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Factor(s) in the culture medium of a slime strain which stimulate DNA uptake.

We reported earlier (Schablik et al. 1977 Acta biol. Acad. Sci. hung. 28: 273) that the Neurospora crassa ragged mutant strain rg;inl a (R2506-5-101) incorporates a substantial amount of ^{3}H labelled DNA under optimal experimental conditions.

Since DNA accumulation was found to be inversely proportional to the age of the culture, we suspected that in older cultures the thickening of the cell wall might interfere with the attachment of DNA molecules to cell membrane receptors.

We report here studies of DNA uptake by slime cells, and present results which suggest: (1) enhanced DNA accumulation at the early stationary phase of growth; and (2) the presence of heat-sensitive factor(s) in the culture medium of 48 h slime cells which stimulates DNA uptake.

The slime strain (FGSC 111118) was obtained from the Fungal Genetics Stock Center. DNA was extracted from wild type strain (RL-3-8 A) obtained from Rockefeller University, New York, utilizing a modified Marmur's method (Aradi et al. 1978 Acta Biochim. Biophys. Acad. Sci. Hung. 13: 259).

Slime cells were maintained and grown on Nelson B medium (Nelson et al. 1975 Neurospora Newsl. 22: 15) containing 1.5% saccharose, 7.5% L-sorbose, IX Vogel's salts with or without 1.5% agar. Liquid medium (80 ml) in 500 ml flasks was inoculated with 2-3 x 105 cells from 6 to 7 day old agar slants and cultures were grown in a New Brunswick incubator at 27°C with shaking (100 rev/min). The isolation of ^{3}H-labelled, high molecular weight

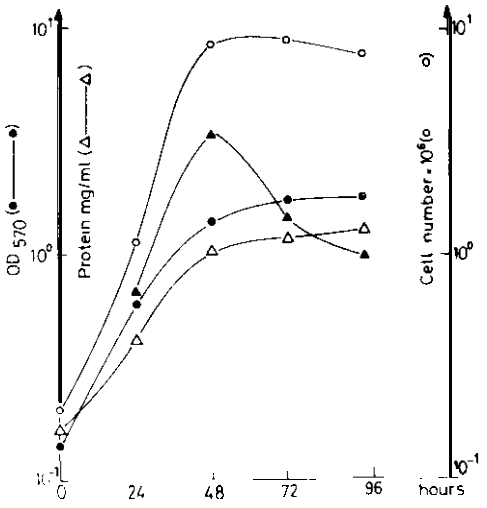

Figure 1. -- DNA uptake by N. crassa slime strain (FGSC #1118). ^{3}H DNA taken up by cells of different ages during 120 min incubation (symbol . — .).

DNA, and optimal conditions for its uptake by the rg strain have been described (Aradi et al. 1978 Acta Biochim. Biophys. Acad. Sci. Hung. 13: 259).

In the present experiments slime cells harvested by centrifugation at 1500-2000 g for 15 min at 27°C. Two ml samples (5 X 106 cells/ml) were treated with 6 to 6.6 µg DNA dissolved in 100 µl liquid medium and incubated at 27°C in a shaking water bath. After appropriate times, samples were digested with 1 ml of DNase I solution (77,000 Dornase units) at pH 7.0 for five min at 27°C, cooled to 0°C, washed three times with 0.5 N NaCl and, then once with 0.5 N $HClO_4$ at 4°C. The remaining radioactivity was extracted from the samples with 0.5 N $HClO_4$ at 100°C for 15 min and measured by scintillation counting (Schablik et al. 1979 Neurospora Newsl. 26: 17).

Fig 1 shows DNA uptake by slime cells different phases of the life cycle. Cells from the early stationary phase (48 h) accumulated significantly more exogenous DNA than cells from the exponential or late stationary phase of growth. DNA uptake was influenced not only by the age of the cells, but also by the amount of washing which they received. Successive washings of 48 h cells resulted in a gradual loss of their capacity to take up DNA (Table 1) as was found earlier in the case of the rg;inl a strain (Schablik et al. 1977 Acta biol. Acad. Sci. hung. 28: 273). These results suggested the presence of a factor(s) in the medium which enhances DNA uptake.

TABLE 1

The effect of washing and media upon DNA uptake by 48 h slime cells

Time of incubation (min)	DNA Uptake in medium: exhausted cpm	exhausted g/cell x x10-14	fresh cpm	fresh g/cell x10-14	after washing 1x cpm	1x g/cell x10-14	2x cpm	2x g/cell x10-14	3x cpm	3x g/cell x10-14
0	39	0,45	38	0.40	32	0,42	29	0,34	32	0.37
60	403	4,50	116	1,35	45	0,53	51	0,59	46	0,53
120	973	11,35	201	2,32	162	1,90	138	1,61	103	1,19

$CaCl_2$: 50 mM
3H DNA concentration : 60 x 10^{-14} g/cell
adenine : 50 µg/ml

TABLE 2

Effect of treatment with DUSF and antibiotics upon DNA uptake

Cultivation time (hours)	Incubation medium	Treatment 10 µg/ml	DNA Uptake at 0 time of incubation dpm	at 0 time of incubation g/cell x in-14	at 120 min dpm	at 120 min g/cell x 10^{-14}
	Fresh		20	0,067	300	1.00
	DUSF		32	0,100	2644	8,90
24	DUSF	Cycloheximide	62	0,200	1205	4,06
	OUSF	Ethidium bromide	68	0,230	2748	9,23
	Culture fluid from R2506-5-101		56	0,200	1361	4,56

$CaCl_2$: 50 mM
3H DNA concentration: 55 x 10^{-14} g/cell
Adenine : 50 µg/ml

In the following experiments cells were harvested at 24 h and resuspended in fresh medium and in medium from a 48 h culture, which supposedly contained the stimulatory factor(s). The results given in Table 2 show that DNA accumulation (i.e. binding of DNA in form resistant to pancreatic DNase and non-extractable by high ionic strength) resulsts from treatment with the 48 h medium. Addition of ethidium bromide (10 µg/ml) to the stimulated cells did not influence DNA accumulation, although Cycloheximide (10 µg/ml) was inhibitory. These results suggest that continuous DNA uptake requires de novo protein synthesis.

To learn about the nature of the DNA uptake stimulating factor(s), we examined its heat tolerance. Treatment of the stimulating culture medium for 5 min at 60°C caused a 50% loss of activity. These preliminary results suggest that some N. crassa strains synthesize a phase-specific substance resembling the bacterial competence factor.

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