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UTILIZATION OF RESPIRATORY SUBSTRATES BY *ANACYSTIS NIDULANS* RICHTER

SOME of the blue-green algae such as *Anabaenopsis circularis, Calothrix grevisima, Chlorogloea fritschii, Nostoc muscorum, Phormidium luridium, Plectonema calothricoides* and *Tolypothrix tenuis* have been reported to grow well in the dark[1]. But, some like *Anacystis nidulans* and *Anabaena variabilis* are obligate photo-autotrophs and they do not show any response in the dark to externally added carbon sources[2]. In the present study we report the response of the dark starved cells of *A. nidulans* to common respiratory substrates.

TABLE I

Utilization of some common respiratory substrates by dark starved cells of Anacystis nidulars

Respiratory substrates[1]	lag period	μ moles of O_2 consumed/ hr/3×10^7 cells
Control[2] (Endogenous rate)	nil	546
Glucose	60 min.	420
α-ketoglutarate	2 min.	504
Succinate	2 min.	588
Pyruvate	2 min.	756

[1] 50 μm of the appropriate substrate were incorporated into the reaction mixture.
[2] When 50 μm $NaHCO_3$ was used 3×10^7 cells liberated 1146 n moles of oxygen/hour.

Pure culture of *A. nidulans* (Nr. 14011, IARI, New Delhi) was grown at 30° C under 12 hr illumination (5,000 lux) and 12 hr dark period. Cells from a 3-day old shake cultures were taken for study. The cells were taken at the end of 12 hr illumination and starved in the dark for 6 hours before the addition of respiratory substrates. Oxygen exchange by these cells was monitored at 30° C using a Beckman oxygen analyser connected to a Heath Servo Recorder (Model EU-20 B). Oxygen evolution and consumption were measured in 3 ml medium containing 100 mM phosphate (pH 7 0), 10 mM $MgCl_2$ and 50 μM of the appropriate substrate. The instrument was calibrated with water saturated with air at 30° C with full scale deflection (237 μM O_2).

The results are presented in Table I. The response of cells was similar to the endogenous rate of O_2 consumption when succinate and α-ketoglutarate were used as substrates. When glucose was used as the respiratory substrate the response was markedly lower. Also the cells required 60 minutes to show any response to the added glucose. A marked increase in the O_2 consumption could be noticed when pyruvate was used as the respiratory substrate. These results indicate that *A. nidulans* can be grown heterotrophically in the dark using suitable substrates such as pyruvate and it can no more be considered as an obligate photoautotroph.

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MUTATION FREQUENCY IN RELATION TO M_1 STERILITY

IT is well known the mutagen treated material show varying degrees of sterility in M_1 generation[1-4]. An attempt was made to see whether the M_1 plants showing comparable degrees of sterility under the influence of ethyl methane-sulfonate (EMS) and X-rays, singly or in combination, yield proportionate amount of mutations.

Dry seeds of black gram (*Phaseolus mungo* L.) and cowpea (*Vigna sinensis* L. Savi) were treated with X-rays and EMS singly as well as in combination as described elsewhere[5]. The M_1 plants were grouped into five sterility groups (Table I) and the mutation rate was determined separately from each group. The M_1 plants obtained after EMS treatment mostly fell under group A, while those obtained after X-irradiation could be grouped under A and B groups, following combined treatments most of the M_1 plants were in A, B and C groups.

TABLE I

Frequency distribution of M_1 plants under different sterility groups (in percentage)

Mutagenic treatment	Sterility groups (in percentage)				
	A 0–20	B 21–40	C 41–60	D 61–80	E 81–100
Black gram					
X-rays	55·7	20·5	10·7	8·3	4·8
EMS	66·6	19·5	7·0	4·4	2 2
X-rays + EMS	33·2	38·0	19·2	12·8	6·8
Cowpea					
X-rays	42·2	22·2	17·8	12·2	5·6
EMS	56·7	22·8	11·4	5·7	3·4
X-rays + EMS	27·7	28·6	20·6	15·5	7·6

TABLE II

Chlorophyll mutation frequency in relation to sterility in black gram and cowpea

Mutagenic treatment	Mutation frequency				
	A 0–20	B 21–40	C 41–60	D 61–80	E 81–100
Black gram					
X-rays	0·26	1·56	2·44	0·61	0·10
EMS	2·86	1·98	0·43	0·12	0·03
X-rays + EMS	1·64	3·83	2·88	1·20	0·18
Cowpea					
X-rays	0·32	1·68	2·36	0·43	0·10
EMS	2·58	1·83	0·54	0·20	0·04
X-rays + EMS	1·78	3·62	3·10	1·68	0·26

The mutation frequency in different sterility groups depended on the nature of mutagen (Table II). In X-irradiated progeny, the mutation rate was very high in B and C groups and was comparatively low in the other sterility groups. After EMS treatment, the mutation rate was very high in A and B, *i.e.*, low sterility groups, as the sterility increased, the mutation rate showed gradual decrease. With combined treatments, the mutations were found in all the groups, the highest being in B and C groups and a decline in A and D groups. For X-rays, there was an obvious 'threshold value' *i.e.*, no mutations appear without a noticeable decrease in M_1 fertility, possibly owing to the fact that the X-ray induced sterility rose more or less linearly with dosage, as do mutations. With EMS, there was a definite 'threshold value', *i.e.*, mutations occur in the offspring of M_1 plants without any decrease in fertility. Combined treatments gave a

picture like the one obtained after EMS. Definitely higher threshold values seem to be obtained with EMS or with combined treatments than with X-rays.

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ISOLATION OF INGENOL FROM THE IRRITANT LATEX OF *EUPHORBIA SERRATA* L.

LIKE other plants of the Spurge family, the plants of *Euphorbia serrata* exude a milky, sticky, caustic and skin irritant latex when the leaves or the stems are cut or broken[1]. From the latex of this plant, which has pronounced cocarcinogenic activity[2], we wish to report the presence of ingenol, the fatty acid esters of which (and of its 16-hydroxy derivative) are known to have cocarcinogenic activity on mice skin[3,4].

The methanolic latex preparation, following the standard method[5], gave an irritant mass; ID_{50}: 1·5 μg/ear[6], which was defatted and hydrolysed with 0·5 M KOH to give the parent diterpene ingenol[5]. Its identity was confirmed by converting it to ingenol-3, 5, 20-triacetate[5]: $C_{26}H_{34}O_8$ (MS), parent ion m/e 474, UV (MeOH): λ_{max} 212, 290; $\epsilon = 16300$, 220; IR (CH_2Cl_2): 1740, 1505, 1640 cm^{-1}, relative retention time on 5% QF_1 column (GLC): 3·8 min[7]. These data are in agreement with those of an authentic sample of ingenol triacetate.

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BREAKING DORMANCY IN POTATOES BY VAPOUR TREATMENT WITH ETHYL ALCOHOL

CHEMICAL treatments to break the dormancy in seed potatoes can be effected either through soaking in aqueous solutions or by exposure to vapours of volatile chemicals. Thiourea, ethylene chlorohydrin and gibberellic acid, singly or in combination, are field proven chemicals for the former method, while ethylene chlorohydrin and carbon disulphide are for the latter[1-4].

Ethylene chlorohydrin, which is preferentially used at this Institute for vapour treatment of dormant seed tubers[5], has pronounced human toxicity; it is also expensive and is not indigenously produced. In the course of a search for alternate chemicals, we found the vapours of the low homologues of aliphatic alcohols to by effective and free from the above defects.

A pilot project study of the efficacy of vapour treatment with ethyl alcohol for breaking seed-tuber dormancy was undertaken in the autumn of 1974 at Kufri Station. Five quintals each, of the freshly harvested seed of 'Kufri Muthu' variety were used in the vapour treatment with ethyl alcohol (95%) and compared with ethylene chlorohydrin; both were used at the rate of 2 ml per kg of tubers in an air-tight chamber[1]. The chemicals were kept in shallow dishes, above which gunny bags containing the seed were stacked and at frequent intervals the air within the chamber was circulated with a fan. The treatment lasted for 48 hours; the temperature within the chamber was maintained around 25° C for the entire duration of the treatment.

The treated tubers were planted at Daurala on 25-9-1974 in randomised blocks of 8 replications with the above two treatments along with an untreated control in plots measuring 7 m by 4·2 m. Periodic plant emergence counts were taken commencing from 45 days after planting. Table I gives the results.

It could be seen that vapour treatment with ethyl alcohol was as effective as the ethylene chlorohydrin.