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Crossing-over and non-disjunction induced by sulfanilamide in Aspergillus nidulans

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Genetica. — *Crossing-over and non-disjunction induced by sulfanilamide in* Aspergillus nidulans. Nota di FRANCESCA AULICINO, ANNA MARIA VACCARO-TORRACCA, WOLFGANG M. ACHTNER e GIUSEPPE RICCIARDI, presentata (*) dal Socio G. MONTALENTI.

RIASSUNTO. — Nel presente lavoro si dimostra che la sulfanilamide aumenta le frequenze di crossing-over e non disgiunzione mitotica in diploidi di *Aspergillus nidulans*.

I risultati di questo lavoro confermano la validità di uno « spost test » messo a punto in questo laboratorio ma anche l'impossibilità di porre su basi quantitative questo semplice saggio della ricombinazione indotta.

In a previous paper [2] it has been shown by using spot tests that sulfag nilamide and some other sulfa drugs are recombinogenic agents inducin- mainly crossing-over.

The present paper has the aim: 1) to control the results obtained with the spot test previously used; 2) to see if it is possible to obtain quantitative data by some further refinements of the spot test.

MATERIALS AND METHODS

Medium.

The minimal agarized medium of Czapek-Dox was used: $NaNO_3$, 3,3 g; $MgSO_4$, 0,5 g; KCl, 0,5 g; $FeSO_4$, 0,01 g; KH_2PO_4, 0,144 g; H_2O, 1000 ml, pH 7. Glucose, 40 g, was added after sterilization.

Strains.

The diploid strain P, whose genetic constitution is indicated in fig. 1, was used to detect induced non-disjunction and crossing-over. The general characteristics of the strain and the procedure for obtaining a quantitative estimate of all kinds of mitotic recombinations have already been described [6]. Briefly, the general procedure is the following. Conidia of the strain

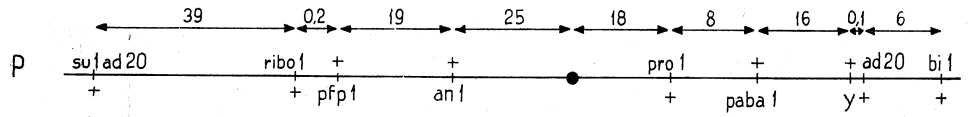


Fig. 1. – Diploid strain P used for detecting non-disjunction and crossing-over. Markers are in linkage group I. Distances are given in meiotic units from Pontecorvo and Kafer [7]. *Abbreviations.* su_1ad_{20} = suppressor of adenine 20 requirement; $ribo_1$ = riboflavine requirement; pfp_1 = parafluorophenylalanine resistance; an_1 = aneurine requirement; pro_1 = proline requirement; $paba_1$ = paraaminobenzoic acid requirement; y = yellow colour of conidia; ad_{20} = adenine 20 requirement; bi_1 = biotine 1 requirement.

(*) Nella seduta del 10 maggio 1975.

cannot grow on minimal medium supplemented with parafluorophenylalanine (PFP), (0,3764 g % *w/v*) because the strain is heterozygous for the gene determining resistance to PFP, a completely recessive mutation. The conidia can grow only if a process of mitotic segregation occurs, i.e., mitotic crossing–over or non–disjunction, producing PFP-resistant colonies.

With strain P, colonies derived by a process of crossing over can be distinguished by the non-disjunctional colonies because the first are green whereas the second are yellow and require paraaminobenzoic acid (PABA). The spontaneous frequency of non-disjunction is of the order of $1 \cdot 10^{-5}$ seeded conidia. The spontaneous frequency of crossing-over in the left arm of the first chromosome (40 meiotic units) is of the order of $1 \cdot 10^{-4}$ seeded conidia.

Treatment with sulfanilamide

The sulfanilamide used in our experiments was purchased from Carlo Erba S.P.A., Milano. The conidia were seeded on Petri dishes containing the minimal agarized medium of Czapek–Dox to which different concentrations (0,6; 1,2; 2,5; 5; 10 mg/100 ml) of sulfanilamide were added. 10 mg/100 ml is the maximum dose which permits sporulation.

Table I

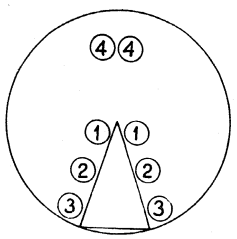
INDUCED RECOMBINATION BY SULFANILAMIDE

SULFANILAMIDE CONCENTRATIONS %		0		0.6		1.2		2.5		5		10	
PFP^r COLONIES	ACTUAL NUMBERS	y	g	y	g	y	g	y	g	y	g	y	g
		0	0	52	24	14	19	12	12	8	33	14	66
	FREQUENCIES 10^{-4}	0	0	3.3	1.5	1.4	1.8	1.2	1.2	0.8	3.5	1.9	9.1

The conidia grown on sulfanilamide medium were collected and seeded on dishes containing the minimal agarized medium of Czapek–Dox supplemented with PFP (0,3764 g % *w/v*) to measure the frequency of induced somatic recombination. The results obtained are illustrated in Table I.

To test the survival of conidia in the spot test, in the experiment shown in Table III, a small sterile triangle of adsorbent paper (3 × 5 cm) was saturated and dried two times with a 2% solution of sulfanilamide in water and layered onto the surface of the agar medium supplemented with PFP in a Petri dish seeded with $1{,}5 \cdot 10^3$ conidia of strain P. The conidia were seeded in the melted agar before it was poured into the dishes. The triangular shape of the paper establishes in the agar a gradient of concentration with a minimum at the top of the triangle. After 4 days small discs of agar were extracted from the area around the triangle and placed on the surface of dishes containing the minimal agarized medium of Czapek–Dox without PFP and sulfanilamide in order to obtain the detoxification and to permit counts of the surviving colonies.

TABLE II



CONCENTRATIONS OF SULFANILAMIDE IN THE DISHES (mg/100 ml)

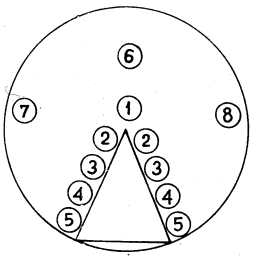
TIME		1 h	5 h	21 h	30 h	48 h	72 h	96 h
DISCS NUMBER	1	5.2	12	18	18	19	20	23
	2	8.1	24	50	50	49	48	49
	3	7.0	24	55	62	61	70	72
	4	0	0	0.7	1.8	2.17	3.54	6.57

QUANTITATIVE ESTIMATE OF THE SULFANILAMIDE IN THE AGAR

Triangles of absorbent paper saturated and dried two times with a 2% solution of sulfanilamide in water were layered onto the surface of dishes containing the minimal agarized medium of Czapek–Dox. The dishes were incubated at 37°, and small discs of agar were extracted at different moments. The agar discs were then eluted quantitatively in water. The sulfanilamide eluted from the agar was estimated with the method of Bratton and Marshall [3] The results obtained are illustrated in Table II.

TABLE III

SURVIVAL OF THE CONIDIA IN THE SPOT TEST



DISCS NUMBER	SURVIVING CONIDIA	SRV %
1	1.5	5
2	8.06	28
3	6.45	22
4	5.5	19
5	4.4	15
6	0	0
7	0	0
8	0	0

RESULTS

Data relevant to the recombinogenic action of sulfanilamide are reported in Table I. It is evident that sulfanilamide increases considerably the frequency of crossing–over. With low doses of sulfanilamide there is also a significant increase in the induction of non-disjunction.

Comparison of the results with those obtained with spot test

In the spot test the induction of recombination is indicated by the apparance of tiny PFP-resistent colonies at a certain distance from a triangle of absorbent paper imbibed with the drug. To quantitize the concentrations of sulfanilamide in the agar, small discs were extracted along the line where the concentration of the PFP-resistant colonies reaches a maximum. The drug with which the agar discs were imbibed was quantitatively eluted and the concentration in the eluate estimated with a method described in Materials and Methods. The estimate of the concentration of the sulfanilamide in the agar was made at different times from the beginning of the experiment. After 21 hours the concentration of the drug in the agar reaches a value which remains almost constant around the triangle, while it continues to increase in the areas distant from it. It can be seen from the data in the Table II that the most effective concentrations in the agar in inducing recombination are somewhat higher but of the same order of magnitude as the concentrations used in the experiment illustrated in Table I. The doses in the discs are actually higher than those used in the experiment relative to Table I. That is not surprising since the doses used in the first experiment are the maximum which permit the sporulation of the mold. On the contrary, colonies near the triangle are usually not sporulated.

Survival of the conidia in the spot test

In a previous paper (2) the frequency of the crossing-overs induced by sulfanilamide seems to reach 30 % of the plated conidia; a frequency which is much higher than that observed among the conidia collected in the media supplemented with sulfanilamide.

In the experiment illustrated in Table III, we have tested the survival of conidia in the agar near the triangle and far from it after 4 days of incubation. The survival of the conidia was tested as previously described. The results demonstrate that, in the presence of sulfanilamide, up to a concentration of about 20 mg/100 ml, the number of surviving conidia is increased. On the other hand a higher concentration of sulfanilamide causes an increased mortality. It is rather easy to explain the diminished lethality: probably sulfanilamide in slowing the metabolism protects the conidia from the harmful effects of PFP. It is, however, to explain the very high incidence of crossing-ovor recovered in the dishes.

One possible hypothesis is that in the dishes there is some residual growth of the conidia surviving the effect of PFP and sulfanilamide and that this last drug exerts its effect on a small population of nuclei and not on a single conidia. In this case a quantitativization of the induced ratio of crossing-over with the spot test would result impossible. Finally we want to discuss briefly

the mode of action of sulfanilamide: sulfa drugs are analogues of paramino-benzoic acid which is part of the folic acid molecule. Folic acid plays an important role in the synthesis of DNA precursors and therefore sulfanilamide inhibits DNA synthesis.

Sulfa drugs therefore could act in the same way as the already known recombinogens, i.e. mitomicin C (5), Fluorouracil and Fluorodeoxyuridine (1 : 4) which also interrupt DNA synthesis. Thanks are due to prof. Giorgio Morpurgo for helpful discussions.

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