ISSN 0103-8478

# Growth of symbiont fungi of some higher attine ants in mineral medium

## Crescimento do fungo simbionte de alguns attine superiores em meio mineral

**Alci Enimar Loeck[1] Carlos Roberto Pierobom[1]**
**Luciana Guerra de Gusmão[2] Ana Paula Afonso[3]**

**ABSTRACT**

*Bioassays were conducted to verify the possibility of culturing the symbiont fungus of some higher attine in mineral medium and finding out the optimum pH value for their satisfactory mycelial growth. Three organic media and one mineral medium were inoculated with isolates from **Atta sexdens piriventris** and **Acromyrmex heyeri**. In mineral medium different values of pH (4.0, 5.0, 6.0 and 7.0) were tested with isolates from **A. laevigata** and **A. laticeps**. The behavior of isolates (colony diameter) was different in the mineral medium. However, even the one which grew the least of all provided enough mycelial for RAPD analysis. The best range of pH for fungal growth in mineral medium was between 4.0 and 5.0*

**Key words:** leaf-cutting ants, symbiont fungus, mycelial growth.

**RESUMO**

*Foram conduzidos bioensaios para verificar a possibilidade de cultivar o fungo simbionte de alguns attine superiores em meio mineral e obter um valor de pH ótimo para o seu crescimento micelial. Três meios orgânicos e um meio mineral foram testados, avaliando-se o crescimento (diâmetro da colônia) de isolados de fungos de **Atta sexdens piriventris** e **Acromyrmex heyeri**. No meio mineral, diferentes valores de pH (4,0, 5,0, 6,0 and 7,0) foram avaliados, por meio do mesmo parâmetro anterior, com isolados de fungos de **A. laevigata** e **A. laticeps**. No meio mineral, os isolados testados apresentaram crescimento diferenciado, entretanto mesmo aquele que menos cresceu, forneceu material suficiente para as análises de RAPD. No mesmo meio, verificou-se que a melhor faixa de pH para o crescimento micelial está entre 4,0 e 5,0.*

**Palavras-chave:** *formigas cortadeiras, fungo simbionte, crescimento micelial.*

## INTRODUCTION

The specific identity of the fungus cultivated by higher attine ants has been a subject of some controversy, moreover, it has not been demonstrated that all ***Atta*** and ***Acromyrmex*** colonies use the same organism for their fungus garden (CAZIN JR et al., 1989). The principal difficulty has been the reluctance - indeed, the near inability – of the fungus to form sporophores, the elaborate fruiting structures required for taxonomic diagnosis (HÖLLDOBLER & WILSON, 1990).

Because the sporulation is rare, the DNA analysis by PCR (Polymerase Chain Reaction) with random primers (RAPD) may be of great help in elucidating the variability existing among this fungus known as ***Agaricus gongylophora***. PAGNOCCA et al**.** (2001) described the interrelationship between both sexual stage and sterile mycelium of fungus cultivated by ***Acromyrmex hispidus fallax*** supported by RAPD analysis.

For Random Amplified Polymorfic DNA (RAPD) analysis it is necessary to culture the fungus in a mineral medium to avoid contamination with DNA organic components.

[1]Engenheiro Agrônomo, Professor do Departamento de Fitossanidade da Universidade Federal de Pelotas(UFPEL), CP 354, 96010-900, Pelotas, RS. E-mail: alcienim@ufpel.tche.br. Autor para correspondência

[2]Engenheiro Agrônomo, Bolsista, Recém-doutor (CNPq) do Departamento de Fitossanidade da UFPEL.

[3]Engenheiro Agrônomo, Doutorando do Programa de Pós-graduação em Agronomia da UFPEL.

**Recebido para publicação 30.10.02 Aprovado em 11.12.02**

Attine fungal symbionts have been cultured *in vitro* by different authors (WEBER, 1957; PAGNOCCA et al., 1990; PIEROBOM et al. 1993; LAPOINTE et al., 1996; PAGNOCCA et al., 1996; SIQUEIRA et al., 1998; RIBEIRO et al., 1998; SILVA et al., 1999), all of whom used organic media for studies of growth requirements and assays for antifungal activity. Despite the use of different media, when cultivated *in vitro* these fungi always exhibit a slow growth-rate.

The purposes of this study were to investigate the possibility of culturing the symbiont fungus of some higher attine in mineral medium and find out the optimum pH value to their satisfactory growth.

## MATERIALS AND METHODS

The symbiont fungi were collected from a laboratory nest of ***Atta sexdens piriventris*** (Rio Grande do Sul State, Brazil) and from field nests of ***A. laevigata*** (São Paulo State, Brazil), ***Acromyrmex heyeri*** (Rio Grande do Sul State) and ***A. laticeps*** (Pará State, Brazil).

Bioassays were carried out in BOD incubator maintained at 25 $\pm$ 1ºC in darkness for 63 days. The fungal growth was estimated macroscopically on the basis of colony diameters, which were measured at weekly intervals.

The following culture media (grams per liter) were used for the assay involving the effect of media: a) Pagnocca (glucose 10.0g, sodium chloride 5.0g, peptone 4.0g, malt extract 16.0g, agar 10.0g, water 1000 ml) (PAGNOCCA et al., 1990), b) $V_8$ Juice Agar ($V_8$ juice 200ml, calcium carbonide 3.0g, agar 15.0g, water 800ml) (TUITE, 1969), c) MURASHIGE & SKOOG (1962) basal salt with agar 20.0g.$L^{-1}$ and celulose-asparagine (ammonium sulfate 0.5g, L-asparagine 0.5g, potassium phosphate 1.0g, potassium chloride 0.5g, magnesium sulphate 0.2g, calcium chloride 0.1g, yeast extract 0.5g, celulose 10.0g, agar 20.0g, water 1000 ml) (TUITE, 1969). Petri dishes containing these media were inoculated with isolates of symbiont fungus of ***A. sexdens piriventris*** and ***A. heyeri***. Each treatment was replicated six times and each plate was considered as one replication.

To investigate the effect of pH on the fungal growth, Petri dishes containing mineral medium with pH values of 4.0, 5.0, 6.0 and 7.0 were prepared. To control the pH HCl 10% (chloridric acid) and 1M KOH (potassium hydroxide) were used. Six replicated plates were inoculated with fungal isolates from ***A. laevigata*** and ***A. laticeps***.

Assays were conducted in a completely randomized design. Means of colony diameters were subjected to analysis of variance and followed by Tukey multiple range test ($P \leq 0.05$) by SANEST (ZONTA et al., 1986).

## RESULTS AND DISCUSSION

All treatments showed the presence of gongylidia (swollen hyphal tips) typical of the higher attine fungus. For both species of leaf-cutting ants the Pagnocca medium allowed the best mycelial growth of their symbiont fungus. However, in mineral medium the fungal isolate from ***Acromyrmex heyeri*** grew significantly better than isolated from ***Atta sexdens piriventris*** (Table 1).

Leaf cutting ants show preferences and select different substracts for the symbiotic fungus they culture. Their foraging activity may be related to the nutritional requirements of each colony (LEWIS et al., 1974). In this respect the results indicate the possibility of the fungi being different, at least, in their nutritional requirements.

Mean total colony diameter showed maximum growth in media between pH 4.0 and 5.0 with no significant differences between ***Atta laevigata*** and ***Acromyrmex laticeps*** symbiont fungus (Table 2). The data are in close agreement with the observations of POWELL & STRADLING (1986) that reported pH values close to 5.0 as the optimum for growth of fungi cultivated by attine ants.

However, BOARETTO et al. (1999) obtained different results to fungus growing by ***Atta capiguara***, which grew best on medium containing pH 7.5. The narrow pH tolerance of symbiont fungus is likely to have been one important factor among conditions, which maximize the nutritional returns to the ants (POWELL & STRADLING, 1986). This fact suggests new investigation of conditions for optimum development of symbiont fungus of grass-cutting ants from ***Atta*** and ***Acromyrmex*** genera.

## CONCLUSIONS

Fungi growing by ***Atta sexdens piriventris***, ***A. laevigata***, ***Acromyrmex heyeri*** and ***A. laticeps*** show satisfactory growth in mineral medium of Murashige & Skoog to obtain sufficient material to RAPD analysis.

## ACKNOWLEDGEMENTS

The authors are grateful to Silvana M. M. Coimbra and Cleide Zanela for technical cooperation and Conselho Nacional de Pesquisa (CNPq) for financial support.

Table 1 – Mean colony diameter ± SE (mm) of ***Atta sexdens piriventris*** and ***Acromyrmex heyeri*** symbiont fungus grew on four culture media.

| Culture media | Colony diameter[1] (mm) of symbiont fungus | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Days after treatment | | | | | | | | |
| | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 |
| ***A. sexdens piriventris*** | | | | | | | | | |
| Pagnocca | 12.6±0.9 a | 25.2±1.7 a | 35.7±2.1 a | 45.7±1.8 a | 54.3±1.4 a | 55.5±1.0 a | 56.7±1.6 a | 57.0±1.0 a | 57.8±1.0 a |
| Murashige & Skoog | 6.8±0.4 a | 10.3±0.9 b | 13.5±1.0 b | 14.8±0.6 b | 15.2±0.8 b | 15.5±0.7 b | 16.2±0.8 c | 16.5±0.5 c | 16.8±0.7 d |
| $V_8$ Juice Agar | 6.3±0.5 a | 8.8±1.5 b | 10.8±2.8 b | 15.8±4.0 b | 20.0±4.1 b | 24.8±3.7 b | 26.0±4.2 bc | 34.5±7.7 b | 34.4±7.7 c |
| Celulose - Asparagine | 4.8±0.6 a | 5.0±0.5 b | 5.8±1.3 b | 12.7±1.8 b | 17.5±2.2 b | 21.0±3.4 b | 28.8±3.9 b | 36.2±5.5 b | 46.2±2.6 b |
| ***A. heyeri*** | | | | | | | | | |
| Pagnocca | 10.0±1.0 a | 21.8±1.8 a | 29.0±1.4 a | 34.8±1.3 a | 39.5±1.5 a | 42.5±1.7 a | 46.2±3.3 a | 50.0±2.2 a | 52.5±2.6 a |
| Murashige & Skoog | 5.7±0.4 a | 12.0±0.9ab | 16.8±1.9 b | 22.5±1.8 b | 28.3±2.1ab | 32.2±2.0 ab | 35.8±1.9 a | 38.8±2.1 a | 40.7±1.6 b |
| $V_8$ Juice Agar | 4.7±0.9 a | 7.2±1.5 b | 8.3±3.4 b | 9.3±4.4 c | 10.8±5.3 c | 12.3±7.1 c | 15.3±8.9 b | 19.0±9.9 b | 21.2±10.5 c |
| Celulose - Asparagine | 5.7±0.3 a | 15.3±0.7ab | 16.2±1.4 b | 22.5±1.4 b | 24.8±1.6 b | 30.2±3.9 b | 42.7±1.8 a | 47.0±2.0 a | 49.2±1.7 ab |
| C. V. (%) | | | | | 32.0 | | | | |

[1]Means in the same column, followed by the same letter are not significantly different by using the Tukey test (P<0.05).

Table 2 - Mean colony diameter ± SE (mm) of ***Atta laevigata*** and ***Acromyrmex laticeps*** symbiont fungus grew on mineral culture in four pH values.

| pH values | Colony diameter[1] (mm) of symbiont fungus | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Days after treatment | | | | | | | | |
| | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 |
| ***Atta laevigata*** | | | | | | | | | |
| 4.0 | 5.0±0.3 a | 7.8±0.7 a | 13.2±1.4 a | 18.5±1.1 a | 25.0±1.4 a | 29.8±1.1 a | 33.7±1.0 a | 39.5±1.6 a | 44.3±1.9 a |
| 5.0 | 5.0±0.3 a | 8.0±0.3 a | 12.8±0.3 a | 17.8±1.2ab | 24.7±1.1 a | 29.5±1.1 a | 33.2±1.9ab | 39.2±2.4 a | 44.5±1.5 a |
| 6.0 | 5.2±0.4 a | 7.1±0.9 a | 11.5±1.3 a | 15.5±1.9ab | 22.3±1.7 b | 26.8±3.3ab | 30.5±2.9bc | 35.7±3.3 b | 40.0±4.2 b |
| 7.0 | 5.5±0.6 a | 7.3±0.7 a | 10.8±1.3 a | 15.0±1.5 b | 21.0±2.0ab | 25.3±3.3 b | 28.0±2.9 c | 31.8±4.4 c | 34.5±5.1 c |
| ***A. laticeps*** | | | | | | | | | |
| 4.0 | 5.2± 0.4 a | 8.5±0.4 a | 15.0±1.3 a | 20.2±1.1 a | 26.8±1.7 a | 32.3±1.9 a | 37.2±1.6 a | 43.0±2.4 a | 46.5±1.5 a |
| 5.0 | 5.2± 0.4 a | 8.0±0.3 a | 13.5±0.8 a | 19.0±1.1 a | 26.3±1.4 a | 32.0±1.9 a | 36.2±1.5ab | 43.0±2.4 a | 47.8±1.2 a |
| 6.0 | 5.3± 0.3 a | 8.0±0.5 a | 12.5±1.2 a | 17.5±1.3 a | 24.2±2.0 a | 28.8±2.3 b | 33.7±1.5 b | 39.2±3.3 b | 43.3±3.5 b |
| 7.0 | 5.2± 0.4 a | 7.3±0.8 a | 12.2±1.2 a | 17.7±1.5 a | 25.0±1.4 a | 30.2±1.1ab | 33.8±1.2 b | 38.5±4.1 b | 42.5±1.9 b |
| C. V. (%) | | | | | 9.3 | | | | |

[1]Means in the same column, followed by the same letter are not significantly different by using the Tukey test (P < 0.05).

## REFERENCES


BOARETTO, M.A.C.; FORTI, L.C.; FENILLE, R.C. Influência do pH e temperatura no crescimento do fungo simbionte de ***Atta capiguara*** Gonçalves (Hymenoptera: Formicidae). **Naturalia**, v.24 (n.esp.), p.41-43, 1999.

CAZIN JR., J.; WIEMER, D.F.; HOWARD, J.J. Isolation, growth characteristics, and long-term storage of fungi cultivated by attine ants. **Applied and Environmental Microbiology**, v.55, p.1346-1350, 1989.

HÖLLDOBLER, B.; WILSON, E.O. **The ants**. Cambridge, Massachusetts : Harvard University, 1990. 732p.

LAPOINTE, S.L.; SERRANO, M.S.; CORRALES, I.I. Resistence to leafcutter ants (Hymenoptera: Formicidae) and inhibition of their fungal symbiont by tropical forage grasses. **Journal of Economic Entomology**, v.20, p.128-136, 1996.

LEWIS, T., POLLARD,G.V., DIBELY, G.C. Microenvironmental factors affecting diet patterns of foraging in the leaf-cuting ant Atta cephalotes (L.) (Formicidae: Attini). **Journal of Animal Ecology**, v.43, p.143-153, 1974.

MURASHIGE, T., SKOOG, F. A revised medium for rapid growth and bioassays with tobacco cultures. **Physiologia Plantarum**, v.15, p.473-497.1962.

PAGNOCCA, F.C.et al. Toxicity of sesame extracts to the symbiotic fungus of leaf cutting ants. **Bulletin of Entomological Research**, v.80, p.349-352, 1990.

PAGNOCCA, F.C.et al. Toxicity of lignans to the symbiotic fungus of leaf cutting ants**. Journal of Chemical Ecology**, v.22, n.7, p.1325-1329, 1996.

PAGNOCCA, F.C. et al. RAPD analysis of the sexual state and sterile mycelium of the fungus cultivated by leaf-cutting ant ***Acromyrmex hispidus fallax***. **Mycological Research**, v.105, n.2, p. 173-176, 2001.

PIEROBOM, C.R.et al. Efeito do diflubenzuron sobre o fungo cultivado pela formiga cortadeira Atta sexdens piriventris Santschi (Hymenoptera: Formicidae). **Anais da Sociedade Entomológica do Brasil**, v.22, n.3, p.623-625, 1993.

POWELL, R.J.; STRADLING, D.J. Factors influencing the growth of ***Attamyces bromatificus***, a symbiont of attine ants. **Transactions of the British Mycological Society**, v.87, n.2, p.205-213, 1986.

RIBEIRO, S.B. et al. Activity of sesame leaf extracts against the symbiotic fungus of ***Atta sexdens*** L. **Anais da Sociedade Entomológica do Brasil**, v.27, n.3, p.421-426,1998.

SILVA, A.C.O. et al. Isolamento, cultivo e indução de basidiomas do fungo simbionte da formiga ***Mycetarotes parallelus***. **Naturalia**, v.24 (n.esp.), p.73-75, 1999.

SIQUEIRA, C.G. de, et al. Metabolism of plant polysaccharides by ***Leucoagaricus gongylophorus***, the symbiotic fungus of the leaf-cutting ***Atta sexdens*** L. **Applied and Environmental Microbiology**, p.4820-4822, 1998.

TUITE, J. Plant pathological methods: fungi and bacteria. Purdue University Lafayette, Indiana : Department of Botany snd Planat Pathology, 1969. 239p.

WEBER, N.A. Fungus-growing ants and their fungi: ***Cyphomyrmex costatus***. **Ecology**, v.38, n.3, p.480-494, 1957.

ZONTA, E.P.; SILVEIRA, P.; MACHADO,A.A. **Sistema de análise estatística (SANEST).** Pelotas : Instituto de Física e Matemática, UFPel, 1986. 399p.