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EVALUATION OF THE ALLERGENICITY OF SPORE AND MYCELIA EXTRACTS OF *Pisolithus tinctorius*

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SUMMARY

The antigenic and allergenic chemical analysis of spore and mycelia extracts of *Pisolithus tinctorius* was carried out. The spores were collected from basidiocarps in plantations of *Eucalyptus* spp and the mycelia from culture in MNM medium. With basis on the fungus growth curve, the mycelia masses were obtained after 10, 20, 30, and 40 days of incubation, which correspond, respectively, to the beginning, middle and end of the log phase, and beginning of the decline phase. The mycelia masses, together with the spores, were submitted to the action of three extractors (Coca, Tris-HCl, and ammonium bicarbonate). The contents of carbohydrates and proteins were determined. The SDS-PAGE electrophoretical analysis revealed separate fractions in these extracts, besides common fractions, in function of cultivation time and extraction methods. The selected extracts for the allergic tests were the ones with the highest number of fractions. The prick-tests were conducted in 374 patients – rural workers, eucalyptus plantation workers, and college students. The positivity to the "prick test" with the antigenic extract of *P. tinctorius* was, respectively, 3.78%, 28.20% and 6.40%. Most prick-test positive patients (82.75%) also presented symptoms of respiratory allergy (asthma and rhinitis). There was no reactivity difference when the spore and mycelia extracts were employed. The analysis of the positive patients' sera revealed the presence of IgE specific to the *P. tinctorius* antigens. Since *Pisolithus tinctorius* is found as mycorrhiza of *Eucalyptus* spp, and this plant is used in reforestation in most countries, the importance of that fungus should be regarded as a possible cause of respiratory allergies, especially in occupationally exposed workers.

KEYWORKS: *Pisolithus tinctorius*; Basidiomycetes; Mold allergens.

INTRODUCTION

WITTCH & STACKMAN (1937) were the first to observe a bronchospasm by inhalation of a basidiomycete. From 1967 on, SALVAGGIO & KLEIN (1967) and later SALVAGGIO et al. (1971) reported massive outbreaks of asthma related to high concentrations of basidiospores in the air of New Orleans (USA), demonstrating that 1/4-1/3 of the patients with bronchospasm exhibited skin tests positive to basidiospores.

The antigenicity and allergenicity to basidiomycetes was first shown by using extracts from culture mycelia and filtrates, both in humans and animals (HERSHEIMER et al., 1969; GIANNINI et al., 1975; LOPEZ et al., 1976). Further researches showed skin reactivity to spore extracts of some basidiomycetes, such as *Agrocybe amara, Amanita muscaria, Armillaria tabescens, Cantharellus cibarius, Chlorophyllum molybdites, Coprinus quadrifidus, Pleorotus ostreatus, Psilocybe cubensis, Boletinellus merulioides, Boletus* sp, *Ganoderma lucidum, Inonotus ludovicianus, Calvatia cyathiformis* and *Pisolithus tinctorius* (GIANNINI et al., 1975; HASNAIN et al., 1985; LEHRER et al., 1986; SPRENGER et al., 1988; HORNER et al., 1993).

The ectomycorrhyzic fungus *Pisolithus tinctorius* (Persoon) COKER & COUCH (1928) has a wide geographic distribution, associating mutualistically with different plants and benefiting them by providing a higher absorption of nutrients.

The *P. tinctorius* spore allergenicity was demonstrated by several authors. DE ZULBIRIA (1990) showed that this fungus presents antigens which are common to other basidiomycetes,

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and that 7%-9% of the individuals with allergic symptoms were positive to the "prick-test" with spore extracts. By using various extraction methods, LIENGSWANGWONG (1987) verified that the allergenicity to basidiomycete extracts changed with the methodology, and that the treatment of the spores with ether to remove fats resulted in the extraction of more allergens, including the *P. tinctorius* spores. In studies accomplished in Europe and USA, LEHRER (1994) found that 5.4% of the patients with symptoms of respiratory allergy (rhinitis, asthma) were positive to *P. tinctorius* spore extracts. In Brazil, there is no report concerning the allergenicity of such basidiomycete. Due to reforestation in Brazil being basically made with *Eucalyptus* spp and *Pinus* spp, normally ectomycorrhized by *P. tinctorius*, it is of great importance to determine the patients sensitized to the fungus in normal populations and in those occupationally exposed.

MATERIAL AND METHODS

Obtainment of the "in vitro" culture of *Pisolithus tinctorius*

Basidiocarps of *P. tinctorius*, preferably small and ones, were collected from *Eucalyptus* spp plantations in Alfenas – MG, in the January-April period. In the laboratory, after being externally cleaned to prevent internal contamination, they were hand-opened, without using any cutting instrument. Internal fragments of the basidiocarps were aseptically collected, inoculated in the MNM solid medium (MARX, 1969), and incubated at 30° C, for the cultures to be daily observed. Upon appearing, the colonies were transferred to other culture plaques to avoid contamination.

Obtainment of the *Pisolithus tinctorius* growth curve

The plaques with the MNM medium were inoculated and incubated at 30° C for 15 days. After growth, colony fragments of uniform size were collected by means of a metal tube (punch) and three of the fragments were inoculated in trial balloons containing 20 mL of MNM liquid medium. The cultures were inoculated in 24 balloons and, every 5 days, filtered and dried for the obtainment of the dry weight. These cultures were then incubated at 30° C, under agitation. The data obtained after 40 days allowed the plotting of the growing curve.

Obtainment of the antigenic extracts

Obtainment of the spore extracts - One gram of spores obtained from the opening of *P. tinctorius* basidiocarps was placed into a test tube, and twice the volume of ethilic ether were added for the removal of lipids.

After 20 minutes, the tube was centrifuged and the supernatant discarded. After ether evaporation at 37° C, the spore mass was placed in the extractor liquids Coca, Tris-HCl (OLIVEIRA LIMA, 1970) and ammonium bicarbonate (O'NEIL, 1988) at a 1:10 ratio (p/v), under agitation at 4° C. The time of contact with the extractor liquid was 24 hours for Tris-HCl and ammonium bicarbonate, and 7 days for the Coca, after which the suspension was centrifuged and dialyzed, against 0.9% physiological saline, and lyophilized.

Obtainment of mycelia extracts - *P. tinctorius* cultures were obtained after 10, 20, 30, and 40 days of incubation, which correspond, respectively, to the beginning, middle and end of the log phase, and beginning of the decline phase. The fungic mass was filtered by a nylon screen and thoroughly washed to remove the culture medium residues.

After washing, the fungic mass was dehydrated at 37° C for 48 hours and powdered in a mortar. The powder was weighed and placed in a tube, as anteriorly proceeded with the spores, and were so characterized:

– Coca extractor liquid – E-Coca (spores); Coca 10 (10-day culture); Coca 20 (20-day culture); Coca 30 (30-day culture); Coca 40 (40-day culture).

– Tris-HCl extractor liquid – E-Tris (spores); Tris 10 (10-day culture); Tris 20 (20-day culture); Tris 30 (30-day culture); Tris 40 (40-day culture).

– Ammonium bicarbonate extractor liquid – EBA (spores); BA 10 (10-day culture); BA 20 (20-day culture); BA 30 (30-day culture); BA 40 (40-day culture).

Biochemical analysis - The protein contents of the spore and mycelia extracts were measured by the method of LOWRY (1951), the carbohydrates by the method of DUBOIS (1956), and total lipids by the method of FRINGS (1972).

SDS-PAGE electrophoresis - The above extracts were analyzed by 12% polyacrylamide gel electrophoresis, according to NICHOLS et al. (1986) in the "Mini Protean II dual lab cell" apparatus (Bio-Rad, USA) and stained with Comassie Blue and silver.

Test for the extract allergenicity

The selected extracts (Tris 10, Coca 10 and E-Coca) were tested in volunteers – 203 college students, 132 rural workers, and 39 eucalyptus plantation workers. The "prick test" technique was used with distilled water and extractor liquids as negative control, and 1:10,000 histamine chloride solution (Sigma) as positive control.

The evaluation of positivity to the "prick test" was made after 20 minutes of extract application, being compared to the positive control (1:10,000) with a 3mm (diameter) or greater (CROCE PORTOCARRERO, 1995).

Western Blotting

The *P. tinctorius* extracts (E-Coca, Coca Tris and Tris 10) were submitted to SDS-PAGE electrophoresis, and the gel, after the run, was transferred to a nitrocellulose membrane in the Mini Trans-Blot apparatus with the respective BioRad (USA) technique. After the transfer, the nitrocellulose membrane strips were placed in contact with the patients'sera, and the presence of IgE was revealed with peroxidase-labelled anti-IgE (Sigma).

RESULTS

P. tinctorius growth curve

In the growth curve (Figure 1), the Log phase extends to the 5th day, wherefrom the Log phase starts and extends to the 30th day. The decline phase is observed starting from the 30th day.

Based on the results obtained from the growth curve (Figure 1), extracts were obtained from cultures on days 10, 20, 30, and 40, which corresponded to distinct plots on the curve, in order to verify possible qualitative and quantitative variations in their chemical and antigenic composition.

Biochemical analysis of the extracts

Table 1 shows that the higher quantity of protein was observed in the extracts from 10-day-incubation cultures with all the extractor liquids. The spores showed higher contents than the mycelia. With Coca liquid and ammonium bicarbonate there was no significant difference between the extracts obtained from cultures with different incubation times. But with Tris-HCl, the protein contents were higher.

The carbohydrate dosage followed the same result patterns of the proteins, i.e., the spore extracts obtained with Tris-HCl showed the highest carbohydrate concentration. The use of ammonium bicarbonate produced extracts with lower carbohydrate contents. In both protein and carbohydrate dosage, as shown by the growth curve, their contents decreased from the log phase, becoming twice or three times lower at the end of the curve.

No lipid was detected by the method used.

SDS-PAGE electrophoresis of the spore and mycelia extracts obtained with the respective extractor liquids.

TABLE 1
Biochemical analysis of the extracts obtained from *P. tinctorius* spores and mycelia.

Extract	protein dosage (mg/mL)	carbohydrate dosage (mg/mL)
E-Coca	6.7	19.42
Coca 10	1.4	4.14
Coca 20	0.6	3.85
Coca 30	0.5	2.14
Coca 40	0.4	2.00
E-Tris	18.0	21.42
Tris 10	2.0	4.28
Tris 20	0.7	2.85
Tris 30	0.6	2.14
Tris 40	0.5	2.00
EBA	6.0	14.28
BA 10	1.3	1.57
BA 20	0.8	1.42
BA 30	0.7	1.42
BA 40	0.5	1.28

Spore extracts obtained with the extractor liquids Coca, Tris-HCl and ammonium bicarbonate:

The spore extract electrophoresis revealed no different bands in the three extracts obtained, respectively, with the liquids Coca, Tris-HCl and ammonium bicarbonate. They all

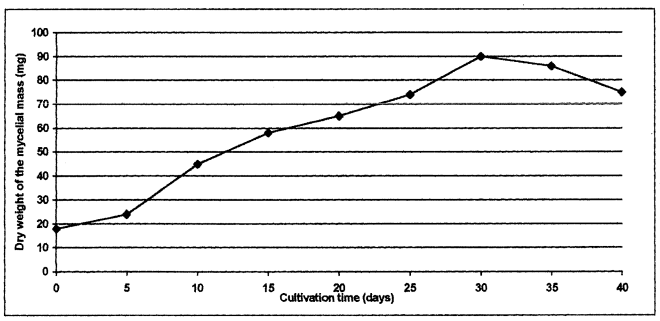

Fig. 1 – *Pisolithus tinctorius* growth curve in MNM medium, observed for 40 days at 30°C.

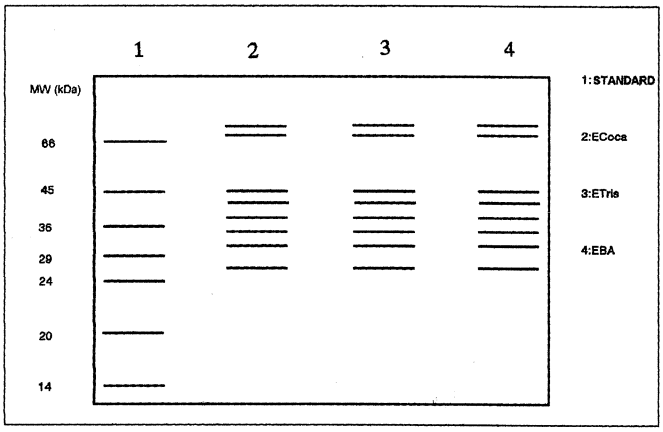

Fig. 2 – SDS-PAGE electrophoresis of samples of *P. tinctorius* spore extracts with the extractor liquids Coca (Ecoca), Tris-HCl (Etris) and ammonium bicarbonate (EBA).

exhibited eight bands with molecular weights ranging from 26.94 kDa to 76.65 kDa. The extracts obtained with Coca were chosen for the tests.

Mycelia extracts obtained with the extractor liquids Coca, Tris-HCl and ammonium bicarbonate on the growth days 10, 20, 30, and 40:

With the mycelia extracts, and the extractor liquids Coca and ammonium bicarbonate, it was shown that all the bands were similar, and only one band more with 20.96 kDa (Coca 10) with 10 days of incubation, also found with ammonium bicarbonate (BA 10). The "prick test" with Coca 10 extract was chosen. As regards the Tris-HCl, the extracts obtained from the different culture times showed the same bands, with exception of the Tris 10, where one band more was detected (26.75 kDa). This extract was chosen for the test. The bands with the respective molecular weights 63.79 kDa, 57.24 kDa, and 30.22 kDa were found in all the extracts.

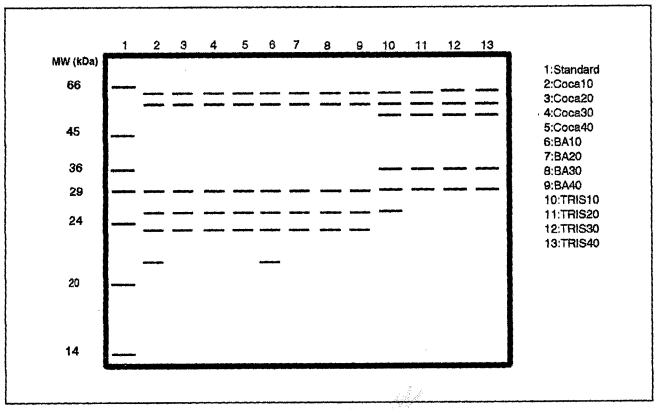

Fig 3 – SDS-PAGE electrophoresis of samples of *P. tinctorius* mycelia extracts in various growth stages (10, 20, 30, and 40 days) with the extractor liquids Coca (Ecoca), Tris-HCl (Etris) and ammonium bicarbonate (EBA).

The extract allergenicity evaluation test

The following extracts were chosen for the test, in accordance with the protein SDS-PAGE electrophoresis analysis:

TABLE 2

Distribution of the results of the "prick-test" evaluation, using *P. tinctorius* extracts according to sex and occupation.

	College Student prick test			**Rural Workers prick test**			***Eucalyptus* spp plantation workers prick test**		
Sex	positive	negative	total	positive	negative	total	positive	negative	total
Male	02	71	73	04	109	113	11	28	39
Female	11	119	130	01	18	19	0	0	0
Total	13	190	203	05	127	132	11	28	39
(%)	(6.4)	(93.6)	(100.0)	(3.8)	(96.2)	(100.0)	(28.2)	(71.8)	(100.0)

TABLE 3

Distribution of the positivity to the "prick-test" with the *Pisolithus tinctorius* extracts, according to the presence or absence of some allergic symptoms in college students, rural workers and *Eucalyptus* spp plantations workers.

	Reactivity to the prick test								
Symptoms	Positive				Negative				Total
	Group 1	Group 2	Group 3	Total	Group 1	Group 2	Group 3	Total	
Asthma	4	5	4	13	6	6	2	14	27
Rhinitis	5	0	5	10	25	18	4	47	57
Asthma and rhinitis	1	0	0	1	10	3	0	13	14
No symptoms	3	0	2	5	149	100	22	271	276
Total	13	5	11	29	190	127	28	345	374

Group 1 – College Students
Group 2 – Rural workers
Group 3 – *Eucalyptus* spp plantation workers

• spore extracts obtained with Coca (Ecoca);

• mycelia extracts obtained after 10 days of incubation with Coca (Coca 10) and Tris-HCl (Tris 10).

The extracts were concentrated for 2 and 4 mg of proteins/mL. The pre-tests have shown that the ideal protein concentration is 4 mg/mL.

The data from Table 2 show that 13 (6.4%) of the 203 student patients and 5 (3.7%) of the 132 rural workers were positive to the "prick test". Among the *Eucalyptus* spp plantation workers, the positivity was 28.2% in 39 patients.

Table 3 shows that 29 out of 374 patients tested were "prick test" positive. From the positive patients, 24 had allergic symptoms.

Among students, the sex-related positivity was shown in 2 males (2.73%) and 11 females (8.48%). Among the 113 male rural workers, 4 (3.53) males were positive, while one (5.26%) female patient was positive among 19 patients tested.

All the patients from the *Eucalyptus* spp areas were male.

The "prick test" positive patients showed a simultaneous reaction to the three tested extracts (Ecoca, Coca 10, and Tris 10).

With respect to the patients'occupation, no significant difference was found through the test of proportion comparison ($P > 0.05$), while 6.4% were found among students, and 3.8% among rural workers (Table 2).

The comparison between rural and *Eucalyptus* spp area workers, and between college students and *Eucalyptus* spp area workers, shows significant differences according to the "prick-test" positivity ($p < 0.001$).

Table 3 shows that all the rural workers, 76.9% of the college students, and 81.2% of the *Eucalyptus* spp area workers, who were reactive to the "prick-test", presented respiratory allergy symptoms (asthma and rhinitis).

The statistical analysis revealed no significant difference between these groups ($p > 0.05$).

Western Blotting

The Western Blotting technique, with the extracts Ecoca, Coca 10 and Tris-HCl 10, revealed the presence of IgE reactive against protein fractions of the extracts in the sera of the patients who were reactive to the "prick-test".

The analysis with the Coca 10 extract allowed the individualization of three IgE-reactive fractions, with molecular weights of about 63.79 kDa, 57.24 kDa, and 30.22 kDa, respectively. Three IgE-reactive fractions also appeared with respect to the Tris-HCl extract, with the approximate molecular weights of 63.79 kDa, 57.24 kDa, and 51.83 kDa. The greatest number of IgE-reactive fractions was observed with the Ecoca extract. Six reactive antigenic fractions were found, with molecular weights of about 44.51 kDa, 40.25 kDa, 36.58 kDa, 33.39 kDa, 30.57 kDa, and 26.94 kDa.

IgE antibodies, reactive to the same fractions of each fungus extract, were demonstrated in all the sera of this study.

DISCUSSION

In Brazil, the occurrence of *P. tinctorius* in plantations of *Eucalyptus* spp and *Pinus* spp has been very frequently reported (YOKOMIZO, 1981; SCHWAN, 1984; MARX, 1977). BARROS et al. (1978) reported that, in Brazil, more commonly in the Center-East and East regions, the fungus genera associated with *Eucalyptus* spp are: *P. tinctorius* and *Scleroderma* sp. In Minas Gerais, the natural occurrence of *P. tinctorius* in the eucalyptus plantations is common, including in Alfenas, a southern town of the State.

The *in vitro* culture of *P. tinctorius* in MNM medium (MARX, 1969) for 40 days at 30° C showed that the log phase of growth occurs between the 6th and 30th day. In order to verify the differences in the biochemical and antigenic composition of *P. tinctorius*, extracts were obtained on the 10th, 20th, 30th and 40th day of incubation, which correspond, respectively, to the beginning, middle and end of the log phase, and start of the decline phase.

A similar growth was obtained by PRADELLA (1990), using the same fungus and the cultivation conditions described above. MARQUES (1997) demonstrated, in cultures of *Pleurotus ostreatus*, that the highest contents of carbohydrates and proteins were determined after 40 days of cultivation.

It is known that the cultivation time of micro-organisms may generally interfere in their chemical composition and metabolic products, as well as in their biological properties. That is the reason why the above mentioned cultivation times were used.

In the preparation of fungus allergenic extracts, the material source is critical. In basidiomycetes, the extracts prepared from triturated total basidiocarps result in a low quality product, whereas those obtained from only spores have a higher quality or better reactivity (LOPEZ, 1976; SANTILLI, 1985 and 1990). The patients'sensitisation normally occurs with the inhalation of spores.

The spore extracts used in this study were obtained from the opening of basidiocarps apparently free from contaminating material and, thus having a higher quality.

As to the extraction methods, three extractor liquids were used (Coca, Tris-HCl and ammonium bicarbonate) to detect possible antigenic differences between the extracts obtained by

different techniques. The posterior analysis by the Western Blotting method, using anti-IgE conjugates, may demonstrate possible variations.

The chemical analysis of the extracts have not revealed detectable levels of lipids, and this may be due to the extraction method, using organic solvents such as ether, which indirectly favours a higher extraction of allergens (LIENGSWANGWONG, 1987). The treatment with this solvent also removes interferers, as observed by CROCE PORTOCARRERO (1995), who detected levels of lecithin in the *Hemileia vastatrix* spores, what might contribute to the appearance of unspecified reactions.

The spore extracts were shown to have higher protein contents when compared to those obtained from mycelia, what was similar to the results obtained by MARQUES (1977) in *Pleurotus ostreatus.*

In spite of the Coca liquid having produced less protein extraction than the Tris-HCl, we chose the former for being widely used by allergists. No significant differences were found in the mycelia extracts obtained by the three extraction methods, but the protein contents were higher at the start of the Log phase at 10 days of cultivation (Table 1).

In the *Hemileia vastatrix* spore extracts, obtained at the 10% concentration (p/v), CROCE PORTOCARRERO (1995) found protein concentration of about 0.087 mg/mL with Coca, and about 0.042 mg/mL with Tris-HCl.

With an extraction method similar to that of CROCE PORTOCARRERO's (1995), we have detected higher contents than those mentioned by this author.

The carbohydrate contents found in the extracts obtained by the Coca and Tris-HCl methods were very similar, but sensitively lower than the ones obtained with ammonium bicarbonate.

CROCE PORTOCARRERO (1995) found carbohydrate contents of about 0.931 mg/mL in the spore extracts of *Hemileia vastatrix*, with both Coca and Tris-HCl, what is comparable to the results of the present study.

The total protein contents present in an extract are not always equivalent to the quantity of allergens, once the allergenic potency may vary between extracts with the same protein contents (LOMBARDERO, 1986), what suggests that the carbohydrate fraction plays a relevant role in the allergenic potency.

The literature has reported protein concentrations which are much higher in the spores than in the mycelia (MENEZES, 1992; QUINCE, 1992; MARQUES, 1997), what is in agreement with the results of this study.

The protein concentration of the extracts was previously tested at the 2 and 4 mg/mL concentrations and the reaction found to be comparable to the 1:10,000 histamine control was 4 mg/mL, as suggested by LEHRER et al. (1986, 1994).

The extracts submitted to SDS-PAGE analysis showed significant differences between the various growth stages of the fungus, as well as between those obtained by different extraction processes.

The *Alternaria* sp allergenic extracts, obtained from spores and mycelia, showed differences in intensity and number of bands when submitted to the same analysis technique (PARIS et al., 1990), what is comparable to our results, where it was found that the mycelia exhibited minimal differences with the Coca and ammonium bicarbonate liquids; however, extracts obtained with Tris-HCl presented two bands with molecular weight of 27.75 kDa and 30.22 kDa, respectively, uncommon to other extracts.

Despite the appearance of these bands, there were no reactivity differences among the patients when the extracts were used in the "prick test", suggesting that these patients probably were not specifically sensitized by them, or the proteins were not allergenic.

As regards the spore extracts, the SDS-PAGE analysis showed no qualitative differences between the methods, once the bands were common to the three extracts.

The fractions Coca 10, Tris 10 and Ecoca were chosen on account of presenting a few fractions which were not common to the other extracts.

The spore bands were compared to the mycelia ones, and it was found that those weighting about 30 kDa were common to both spore and mycelia extracts with the different extractor liquids. However, those with molecular weight of about 26 kDa were found in the mycelia extracts with Tris-HCl, after 10 days of incubation (Tris 10).

"Prick-test" positive patients had similar reactions with the same intensity for the three extracts. Some fractions, such as the ones above mentioned, are probably common to all the extracts. According to LOPEZ (1976) and GAMBALE (1988), the basidiomycetes allergenicity may occur with both the spores and the mycelia, and it is common to observe cross reactions between them, in the same genus, and the same species.

According to WEISMANN et al. (1987), spore and mycelia extracts of *Pleurotus* sp share antigenic determinants, but some antigenic fractions are found only in the spore extracts. Several authors have suggested, or even demonstrated, the cross allergenicity between members of various fungus species (O'NEIL, 1988; BURGE, 1985).

Aqueous extracts from selected species of basidiomycetes and deuteromycetes were evaluated (O'NEIL, 1988) as to the presence of shared antigenic determinants, by the "prick test"

and RAST inhibition test. In this study, significant cross reactions were found between various fungi. Though the cross reactivity exists, their clinical significance has not been clearly explained (O'NEIL, 1988).

The data previously described suggest that antigens representative of the largest fungus groups (basidiomycetes and deuteromycetes) should be used for the diagnosis of fungic allergies, due to the antigenic similarity of such groups (O'NEIL, 1988).

In the extract allergenicity evaluation tests carried out with students and rural workers, positivity was observed in both sexes, but more often in women. These data, however, when submitted to the tests of proportion comparison did not present statistically significant differences ($P > 0.05$), as shown in Table 2.

In the *Eucalyptus* spp area workers'group, the tests were carried out only in males. The percentage medium of positivity between the rural workers and college students was 5.67%, which is close to that found DE ZULBIRIA (1990) in New Orleans (USA), with 5-9% positivity, and the found by LEHRER (1994), in tests performed in the USA and Europe, with 5.4% positivity. Considering the *Eucalyptus* spp area workers, it is clear the relatively high percentage of "prick-test" reactors – 28.21%, with the *P. tinctorius* antigens, correspond to the highest degree of exposition to such fungus.

The Western Blotting reaction used by most authors (MENEZES, 1992; CROCE PORTOCARRERO, 1995; MARQUES, 1997) in the characterization of allergenic fractions revealed, in the present study, the presence of IgE specific to various antigenic fractions of *P. tinctorius* spore and mycelia extracts, at the analysis with the sera of patients who were reactive to the "prick-test". Three fractions of the Coca 10 and Tris-10 extracts (mycelium) showed reactivity to IgE from the patients'serum, two of them with similar molecular weights. The Ecoca extract (spore) exhibited a higher number of reactive fractions to the same sera than the other extracts, and no fractions were similar to those of the mycelia extracts. The presence of IgE reactive to fungus mycelia and spore antigens demonstrate that the sensitization probably occur with the inhalation of both antigens spread in the environment.

As *Pisolithus tinctorius* is found as *Eucalyptus* spp mycorrhiza spp, and as reforestation is, in most countries, made with this plant, the importance of such fungus should be pointed out as a possible cause of respiratory allergies, especially in occupationally exposed workers.

CONCLUSIONS

1. Protein and carbohydrate contents of *P. tinctorius* spore and mycelia extracts were higher after 10 days of cultivation.

2. Spore extracts showed higher contents of proteins and carbohydrates than those from mycelia.

3. The number of protein fractions revealed by the SDS-PAGE was higher in the spore extracts.

4. There were no differences in band number in the spore extracts obtained with the liquids Coca, Tris-HCl and ammonium bicarbonate, when submitted to the SDS-PAGE analysis.

5. The mycelia extracts obtained from the ten-day incubation culture presented the highest number of bands when analysed by SDS-PAGE.

6. The positive test prevalence in the population studied was 5.6%, and almost all exhibited allergic symptoms (asthma and/or rhinitis).

7. Reactivity to spore and mycelia extracts was simultaneously observed in "prick test" positive patients.

8. The ideal protein concentration in the mycelia and spore extracts was 4.0 mg/mL.

9. The results revealed the presence of IgE, in the sera of "prick test" positive patients, which was specific to the *P. tinctorius* mycelia and spore extracts.

RESUMO

Avaliação da alergenicidade de extratos de esporo e micélio de *Pisolithus tinctorius*

Foi realizada a análise bioquímica, antigênica e alergênica de extratos de esporos e micélios de *Pisolithus tinctorius*. Os esporos foram coletados de basidiocarpos em plantações de *Eucalyptus* spp e os micélios obtidos de cultura em meio de MNM. Baseado na curva de crescimento do fungo, obteve-se massa miceliana aos 10, 20, 30 e 40 dias de incubação, que correspondem respectivamente ao início, meio, final da fase log e início da fase de declínio. A massa miceliana, juntamente com os esporos, foram submetidos a ação de três líquidos extratores (Coca, Tris-HCl e bicarbonato de amônio). Determinou-se os teores de carboidratos e proteínas. A análise eletroforética por SDS-PAGE revelou, além de frações comuns, frações distintas nestes extratos, em função do tempo de cultivo e métodos de extração. Os extratos selecionados para os testes alérgicos foram aqueles com maior número de frações. Realizou-se o "prick-test" em trabalhadores rurais, trabalhadores de áreas de plantações de *Eucalyptus* spp e em estudantes universitários, totalizando 374 pacientes. A positividade ao "prick-test" com extrato antigênico de *P. tinctorius* foi, respectivamente, 3,78%, 28,20% e 6,40%. A maioria dos pacientes positivos ao "prick-test" (82,75%) apresentava também sintomas de alergia respiratória (asma e/ou rinite). Não houve diferença de reatividade quando do emprego de extratos de esporos e de micélio do fungo. A análise dos soros dos pacientes positivos, revelou a presença de IgE específica aos antígenos do *P. tinctorius*. Considerando que o *Pisolithus tinctorius* é encontrado como micorriza de *Eucalyptus* spp e que

o reflorestamento, na maioria dos países, é feito com esta planta, deve ser relevada a importância deste fungo como possível causador de alergias respiratórias, principalmente em trabalhadores ocupacionalmente expostos.

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