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THE USE OF *Aspergillus nidulans* TEST SYSTEM FOR THE RECOMBINOGENIC ACTIVITY OF ENVIRONMENTAL FACTORS

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ABSTRACT

A test system, based on the parasexual cycle of *Aspergillus nidulans* for the detection of the recombinogenic activity of enviromental factors is described. The use of diploid strain heterozygous for nutritional requirements and spore colour enables the identification of recombinants formed by mitotic crossing-over or non-disjunction. A diploid strain is especially described which provides information about the mechanisms of the recombinogenicity of various chemical factors by simple genetic analysis of the colour segregants induced by such factors.

RESUMEN

Se describe un sistema de pruebas, basado en el ciclo parasexual de *Aspergillus nidulans*, para determinar la influencia de los factores del ambiente en la recombinación genética. La utilización de variedades diploides heterocigóticas para algunos requerimientos nutritivos y para el color de las esporas hace posible el reconocimiento de los segregantes de las colonias en desarrollo formadas por entrecruzamiento ("crossing-over") mitótico o por no-disyunción. Se describe especialmente una variedad diploide que proporciona información sobre los mecanismos de recombinación genética de varios factores químicos por el simple análisis genético de los segregantes cromáticos inducidos por tales factores.

INTRODUCTION

During the last few years a new field in genetics has been developed, that of environmental mutagenesis. The realization that several man-made chemicals may pose a genetic hazard in our environment has stimulated interest in this field.

One of the main objectives of research in the area of environmental mutagenesis is to develop systems suitable for reliable, rapid, and inexpensive test of the possible genetic activity of the various environmental pollutants.

Several test systems are now recommended for screening different types of genetic damage caused by environmental mutagens. Most of them use microbes

Abbreviations: CM, complete medium
MM, minimal medium
PFP, parafluorophenylalanine

as test organisms (de Serres, 1971; Bridges, 1972) but others use higher organisms such as plants (Sparrow *et al.*, 1971, 1974) as well as mammalian tissue cultures (Chu, 1971). In some cases a micobial system combined with a metabolic activation one, provides information about the genetic effect of chemicals which are metaboliz ed in higher organisms (Ames *et al.*, 1973). The main types of genetic damage for which information is obtained by the different test systems are: point mutations, chromosomal aberrations, and genetic recombination.

In this article a test system, based on the parasexual cycle on the fungus *Aspergillus nidulans* is described which enables the detection of the recombinogenic activity of environmental factors.

THE TEST ORGANISM

Aspergillus nidulans (Eidam) Winter is an ascomycete with a vegetative and a sexual cycle showing colonial mycelial growth.

Uninucleate vegetative coloured haploid spores (conidia) on a proper culture medium germinated and form colorless multinucleate mycelium vertical branches of which consist the conidiophores with many parallel chains of conidia. Germination and growth of conidia into colonies bearing new conidia occurs in about two days at 38°.

A. nidulans is a homothallic fungus. The absence of different mating types enables crosses between any two strains. Self or cross fertilization may occur. The ascospores thus obtained from the asci of the cleistothecia will either be of one parental type or segregate for the characters of both parents. The eight ascospores of the asci are unordered.

Details on the life cycle, culture media, and the genetics of *A. nidulans* were given by Pontecorvo *et al.*, (1953).

THE PARASEXUAL CYCLE

The parasexual cycle of *A. nidulans* was discovered by Pontecorvo *et al.* (1954). The main steps of the parasexual cycle are: heterokaryosis, diploidization, and mitotic segregation and recombination (Roper, 1966, 1971).

Heterokaryons can be formed when anastomoses occur between hyphae carrying nuclei of unlike genotype. By inoculating conidia of the haploid parent strains, usually different auxotrophs, on solid minimal medium (MM) supplemented with suficient of the required nutrients to permit germination but not full growth, heterokaryons can be obtained shown from their full growth. When mutants with conidia colour are used, the heterokaryons can be detected under the microscope by the chains of different colour conidia in the same conidiophore.

In heterokaryotic hyphae fusions occur occasionally of unlike haploid nuclei and heterozygous diploid nuclei are formed. These can easily be selected if a large sample of conidian from a heterokaryon is plated at high density on MM. Haploid conidia because of their nutritional requirements will not grow but heterozygous

diploid will grow since the nutritional mutations are always recessive. Diploid conidia can also be recognized by their size which is 1.3 times larger in diameter than haploid (Roper, 1952). Heterozygous diploids can also easily selected when the parental haploids used had the mutations for white and yellow spore colour. The diploids are green because both colour mutations are recessive to wild green colour.

The heterozygous diploid strains of *A. nidulans* are stable. Occasionally however mitotic recombination of the genetic material followed by the seperation of the recombinant genotypes into seperate nuclei occurs resulting in the formation of recombinantes of either diploid or haploid genotype If the heterozygous diploids carry the mutations for white yellow conidia the recombinants are shown in growing green colonies as colour "areas" in the form of sectors, patches or spots. Under the microscope they can be seen even as single conidial heads.

The mechanisms by which mitotic recombinants are formed spontaneously in *A. nidulans* are: mitotic crossing-over and non-disjunction (Käfer, 1961).

Mitotic crossing-over occurs at the four-strand stage of mitosis and at any one event is confined to a single exchange in one chromosome arm (Roper, 1966). The crossover diploid recombinants formed are identified either by colour (Pontecorvo *et al.*, 1953, 1954) or by resistance to inhibitors (Roper and Käfer, 1957) when homozygosity of these recessive mutations happens because of the crossing-over process. The frequency of spontaneous mitotic crossing-over is about one in every fifty mitotic divisions (Käfer, 1961).

Non-disjunction, occuring spontaneously in *A. nidulans* at about the same frequency with crossing-over, involves abnormal segregation of single or few chromosomes resulting in the formation of 2n+1 on 2n—1 primary products (Käfer, 1977). These aneuploids are unstable and it seems that by subsequent non-disjunctions new diploid and haploid recombinants are formed which are the final stable products of the process of non-disjunction.

THE TEST FOR INDUCED RECOMBINATION

It was first shown by Morpurgo (1961) that the aminoacid analogue PFP (parafluorophenylalanine) induced haploidization in diploid strains of *A. nidulans*. Since then several agents, mainly known mutagens, have been tested and some were found to induce mitotic recombination in the fungus (Fratello *et al.*, 1960; Käfer, 1963; Morpurgo, 1963; Beccari *et al.*, 1967; Shanfield and Käfer, 1971).

Hastie (1970), Kappas and Georgopoulos (1974) and Kappas *et al.* (1974) have found that chemicals of common use, such as fungicides, greatly increased the mitotic recombination of heterozygous diploid strains of *A. nidulans* shown as colour segregants in green colonies. Interest in using the fungus as a test system in the field of environmental mutagenesis was generated by this finding.

A technique suitable for such a test (Kappas *et al.*, 1974; Kappas, 1978) will be briefly described.

A prototrophic diploid strain was synthesized with markers in all eight chromosomes producing green conidia but carrying the mutations for yellow (*y*) and

white (w) colour. In chromosomes I and II markers are on both arms for the distinction between the products of crossing-over and non-disjunction. The genotype of the diploid strain is shown in figure 1.

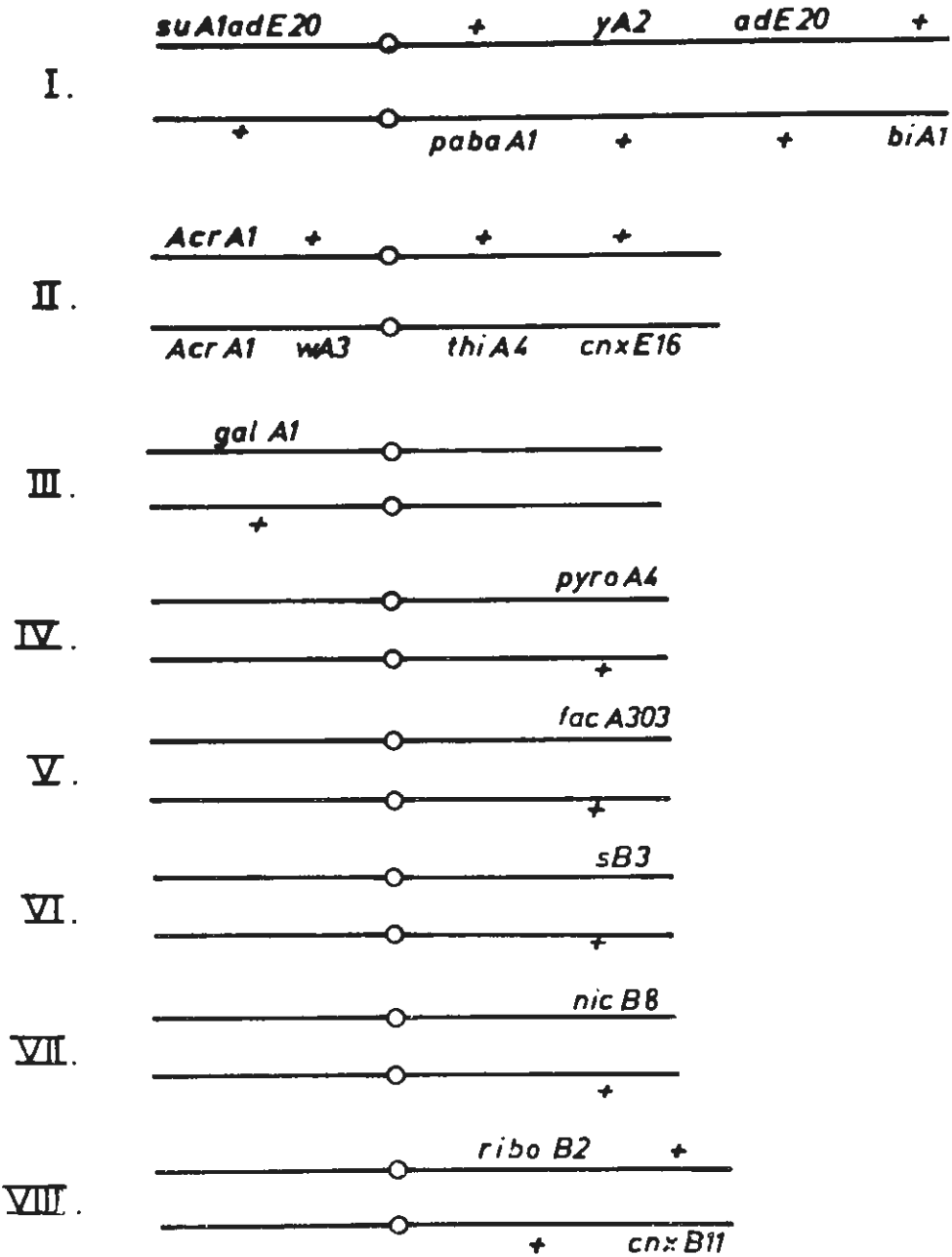

Fig.1. Genotype of the heterozygous diploid strain of *Arpergillus nidulans*.

Conidia of the diploid strain on MM are incubated overnight. Blocks of inoculum (1 mm²) are then transferred and equidistantly arranged on plates (5 on each plate) containing complete medium (CM) and the chemical under test. The plates are incubated at 38° for 4-5 days and then are scored for white and yellow areas in the green colonies. About 80-100 colonies are tested on each chemical concentration. An figure 2 control and treated colonies (with the fungicide benomyl) are shown after 5 days incubation.

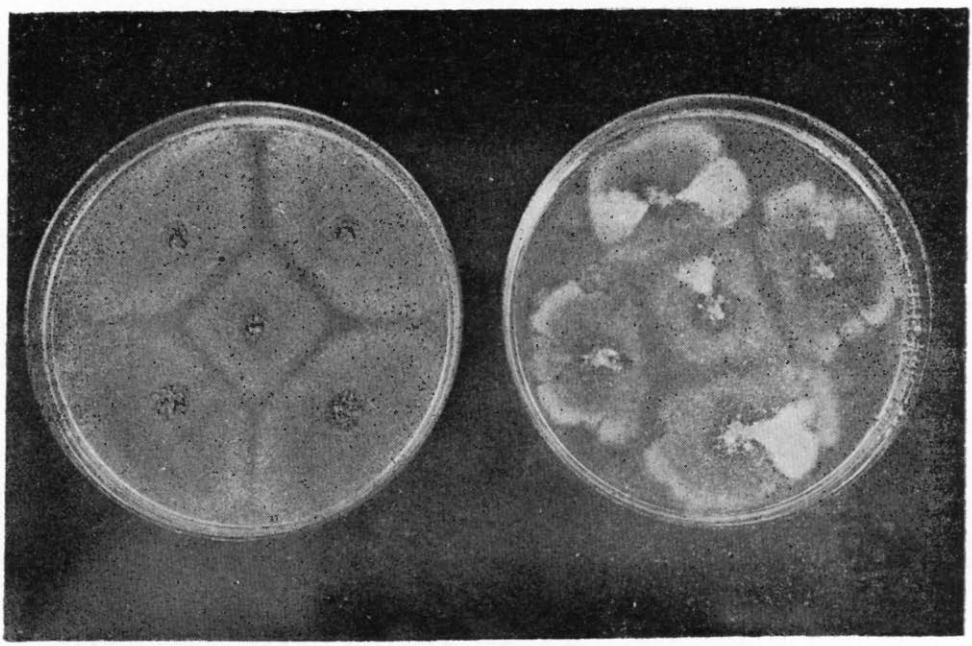

Fig. 2. Control and treated colonies with 0.4 μg/ml of benomyl showing colour sectors 5 days after inoculation.

The colour segregants are transferred on CM and templates are formed (Roberts 1959). The test for their auxotrophies is made by growing them on MM and MM supplemented with the different nutritional requirements in all possible combinations.

As can be seen from figure 1 yellow mitotic cross-over diploids must require adenine (*ad*) whereas non-disjunctionals must be protothrops. White mitotic cross-over diploids must be protothrops, but non-disjunctionals must require thiamine (*thi*) and ammonium (*cnx*). Yellow haploids must require riboflavin (*ribo*) or ammonimum (*cnx*) from chromosome VIII and may be one or more of the requirements in the other chromosomes; white haploids must always require thiamine (*thi*) and ammonium (*cnx*). Where a class of haploids is expected to be of the same phenotype as some diploids, the dimensions of conidia from a sample is examined microscopically.

By using the described diploid strain (Fig. 1). it was also possible to detect segregation due to breakage deletion (Kappas, 1978). The metabolic activation technique, using microsomes from rats, has also been combined with this system for the detection of the recombinogenicity of fungicides (Kappas. 1977).

Diploid strains heterozygous for resistance to PFP can also be used for the recombinogenicity of chemicals (Bignami *et al.*, 1974; 1977). Conidia of these strains cannot grow on MM supplemented with PFP, but homozygous for PFP resistance diploids as well as wild type haploids will grow and the different types of recombinants can be selected by the appearance of different colour.

Käfer *et al.* (1976) has recommended two strains suitable for scoring the recombinogenic activity of environmental factors. With one of the three types of recombinants, diploid cross-over, diploid non-disjunctional and haploid can be easily recognized by the different colour they show (green, yellow, and light colour respectively). With the other one crossing over is detected by the appearance of colour sectors as "twin spots".

Other chromosomal effects such as translocations or deletions, can also be discovered in proper diploid strains of *A. nidulans* (Käfer, 1977; Morpurgo *et al.*, 1978).

CONCLUSION

The parasexual cycle of *A. nidulans* provides an ideal system for the detection of somatic recombination by environmental factors. It is inexpensive and can be compared and combined with other test systems.

Depending on the strain it takes 3-10 days for a complete answer about the effectiveness of a chemical on recombination and the types of induced recombinants.

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