Fungal Genetics Reports

Volume 7 Article 10

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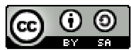

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Recommended Citation
Grigg, G. W. (1965) "Determination of nuclear ratios of microconidial strains," *Fungal Genetics Reports*: Vol. 7, Article 10. https://doi.org/10.4148/1941-4765.2097

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Abstract

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This technical note is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol7/iss1/10

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Both the methods discussed here are plating methods and although they are subject to those biases and errors which may result from interactions between cells of different genotypes and the growth medium used, they have the virtue of simplicity and rapidity. The first and most obvious method is what we could describe as the microconidial plating method.

Assuming that microconidia contain a random sample of the nuclei in the heterokaryon on which they are produced, the nuclear ratio may be estimated by plating the conidia on suitable media and determining the ratio of the types of colonies which appear. This apparently straightforward (and unoriginal) procedure is particularly subject to errors which can invalidate it as a useful method. They will be apparent when we compare it with the following method which we call the macroconidial plating method which works particularly well when the nuclear ratio is low. Microconidial cultures usually produce a low proportion of macroconidia. These latter germinate faster than microconidia and in colonial strains at least form macroscopic colonies an appreciable time before microconidia do (18 hours in the case of cms 22 (pe, su-pe; col-l; acon-t) in Horowitz's "conidiating" medium at 25°C). Hence, the proportion of viable macroconidia can be determined readily by counting the number of "early" colonies following the plating of a mixture of macro- and microconidia. In the example described below, the nuclear ratio was rather low and, since one component of the heterokaryon was relatively rare, we can assume that the colonies resulting from the plating of macroconidia would be either heterokaryons or homokaryons of the more common genotype. The frequency of the rarer nuclei of the heterokaryon is given then by the frequency of the heterokaryotic colonies. The distribution of nuclei in the macroconidial populations of the heterokaryotic culture which was required was obtained by staining with Azure A according to Huebschmann's recipe: this staining method has always given good results in our laboratory.

The nuclear ratio could be estimated when the nuclear ratio is low by $p = \frac{N_H}{N_T \times \sigma n - yN_T} - \frac{N_H}{N_T \times \sigma n}$ when y is low, as it is when the conidia are reaped from rich media. N_H = number of heterokaryotic colonies; N_T = total number of "macroconidial" colonies; y = proportion of macroconidia having only one nucleus; and σn = mean number of nuclei per macroconidium.

The two methods of estimating nuclear ratios do not always give the same results. For example, we can look at some data collected from the cms 22 + hist-3, al-2 heterokaryon. Of 654 colonies counted on "conidiating" medium and derived from macroconidia (which formed about 1% of the total conidial population plated), 24 were heterokaryons. The mean number of nuclei per macroconidium on this complete medium was 6.0 and since the proportion having 1 nucleus was negligible, the nuclear ratio p - 24/654 or 0.037. When microconidia were plated in histidine medium (30 µg/ml) only one hist-3, al-2 colony was detected among 158 colonies counted. All the others were cms 22 in appearance. In subsequent plating experiments using the same histidine medium, only one further hist-3, al-2 colony was detected amongst some thousands

scored. Thus the nuclear ratio estimated by this microconidial plating method was lower by at least an order of magnitude than the other. The reason for this discrepancy lies in an inaccuracy in estimating the frequency of the *hist-3, al-2* microconidia. Although the medium contained an amount of histidine which allowed a maximum growth rate of *hist-3* inoculated on it, the 30 to 50 *hist*$^+$ (*cms 22*) microconidia present in a petri plate of the histidine agar germinated more rapidly than the *hist* ones and removed enough histidine from the medium to prevent the *hist* conidia from growing to form macroscopic colonies. This type of suppressive interaction between small numbers of prototrophic and auxotrophic microconidia may be quite common and should be watched for. In the case of the *cms 22* + *hist-3* heterokaryon, an increase in the concentration of histidine in the medium imrpoved matters, but since histidine concentrations higher than 100 μg/ml are inhibitory to wild type and could cause another type of selective inhibition, we concluded that in this particular case the simple microconidial plating method was not as reliable as the alternative macroconidial method. The important point about the latter method, as used in this example, is that we were estimating the relative frequencies of two types of macroconidia which were phenotypically prototrophic and which competed only for common nutrients present in comfortable excess. It is worth underlining Pittenger's recommendations (1964 NN#6: 23) that in any plating experiment even obvious assumptions should be validated for each system being studied before relying on these assumptions to derive further principles. - - - C.S.I.R.O., Division of Animal Genetics, Sydney, N.S.W., Australia.