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Humus Acids as a Nutrition Source for Some White-Rot Fungi

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Abstract. The rate of growth of 5 species of white-rot fungi on an agar medium with additions of humus acids was used for assessing the ability of these fungi to utilize humus acids as a sole source of nutrition. Differences in the effect of the various fractions of humus acids and their concentrations, as well as in the reaction of the fungi tested were observed. During the growth, loss of colour of agar media with humus acids was observed. Activity of laccase in the discoloured areas was highest

Literary data on the ability of microorganisms to utilize humus acids as sources of nutrition are varied. Whereas some authors ascribe this ability to a broad spectrum of microorganisms (NIKITIN 1961, VOLKOVA 1961 and others), other authors (like VINOGRADSKY 1952) restrict it to a specific microflora. Experiments with lower fungi (ALEKSANDROVA 1953, FLAIG and SCHMIDT 1957, KUDRINA 1961), with higher fungi (SUNDMAN et al. 1964), actinomycetes (SCHONWALDER 1958, VOLKOVA 1961) and bacteria (KÜRSTER 1950, NIKITIN 1960, MRYSHA 1966) showed that humus acids can be utilized by these organisms as the principal source of nutrition only to a limited extent.

The present study is devoted to the growth of white-rot fungi on media containing different concentrations of humic acid, hymatomelanic acid, fulvic acid and residual substances. Simultaneously, the production of laccase was examined. It is probably that this enzyme takes part under certain conditions in the decomposition and synthesis of humus like substances (TROJANOWSKI 1962, SCHÁNĚL 1962).

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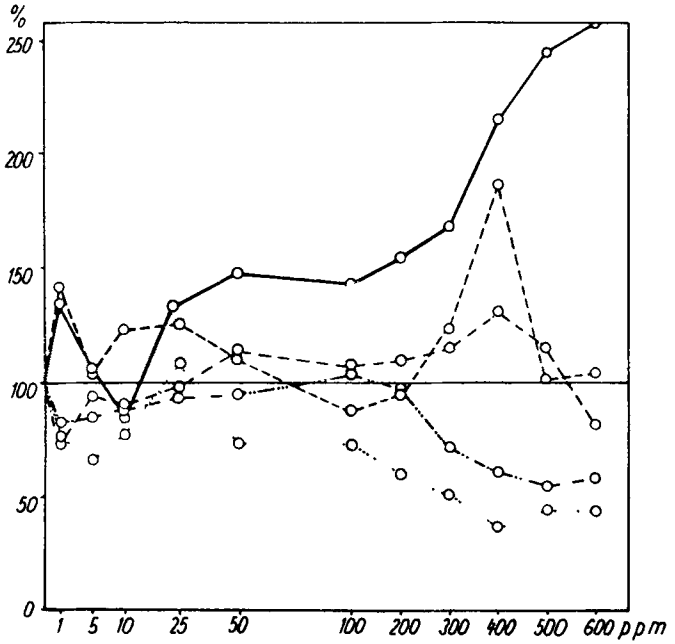
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Fig. 1.

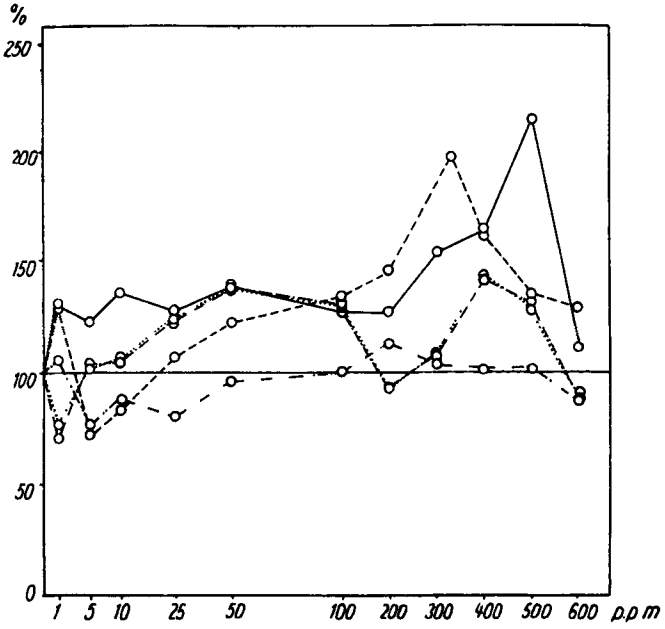
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Fig. 2.

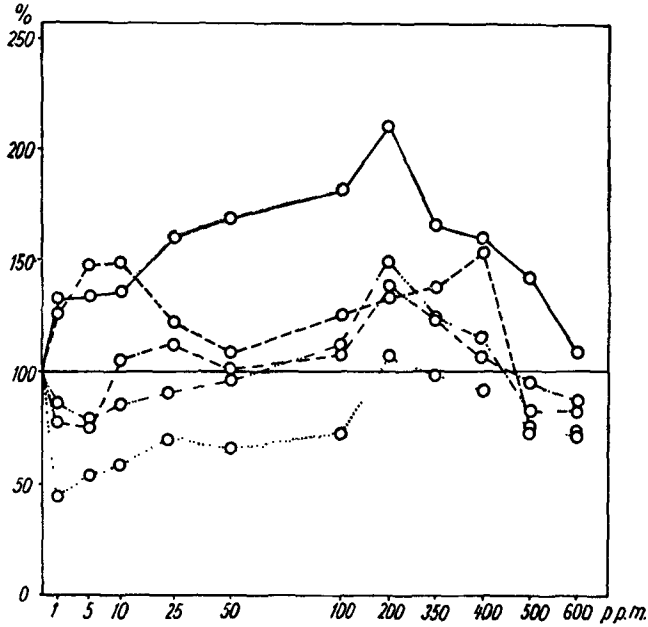

Fig. 3.

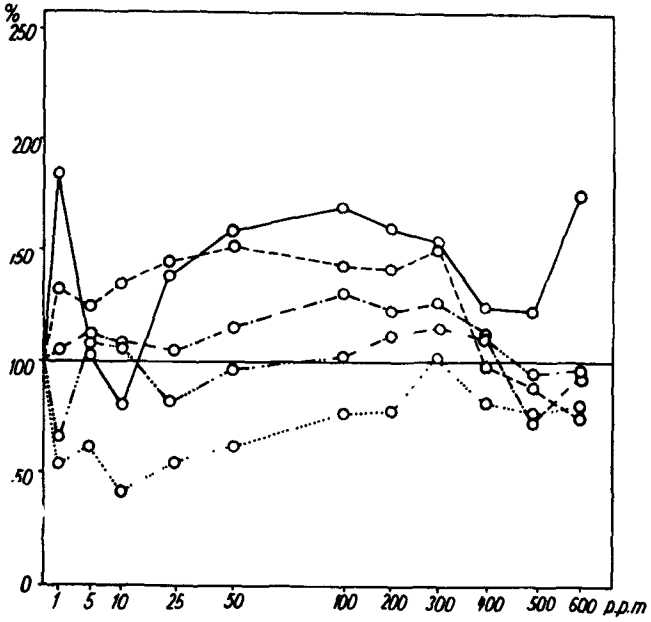

Fig. 4.

Fig. 1—4. Dependence of the growth rates of *Stereum hirsutum* — — —, *Inonotus hispidus* —.—.—.. *Pleurotus ostreatus* ————, *Trametes gibbosa* —...— and *Trametes hirsuta* — — — —, on the concentration of humic acid (1), hymatomelanic acid (2), fulvic-acid (3) and the residual substances (4). Abscissa: amount of humus acids in mg/l; ordinate: growth rate in % of control.

Material and Methods

For the experiments we used the white-rot fungi *Stereum hirsutum* (WILLD.) GRAY, *Inonotus hispidus* (BULL.) KARSTEN, *Pleurotus ostreatus* (JACQ.) FRIES, *Trametes gibbosa* (PERS.) FRIES, *Trametes hirsuta* (WULF.) PILÁT, from the collection of pure cultures of wood-rotting fungi of the Laboratory of Plant Physiology, J. E. Purkyně University, Brno.

The humus acids, i.e. humic, hymatomelanic, and fulvic acids and the residual substances were isolated from beech wood (decomposed in vivo by the cellulose-digesting fungus Fomes marginatus) according to the procedure of TICHÝ (1961).

The white-rot fungi were grown on 3% aqueous agar with additions of individual humus acids in concentrations of 1, 5, 10, 25, 50, 100, 200, 300, 400, 500 and 600 mg/l. The pH value of the nutrient medium was between 6·5 and 6·8. Petri dishes with the nutrient medium were inoculated by a square inoculum of the fungi tested which was removed from a marginal part of a colony grown on 3% malt agar; 3% aqueous agar served as control. All the fungi were grown at their optimal temperatures: *Stereum hirsutum* and *Pleurotus ostreatus* at 25° C, *Inonotus hispidus*, *Trametes gibbosa* and *Trametes hirsuta* at 28° C. The growth of the individual cultures was examined only after the fourth transfer on the corresponding medium. Beginning with the third day of cultivation, the increment of every culture in mm was registered daily for ten days. Each experimental variant was run in three replicates. From the values obtained the growth rate of the individual cultures in $\mu m \cdot h^{-1}$ was calculated.

After a 13-day growth of the fungi on the media tested an oxidase test according to TICHÝ and KLABANOVÁ (1962) was carried out.

Results and Discussion

The values of growth rates of the white-rot fungi studied differed depending on the type of acid employed and on its concentration. The differences depend on the fungus species (Table 1). To compare mutually the growth rates of the individual fungi, they were referred to the control represented by 3% aqueous agar medium (Fig. 1—4). A positive effect of most concentrations of humus acid fractions was found with *Pleurotus ostreatus*, *Stereum hirsutum* and *Inonotus hispidus*. An insignificant effect could be observed with *Trametes gibbosa* while with *Trametes hirsuta* the effect was rather one of inhibition.

The curves of dependence of the biological effect of humus acids on their concentration rarely follow typical toxicity curves. The stimulation phase is generally rather broad and passes over to a gentle inhibitory effect.

In the course of a 13-day cultivation of fungi on media with additions of

Table 1
Growth rates of white-rot fungi in μm/h

Fungus		Concentration of humus acids in mg/l^{-1}										
		1	5	10	25	50	100	200	300	400	500	600
Stereum hirsutum	HU	450	333	391	400	350	279	300	394	587	347	333
	HY	412	225	266	345	385	425	462	591	512	433	408
	FU	420	470	471	387	345	400	421	438	478	233	232
	Re	418	400	437	458	475	460	454	479	312	279	242
Inonotus hispidus	HU	200	261	250	273	308	300	304	321	362	320	225
	HY	200	283	291	340	375	357	258	295	390	358	247
	FU	215	210	291	312	279	300	383	341	295	266	244
	Re	291	310	300	302	317	361	341	352	312	206	262
Pleurotus ostrateus	HU	425	333	271	416	441	429	462	525	679	766	812
	HY	407	383	429	400	379	404	375	479	500	666	358
	FU	415	420	425	504	529	566	660	521	500	441	343
	Re	579	320	250	431	500	528	500	483	390	387	550
Trametes gibbosa	HU	433	450	466	490	500	550	504	383	325	304	309
	HY	557	408	462	420	504	525	577	541	533	530	466
	FU	423	420	441	479	512	591	745	654	612	445	445
	Re	354	342	332	424	511	537	600	616	587	500	512
Trametes hirsuta	HU	375	341	395	550	390	371	270	258	200	226	200
	HY	395	321	371	887	400	415	395	340	325	331	321
	FU	231	268	295	350	333	366	550	500	458	311	375
	Re	270	312	210	270	311	395	402	512	412	395	408

HU = Humic acid, HY = hymatomelanic acid, FU = fulvic-acid, Re = residual substances

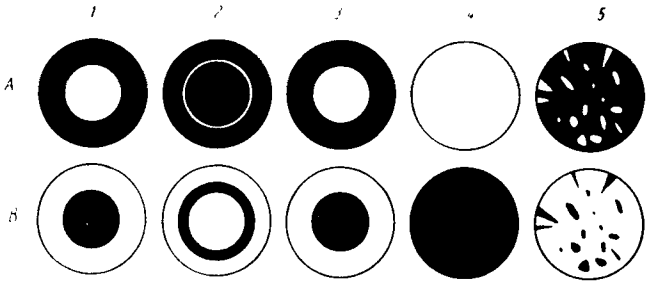

Fig. 5. Types of discoloration of an agar medium with additions of humus acids (series A) and types of guaiacol tests (series B) with *Stereum hirsutum* (1), *Inonotus hispidus* (2), *Pleurotus ostreatus* (3), *Trametes gibbosa* (4) and *Trametes hirsuta* (5).

humus acids a loss of colour of the medium was observed five or six days after inoculation (in the case of humic and hymatomelanic acids) or seven to eight days after inoculation (in the case of fulvic acids and of the residual substances).

On the basis of evaluating an average of 180 plates for each fungus the nature of the colour loss could be estimated. Agar media with humus acids were discoloured always in the same fashion for a given fungus species. *Stereum hirsutum* discoloured the area below the growing mycelium and 30—40 mm around the fungus colony (Fig. 5, A1). A similar situation was obtained with *Pleurotus ostreatus* (Fig. 5, A3). The nutrient medium with a growing mycelium of *Inonotus hispidus* was discoloured in the form of a ring near the edge of the mycelium (Fig. 5, A2), with *Trametes hirsuta* there were colourless spots and streaks (Fig. 5, A5), with *Trametes gibbosa* the nutrient medium below and in front of the growing mycelium was discoloured (Fig. 5, A4).

The loss of colour of humus acids due to the action of various microorganisms is usually considered as indirect an evidence of their decomposition (THIELE and ANDERSON 1953, BURGES and LATTER 1960, MISHUSTIN and NIKITIN 1961, FEDOROV and ILYINA 1963). The finding that the loss of colour of humus acids is an oxidative process (HURST and BURGES 1967) permits us to evaluate to a certain extent the discolouration of the nutrient medium in our experiments in connection with the results of oxidase tests. In places where the medium was discoloured, the oxidase test was highly positive, the activity of laccase was highest (Fig. 5, series B). One can thus assume a certain relationship between the loss of colour of humus acids and laccase activity which is excreted by the white-rot fungi into the medium and represents one of the important components of oxidation-reduction enzyme systems.

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Kyseliny huminová, hymatomelanová, fulvokyselina a zbytkové látky, představující jediný zdroj výživy, ovlivňovaly většinou positivně růst lignivorních hub *Pleurotus ostreatus*, *Stereum hirsutum* a *Inonotus hispistus*, nevýrazně působily na růst *Trametes gibbosa* a spíše inhibičně na růst *Trametes hirsuta* Během růstu hub se charakteristicky pro každou houbu odbarvovalo médium s přídavky humusových kyselin. V místech odbarvení byla zjištěna nejvyšší aktivita lakkásy.

Марта Тесаржова, Л. Сханел, Ботанический институт ЧСАН, Брно, Лаборатория физиологии и анатомии растений университета им. Я. Е. Пуркинье, Брно: **Гуминовые кислоты как источник питания для некоторых грибов вызывающих белую гниль древесины.** — Biol. Plant. **10** : 135—141, 1968.

Гуминовая, гиматомелановая кислоты, фульвокислота и остаток, в качестве единственного источника питания, влияли в большинстве случаев положительно на рост грибов вызывающих белую гниль древесины *Pleurotus ostreatus*, *Stereum hirsutum* и *Inonotus hispidus*, но имели не очень выраженное влияние на рост *Trametes gibbosa* и скорее тормозили рост *Trametes hirsuta*. Во время роста грибов происходило обесцвечивание агаровой среды с гуминовыми кислотами способом, характерным для отдельных видов. В местах обесцвечивания установлена наибольшая активность лакказы.