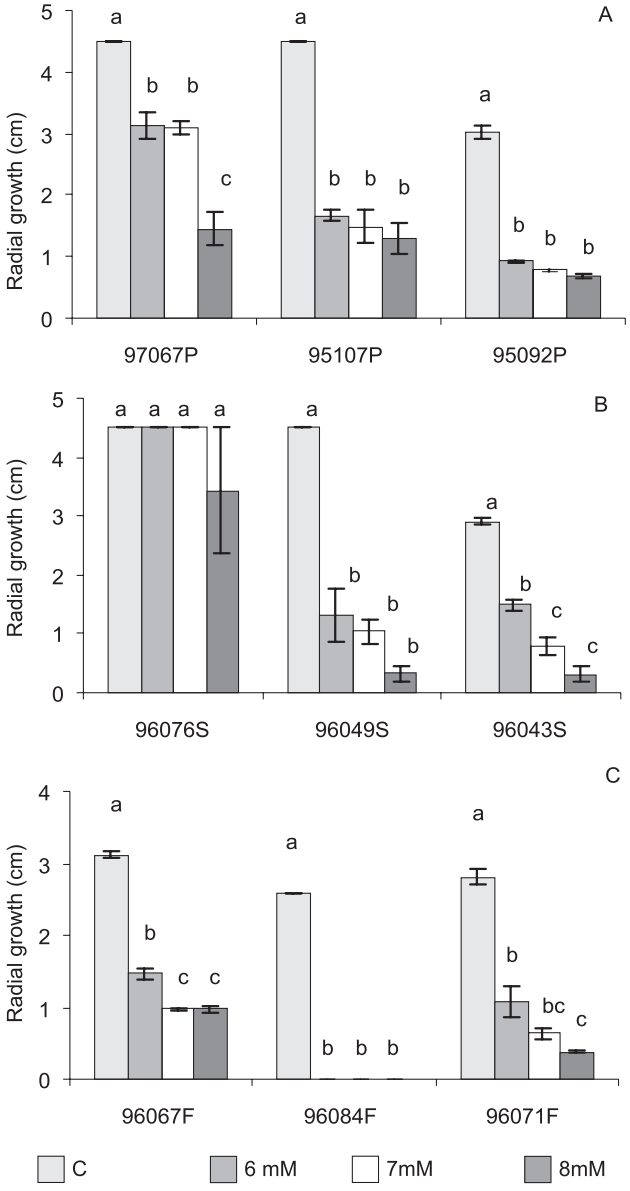

Fig. 2. Radial growth of *Heterobasidion annosum* P-strains (A), S-strains (B) and F-strains (C) after 14 days on malt agar at pH 4.0 to which $AlCl_3 \cdot H_2O$ was added. Means designed by the vame latter did not differ at the 5%level using. Tukey's test for each strain separately

others were tolerant to low pH, but sensitive to Al (Table 2).

Discussion

The results of the study are in an agreement with the investigations concerning metal tolerance of fungi *in vitro*. Blaudez et al. (2000) reported that there is a high inter- and intraspecific variation in metal tolerance among ectomycorrhizal fungi. Most of the studies were focused on variation in the tolerance of the fungi to heavy metals and aluminium (Egerton-Warburton and Griffin 1995; Marschner et al. 1999), and only a few data exist on the effect of toxic metals to root pathogenic fungi (Wargo and Carey 2001). In the present study, *H. annosum* appeared to be more sensitive to Al than the mycorrhizal and saprotrophic fungi regarded as highly tolerant (Hintikka 1988; Kawai et al. 2000). Hintikka (1988) reported additionally that basidiomycetes such as *Fomes fomentarius* (L.) Fr., *Fomitopsis pinicola* (Sw.) Karst., *Phlebiopsis gigantea* (Fr.) Jül., *Piptoporus betulinus* (Bull.) Karst. and numerous saprotrophic fungi were much more sensitive to Al than investigated mycorrhizal fungi.

The effect of pH on growth of *H. annosum* in pure cultures and *in vivo* is well known. *In vitro*, *H. annosum* mycelia grow in a wide range of pH with optimum at 4.0 – 5.7 and some strains show high variation in response to pH. *Heterobasidion annosum* occurs on both acid and alkaline soils, but both high Ca content and pH are favourable to the disease (Korhonen and Stenlid 1998).

In our experiments, the studied strains of *H. annosum* differed in response to pH and aluminium. Inter-, but not intraspecific, variation in response to low pH and high Al concentrations of ectomycorrhizal fungi was described by Jongbloed and Borst-Pauwels (1992). The discussed phenomenon requires more detailed investigations to explain (i) whether the fungal response concerns H^+ and Al^{3+} ions only or Cl^- ions as well, (ii) how Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$ and Al_{13} ions influence fungal growth, (iii) whether two different mechanisms of tolerance to hydrogen and aluminium ions are involved in fungal cells. In our experiments an effect of Cl^- ions was not considered, because in separate experiments Cl^- added as NaCl had no significant effect on the fungus (P-97067) on agar medium (data unpublished). The rhizotoxicity of different aluminium ions was investigated (Kinraide 1991), but the informations on their influence on the fungal growth are scarce. The reasons of various response of *H. annosum* strains to HCl and $AlCl_3$ also remains unknown. Since in our experiments slow growing strains were often more sensitive to low pH and aluminium stress, one can assume that the tolerance to these stress factors may be related to the strain vitality.

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