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EFFECTS OF CAMPHOR ON HAPLOID AND DIPLOID CELLS OF *PROTOMYCES INUNDATUS**

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Abstract

The effect of camphor on haploid and diploid cells of *Protomyces inundatus* has been studied by using liquid culture method. Camphor reaction of haploid cells or diploid cells was identical and it induced gigas cell forms at different concentration with varying degree of intensity. The gigas cells reverted to normal cell form after subculture.

There was no indication that camphor caused mutation of gene that controls sexual compatibility in *P. inundatus*.

Introduction

Many studies on the camphor reaction of fungi have been made. Some have reported the production of permanent gigas cell forms in *Torulopsis utilis* (Thaysen & Morris, 1943), *Saccharomyces cerevisiae* (Bauch, 1941; Subramaniam, 1945), *Penicillium notatum* (Sansome, 1949) and *Neurospora* (Sansome, 1956), however; other workers have reported failure to obtain permanent gigas cell forms as reaction to camphor (Skovsted, 1944; Levan, 1947).

It has been shown that camphor reaction of *Protomyces inundatus* haploid cells could produce permanent gigas type diploid cells (Valadon, Myers & Manner, 1962). The life history of *P. inundatus* has been reinvestigated (Valadon, Manner & Myers, 1962). The fungus *P. inundatus* is a parasite on *Apium nodiflorum* and produces chlamydospores, which germinate to give rise to two opposite mating types of haploid endospores. The haploid cells, of opposite mating type, fuse in pairs and produce diploid cells. It was considered that gigas cell types production is limited to the camphor reaction of haploid cells only and the diploid cells surprisingly have no camphor reaction; and for this reason, it was postulated that camphor may have produced mutation of the opposite mating type gene in haploid cells (Valadon, 1961).

This report describes the camphor reaction of haploid cells as well as diploid cells of *P. inundatus*. It has been shown that both haploid and diploid cells produce gigas cell forms as a result of camphor reaction, but these gigas cell forms reverted to normal form and there was no evidence that camphor acted as a mutagenic agent of compatibility gene.

Materials and Methods

Three strains of *P. inundatus*, a diploid 2nB and two opposite mating types 18+ and 2—, were used for this study and the isolation of these strains had been

*This research work was carried out in Botany Department Southampton University, England.

described previously (Ahmad, 1973). The strains were maintained on 2% malt agar.

The effects of camphor on *P. inundatus* cells were studied at various concentrations of camphor in 2% malt agar medium. It was difficult to dissolve camphor to the medium, however, when it was dissolved in alcohol and dispersed in the agar medium; alcohol alone proved lethal to *P. inundatus* even at 1% concentration level. So, *P. inundatus* inocula (each inoculum consisted of 1000 cells) were taken from 3-4 day old 2% malt agar slant in 10 ml portions of malt agar, and were spread out in Petri plates; and known quantities of camphor were placed in the centre of each plate. The plates were 100 mm BS611 and incubated at 22°C under bell jars. The colonies produced in different plates were counted after 15 day incubation and each colony was examined for gigas cell forms. The colonies produced in plates of 18+ and 2— strains were tested for compatibility.

For liquid culture method, *P. inundatus* cells were grown in 2% malt broth using 350 ml conical flasks with various concentration of camphor. Each flask had 100 ml of broth. Preliminary experiments showed that growth was only slightly retarded by 0.3% alcohol in liquid cultures so the camphor was added as a solution in 0.3% alcohol. At higher concentrations the camphor was precipitated out when added in the broth medium but remained dispersed homogenously. The flasks were incubated at 20°C on a mechanical shaker. Samples from camphor treated cultures were taken after appropriate intervals for cell counts and growth. The cells were counted using a Fuchs—Rosenthal haemocytometer, gigas cell forms were scored separately and each snake form was counted as an individual cell. Colonies grown from the plated samples were examined for gigas cell forms and tested for compatibility.

The crosses were made by mixing cells of any two cultures, for testing compatibility, on the surface of 2% malt agar slant; and incubated at 20°C. After 24 hr incubation the cells were suspended in water and examined for criss-cross bodies under the microscope and the prescence of such bodies was considered as a positive test for compatibility.

TABLE. 1. Survival of cells of *Protomyces inundatus* strains 18+, 2— and 2nB in solid media under different camphor concentrations.

Camphor concentration (mg)	Percentage survival		
	Strain		
	18+	2—	2nB
Check	100	100	100
5	73	75	71
10	30	32	25
15	15	16	13
20	3	4	4
25	0.4	0.6	0.5

Results

The viability of cells of *Protomyces* strains 18+, 2— and 2nB under varying concentration of camphor on malt agar plates in shown in Table 1. Number of colonies formed by cells survived in Petri plates containing 25 mg of camphor was less than 1 per cent of the original inoculum. The microscopic examination of cells from colonies grown in camphor plates rarely showed up gigas cell forms. However, these cells were found to be true to their parents in sexual compatibility, that is; the colonies produced from camphor treated plates of 18+ and 2— were always sexually compatible with each other and the colonies from 2nB were incompatible with either 18+ or 2—. The gigas cells when subcultured reverted to normal size cells.

The strains 18+, 2— and 2nB responded to the camphor treatment in liquid malt in identical manner. Typical camphor form cells were produced under the influence of camphor, Fig. 1, A to F. It is evident that the incidence of camphor forms, cell volume and the property of adhering to form snakes increases as comphor concentration increases in a given treatment.

Total growth for the camphor treated cultures was reduced and the cell number at concentration above 0.04% (40 mg/100 ml) was less than that of the original inoculum. From the cell counts made after 24 and 48 hr camphor treatment it appeared that growth was never completely stopped by camphor even at the highest concentration of 0.07% that was used in 2nB strain. (Table 2). Similar data were

TALBE 2. Effect on total growth and induction of camphor forms at various camphor concentrations in cultures of *P. inundatus* 2nB.*

Camphor concentration (mg/100 ml)	Total cells per ml ($x10^5$)		Camphor forms per ml ($x10^5$)	
	After 24 hr	After 48 hr	After 24 hr	After 48 hr
Check	148	1075	0	0
10	129	825	2.50	3.12
20	89.6	255	8.25	2.30
30	32.6	97.1	7.20	2.20
40	5.15	10.2	2.40	6.45
50	4.21	4.87	0.05	0.67
60	3.11	3.56	0.13	0.08
70	3.11	3.56	0.13	0.08
70	3.00	3.30	0.08	0.01

*The cultures were grown in 2% malt broth: number of cells and camphor cell forms were counted after 24 and 48 hr incubation periods. About $5.51x10^5$ cells were seeded per ml. Similar results were obtained for haploid 18+, 2— strains.

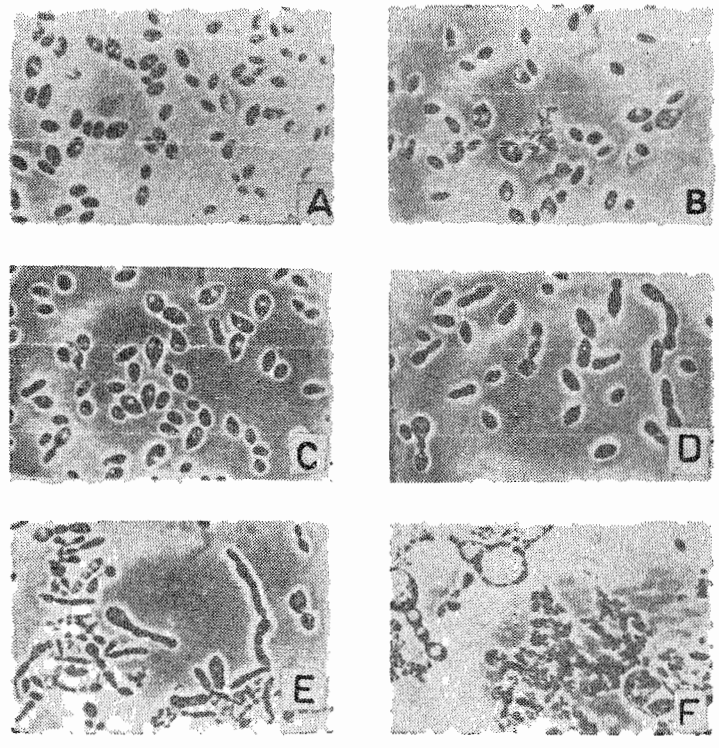

Fig. 1. Phase contrast micrographs of cells of *P. inundatus* cultures treated with camphor at concentrations; check (A), 10 mg (B), 20 mg (C), 30 mg (D), 40 mg (E) and 50 mg (F), per 100 ml; (x800).

obtained with sexually compatible strains 18+ and 2—. The number of camphor cell forms were highest at 0.04% concentration of camphor and at this concentration there was little growth augmentation. A surprising feature of the result was that the percentage of camphor forms had normal distribution when plotted against camphor concentration instead of increasing as camphor concentration increased in the broth culture. (Fig. 2A). This may be partly because of distintegration of gigas cell forms and partly because of acute inhibition of growth at lethal camphor concentration. (Fig. 2B).

The colonies produced by plating cells taken from camphor treated cultures always consisted of normal size cells, and never deviated from the inherent pattern of their compatibility. There was no documentation of production of opposite mating type mutation in *Protomyces* under the influence of camphor.

Discussion

Camphor reaction of mating type haploid cells of *Protomyces inundatus* produced gigas type cells which were found to be diploid in nature and sexually incom-

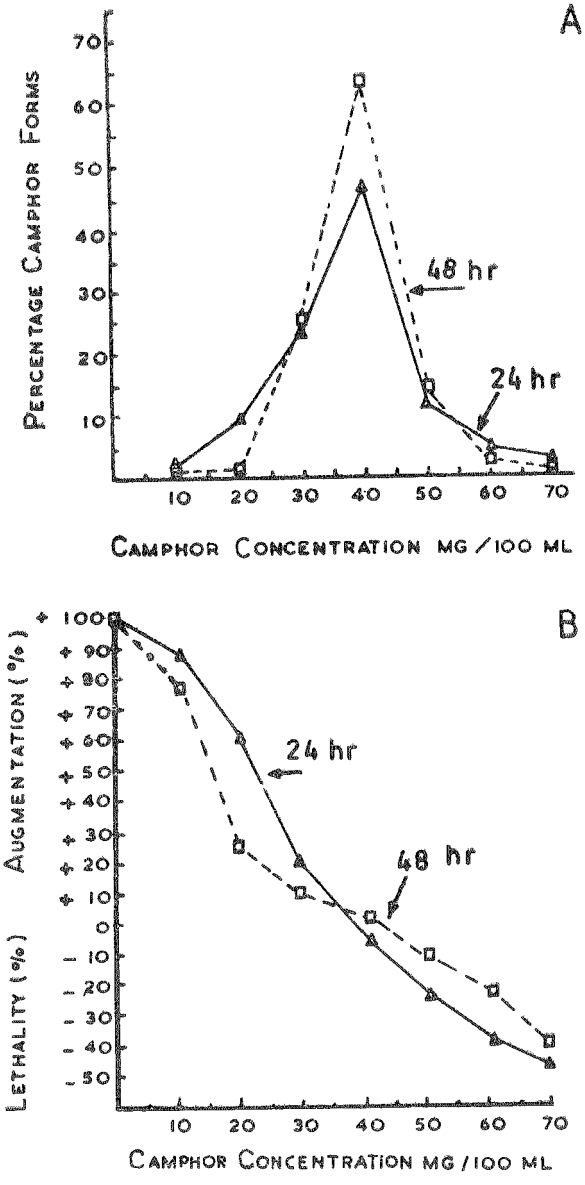

Fig. 2.A. Percentage camphor forms observed in 24 and 48 hr old cultures of *P. inundatus* that were grown in 2% malt broth containing various camphor concentrations.

Fig. 2.B. Apparent percentage augmentation and apparent percentage lethality.

Augmentation = Cell number in camphor treated culture-cell number seeded/cell number in control culture-cell number seeded.

Lethality = Cell number seeded-cell number in camphor treated culture/cell number seeded

patible, moreover; the camphor derived diploid cells were double in size as compared on normal diploid cells derived from the fusion of opposite mating type haploid cells (Valadon, 1961; Valadon, Myers & Manners, 1962). For the reason that normal diploid cells did not show camphor reaction, it was considered that mating type gene might have got mutated in haploid cell and thus produced the gigas diploid that was sexually incompatible (Valadon, 1961). On the assumption that diploids produced from strains 18+ and 2—, would be incompatible with parent strains, cross fusions of colonies were made as a matter of routine but present studies never revealed incompatible colonies derived from compatible parents. However, the possibility of the production of sexually compatible diploids has not been ruled out. All the three strains produced gigas cell forms under the influence of camphor in liquid cultures. The cell volume of camphor cell forms increased as camphor concentration in a given treatment is increased from 0.01 % to 0.05 % though the number of camphor forms produced in the active range of camphor concentrations 0.01 to 0.07 % appears to be gaussian in spread. Cells seem to lyse or disintegrate at 0.04 to 0.07 % camphor concentration but growth is never stopped and cell counts made after 48 hr of camphor treatment show increase over that of 24 hr.

Those who claimed that gigas cells produced as a reaction to camphor are polyploids have provided no convincing cytological proof, and it has been suggested from cytological studies of *Torulopsis utilis* var. major that gigas forms are due to cytoplasmic effects (Thomas, 1945). Genetical studies on *Neurospora crassa* showed that camphor diploids revert to the haploid state after subculture (Sansome, 1956). Levan & Sandwall (1943) and Levan (1947) have called the reaction of yeast to camphor poisonous and reversible. Levan (1947) has shown that the camphor reaction is not peculiar to camphor alone and has given a list of other chemicals which produce similar reactions. Camphor has been shown to be really a c-mitotic substance like colchicine (Ostergren & Levan, 1943), and affects mitosis in onion root tips in the same way as colchicine. In the process of c-mitosis the chromosomes divide but the cell remains undivided and anaphase movements are disturbed. It has also been shown that c-mitotic substances are c-tumor substances as well (Ostergren, 1944). In the c-tumor process cell volume increases and chromosomes divide within the nuclear membrane by endomitosis. It may be noted that c-mitotic substance does not necessarily have the same effect on different organisms and colchicine which is a c-mitotic substance for onion root tips has no effect on yeast (Levan, 1947). The camphor reacton of *P. inundatus* seems more like c-mitotic or c-tumor and there was no evidence that suggested that camphor reacted as a mutagenic agent of compatibility gene.

References

Ahmad, M.S. 1973. Some growth properties of haploid and diploid strains of *Protomyces inundatus*. Pak. J. Bot., **5**: 33-43.

Bauch, R. 1941 Experimentelle Mutation sauslosung bei Hefe und anderen Pilzen durch Behandlung mit Camphor, Acenaphthen und Colchicin. Naturwiss., **29**: 503-504.

Levan, A. 1947. Studies on the camphor reaction of yeast. Hereditas, **33**: 457-514.

Levan, A. and C.G. Sandwall. 1943. Quantitative investigation on the reaction of yeast to certain biologically active substances. Hereditas, **29**: 164-178

Ostergren, G. 1944. Colchicine mitosis, chromosome contraction, narcosis and protein chain folding. Hereditas, **30:** 429-467.

Ostergren, G. and A. Levan. 1943. The connection between c-mitotic activity and water solubility in some monocyclic compounds. Hereditas, **29:** 496-498.

Sansome, E.R. 1949. Spontaneous mutation in standard and 'gigas' forms of *Penicillium notatum* strain 1249 B21. Trans. Brit. Mycol. Soc., **32:** 305-314.

Sansome, E.R. 1956. Camphor-induced 'gigas' forms in *Neurospora*. Trans. Brit. Mycol. Soc., **39:** 67-68.

Skovsted, A. 1947. Induced camphor mutations in yeast. Fourth Int. Congress for Microbiology, p. 120.

Subramaniam, M.K. 1945. Induction of polyploidy in *Saccharomyces cerevisiae*. Curr. Sci. **14:** 234.

Thaysen, A.C. and M. Morris. 1943. Preparation of a giant strain of *Troulopsis utilis*. Nature, **152:** 526.

Thomas, P.T. 1945. Experimental imitation of tumor conditions. Nature, **156:** 738-740.

Valadon, L.R.G. 1961. Studies on the life history and relationship of *Protomyces inundatus* Dangeard. Ph. D. Thesis, University of Southampton.

Valadon, L.R.G., A. Myers and J.G. Manners. 1962. The behaviour of nucleic acids and other constituents in *Protomyces inundatus* Dangeard. J. Exp. Bot., **13:** 378-389.