1 Acknowledgments. We are indebted to Dr Regina R. Skelly for comments on manuscript and to Drs Z. Pojda and C. Szczylik for fruitful discussion. This work was supported by grant 10.5 from the Polish Academy of Sciences (P.A.S.). M. Ratajczak is the recipient of a Doctoral Fellowship of P.A.S.

2 Silvers, W.K., The Coat Colors of Mice. A Model for Mammalian Gene Action and Interaction, p. 242. Springer-Verlag, New York 1979.
3 Russell, E.S., Adv. Genet. *20* (1979) 357.
4 Kitamura, Y., and Go, S., Blood *53* (1979) 492.
5 Kitamura, Y., Go, and S., Hatanoka, K., Blood *52* (1978) 441.
6 Ruscetti, F.W., Boggs, D.R., Torok, B.J., and Boggs, S.S., Proc. Soc. exp. Biol. Med. *152* (1976) 398.
7 Chervenick, P.A., and Boggs, D.R., J. cell. Physiol. *73* (1968) 25.
8 Ebbe, S., Phalen, E., and Stohlman, F. Jr, Blood *42* (1973) 865.
9 Wiktor-Jedrzejczak, W., Ahmed, A., Szczylik, C., Sharkis, S.J., Sell, K.W., and Siekierzynski, M., Bull. Acad. Pol. Sci. (B) *27* (1979) 895.
10 Metcalf, D., Hemopoietic colonies, p. 140. Springer-Verlag, Berlin 1979.
11 Wiktor-Jedrzejczak, W., and Kaczmarek, L., J. Heredit. *75* (1984) 73.
12 Gross, R., in: Physiologie und Physiopathologie der weissen Blutzellen, p. 15. Ed.H. Braunsteiner. Georg-Thieme-Verlag, Stuttgart 1959.

0014-4754/85/121596-03$1.50 + 0.20/0


## Abnormal development of phialides in a strain of *Aspergillus niger*[1]

R. Bonatelli Jr, G. U. Valent, E. A. Luna and W. Gadelha

*Department of Genetics and Evolution, IB, UNICAMP, P.O. Box 1107, 13100 Campinas, SP (Brasil), and Department of Mycology, CCB, UFPE, Recife (Brasil), 27 February 1985*

*Summary.* An *Aspergillus niger* mutant strain *(hpp)* produces an average of 4.1% of conidiophores with phialide proliferations. Increased frequency of proliferations could be induced on all studied strains by growth on potato dextrose agar. The characteristic is recessive and seems to be due to a pleiotropic effect of the mutation for olive conidia color.
*Key words. Aspergilus niger;* conidiophores; phialide proliferation.

*Aspergillus niger* strains are used in the industrial production of citric acid, as well as others metabolites of industrial interests[2,3]. These strains and others isolated from nature generally have a normal development of their asexual reproducing structures, but in some adverse conditions, phialides proliferate into conidiophores. These conditions are: growth in malt agar[4], nearly anaerobic conditions[5] or transfer from low nitrogen media to nitrogen containing media with citrate as a carbon source[6]. Other authors[7-9] observed proliferations in others species of *Aspergillus* and in *A. proliferans;* these proliferations are considered the most conspicuous species characteristic[10]. In this paper we will describe the isolation of a mutant from an industrial strain of *A. niger* with a high frequency of phialide proliferations, and the influence of culture media and the genetic study of this characteristic.

*Materials and methods.* The parental strain, HCA, was identified as *Aspergillus niger,* according to Raper and Fennel[10], and mutants were obtained by Bonatelli Jr et al.[11]. The minimal medium (MM) used was Czapek-Dox with 1% of glucose. The complete medium (CM) contains all the substances of MM plus yeast extract, hydrolyzed casein, peptone, hydrolyzed nucleic acid and vitamins[12]. Potato Dextrose Agar (PDA) was prepared with 200 g of peeled potatoes, 10 g of dextrose and 15 g of agar in 1 l of distilled water. The vitamins and adenine were added to MM and PDA in final concentrations of 5 µg/ml and 50 µg/ml, respectively. The number of proliferations was recorded by counting 400–800 conidiophores after 7 days of incubation at 28 °C. In some cases, the presence or absence of proliferations was observed with a stereomicroscope ( × 40) in plates containing MM, CM or PDA. In these cases, the number of conidiophores with proliferations was not recorded. The general genetic techniques were those of Pontecorvo et al.[12] and Lhoas[13]. Heterokaryons were formed in liquid MM with 4% (v/v) of CM. Diploids $pur_1//pdx_1olv_1$ and $pab_1fwn_1//pdx_1olv_1$ were isolated by the technique of Roper[14]. The segregants were isolated by inoculating conidia of the diploid strains on solid CM containing 1–3 µg/ml of Benlate[15]. The ploidy of the strains was determined by measuring the conidial diameter[13].

*Results and discussion.* The presence of proliferations was observed when the strain $pdx_1olv_1$ was analyzed cytologically, because it has no morphological alteration with the naked eye. Consequently this strain was denominated as *hpp* (high phialide proliferations). Later, it was observed that proliferations were visible under a stereomicroscope and this fact permitted the screening of several colonies with little work. On MM the frequency of proliferations of *hpp* strain is 4.7–16.4 times greater

Table 1. Effect of culture media on the frequency of proliferations in *Aspergillus niger* strains

| Culture media | Strains | Total number of conidiophores scored | Number of observed proliferations/ conidiophore | Conidiophores with proliferations (%) | Conidiophores with one proliferation (%) |
|---|---|---|---|---|---|
| PDA* | HCA | 400 | 1–2 | 15.0 | 14.5 |
| | $pab_1fwn_1$ | 800 | 1–3 | 23.4 | 22.8 |
| | $pdx_1olv_1$ | 800 | 1–4 | 41.4 | 33.1 |
| | $pdx_1olv_1//pab_1fwn_1$ | 656 | 1–3 | 23.8 | 21.8 |
| MM* | HCA | 400 | 1 | 0.3 | 0.3 |
| | $pdx_1olv_1//pab_1fwn_1$ | 800 | 1 | 0.6 | 0.6 |
| MM* supplemented with | | | | | |
| pab** | $pab_1fwn_1$ | 800 | 1 | 0.9 | 0.9 |
| pdx** | $pdx_1olv_1$ | 800 | 1 | 4.1 | 4.1 |

*PDA: potato dextrose agar; MM: minimal medium. ** pab: *p*-aminobenzoic acid; pdx: pyridoxine.

than the control strains. On PDA, which increases proliferations in all studied strains, this increment was 1.8–2.7 times and concerns more the number of conidiophores with proliferations than the number of proliferations per conidiophore (table 1). On CM only *hpp* strain showed an increase of the frequency of proliferations (table 2). These data indicated interaction between strains and culture media, and we suggest that the study of the components of the culture media could be useful for the elucidation of the conditions that induce phialide proliferations.

Table 2. Effect of complete medium (CM) and potato dextrose agar (PDA) on the frequency of proliferations in *Aspergillus niger* strains

| Strains | Culture media | |
|---|---|---|
| | CM | PDA |
| $pur_1$ | + | ++ |
| $pdx_1olv_1$ | +++ | +++ |
| $pab_1fwn_1$ | + | ++ |
| $pur_1//pdx_1olv_1$ | + | ++ |
| $pab_1fwn_1//pdx_1olv_1$ | + | ++ |
| HCA | + | ++ |

+, ++, +++ indicate low, media and high frequency of proliferations respectively, as observed with a stereomicroscope.

The diploids synthesized between $hpp^+$ ($pur_1$ or $pab_1fwn_1$) and *hpp* ($pdx_1olv_1$) strains showed that the characteristic is recessive (table 1). The segregants obtained from diploids suggested the existence of a pleiotropic effect of the $olv_1$ gene because all the olive ones were *hpp*. An alternative explanation could be strongly linked genes, and to test this hypothesis, revertants to wild type conidial color were isolated from the *hpp* strain. These revertants, including a mutant with light brown conidia (fawn), showed a frequency of proliferations similar to $hpp^+$ strains, indicating that the mutation in the $olv_1$ gene is responsible for the *hpp* phenotype.

To the authors' knowledge this is the first time that a mutant of this type has been reported, and the *hpp* strain could be used in order to understand better the morphogenetic processes of conidiation in *A. niger*.

1 Acknowledgments. The authors are indebted to CNPq for financial assistance provided with grant PIG/SIP 04/053 as well as the scholarships Pesquisador Científico (R.B.Jr) and Iniciação Científica and Aperfeiçoamento (G.U.V.).

2 Prescott, S. C., and Dunn, C. G., Industrial Microbiology. McGraw-Hill, New York 1959.
3 Lockwood, L. B., in: The Filamentous Fungi, vol. 1, p. 140. Eds J. E. Smith and D. R. Berry. Edward Arnold, London 1975.
4 Batista, A. C., and Maia, H. S., Ann. Soc. Biol. (Brasil) *15* (1957) 206.
5 Miller, C. W., and Anderson, N. A., Mycologya *53* (1961) 433.
6 Anderson, J. G., and Smith, J. R., Trans. Br. mycol. Soc. *56* (1971) 9.
7 Thom, C., and Raper, K. B., U.S.D.A. Miscellaneous Publications (USA), p. 426.
8 Thielke, C., Planta *51* (1958) 308.
9 Thielke, C., and Paravancini, R., Arch. Mikrobiol. *44* (1962) 75.
10 Raper, K. B., and Fennel, D. I., The genus *Aspergillus*. Williams & Wilkins, Baltimore 1975.
11 Bonatelli R. Jr, Azevedo, J. L., and Valent, G. U., Braz. J. Genet. *5* (1982) 483.
12 Pontecorvo, G., Roper, J. A., and Forbes, E., J. gen. Microbiol. *8* (1953) 198.
13 Lhoas, P., Genet. Res., Camb. *10* (1967) 45.
14 Roper, J. A., Experientia *8* (1952) 14.
15 Hastie, A. C., Nature *226* (1970) 771.

0014-4754/85/121598-02$1.50 + 0.20/0


## Preimplantation mouse embryos cultured in mouse, rat and human sera: differentiation and sister chromatid exchange

C. Krüger, R. Vogel and H. Spielmann*

*Max-von-Pettenkofer-Institut des Bundesgesundheitsamtes (BGA), P.O. Box 330013, D-1000 Berlin 33 West, 26 February 1985*

*Summary.* Development of 4-cell and of 8-cell mouse embryos and of morulae and blastocysts is inhibited in vitro by mouse serum but not by rat or human sera which also do not influence sister chromatid exchange in cultured morulae and blastocysts.
*Key words.* Mouse embryo; preimplantation; in vitro culture; heterologous sera; sister chromatid exchange.

Rat and human sera have successfully been used in whole embryo culture during organogenesis both in the rat[1-3] and in the mouse[4]. However, there are no comparable data on the culture of preimplantation embryos in homo- or heterologous sera. A systematic evaluation of the effects of homo- and heterologous sera on the development of preimplantation mouse embryos in culture may help to establish test systems in which sera from patients or animals can be tested for embryotoxic effects on early embryos in vitro. We, therefore, cultured preimplantation mouse embryos in mouse, rat and human sera and we studied subsequent development of the embryos in culture during implantation[5] as well as the rate of sister chromatid exchanges (SCEs) in exposed embryos, since the SCE test has successfully been used to determine persistent DNA lesions at the earliest stages of embryonic development[6].

*Materials and methods.* Culture of preimplantation mouse embryos in homo- and heterologous sera. Nullipara NMRI mice were caged with males overnight. The morning on which a vaginal plug was found was considered to be day 0 of pregnancy. 4-cell and 8-cell mouse embryos flushed from the oviducts on day 2 were cultured for 48 h in Whitten's medium[7] supplemented with 0.3% BSA (W-BSA) to the blastocyst stage and then transferred to medium NCTC-109 (M.A. Bioproducts, Walkersville, Md, USA) supplemented with 10% fetal calf serum (NCTC). After 96 h of culture in NCTC, development and outgrowth of the inner cell mass (ICM) of the blastocyst were recorded as described previously[5,8]. Morulae and blastocysts flushed from the uteri on day 3 were cultured for 24 h in W-BSA to the late blastocyst stage and were then transferred to NCTC and cultured for 96 h[5,8].

To determine the effects of homo- and heterologous sera on development of preimplantation mouse embryos, 4- and 8-cell embryos and also morulae and blastocysts were cultured for 24 h in Whitten's medium supplemented with increasing concentrations of the sera to be tested. Thereafter, 4- and 8-cell embryos were transferred for another 24 h to W-BSA and then to NCTC whereas morulae and blastocysts were immediately transferred to NCTC. Development of the exposed embryos was recorded after 96 h of culture in NCTC[5,8] and compared to controls.

Preparation of sera. Homo- and heterologous sera from mice,