Tests for alloxantin in the Hill reaction

Data reference	Reaction vessel[a]						
Figure	1	2	3	4	5	6	7
1	+(○)	+(▲)	+(◑)	−(●)	−(◓)		
2	+(▲)	+(○)	−(△)	−(◑)	−(●)	−(◓)	−(□)

[a]Color test for alloxantin with $Ba(OH)_2$: +, positive, blue color observed; −, negative, no blue color observed.

of a Warburg flask. The center well contained 0.1 ml of 2 *N* KOH solution and a fluted filter paper. The side arm contained 0.2 ml of chloroplast suspension. A control flask containing boiled chloroplast suspension was always prepared in each experiment. The reaction was initiated by tipping in the chloroplast suspension into the main compartment and turning on the illuminating lamps.

Results. As indicated in Figure 1, significant oxygen evolution was delayed until illumination was begun. Alloxantin (III) was determined to be present, by a qualitative test, in runs where significant oxygen evolution was observed. This test involves the formation of a characteristic blue-colored complex when (III) is treated with $Ba(OH)_2$[6]. The results for the sets of runs presented in Figures 1 and 2 are presented in the Table. Hill activity was not inhibited by (II) or (III) as determined by experiments where (III) was placed in the reaction medium prior to reaction initiation. An equilibrium quantity of (II) would become available from dissociation of (III) in this case.

We observed inhibition of the Hill reaction by oxygen in high concentrations as determined by experiments performed in an air atmosphere and also a slight volume decrease near the end of the experiment (Figure 2). Oxygen inhibition is a characteristic of the Hill reaction[7] and a volume decrease is similar to observations made previously by MEHLER[7a] using other oxidants.

Our observations are consistent with a system as depicted in the Scheme. Oxygen and dialuric acid (II) are produced by a light-dependent reaction involving the chloroplasts. The amount of alloxantin (III) formed and present at any given time will be described by the equilibrium constant for the reaction between (I) and (II)[8]. An equilibrium quantity of (II) will always be available for reaction with oxygen to spontaneously re-form (I). If (II) were the sole product, then the theoretical quantity of oxygen produced under the conditions of the experiments presented in Figures 1 and 2 would be 224 µl. However, if all of (II) reacted with (I) to form (III), then the theoretical volume of oxygen would be 112 µl. In all experiments the observed oxygen volumes were considerably less than 112 µl (see Figures 1 and 2). As the light-dependent production (II) and oxygen increases, their rate of reaction with each other to produce (I) will also increase. The reaction scheme then begins to approach a steady-state where oxygen will react as fast as it is produced. This will appear as a decrease in the rate of oxygen production with an eventual cessation when in reality oxygen continues to be produced.

We regard this behavior in the Hill reaction to be novel and interesting with respect to the light induced cyclic electron flow and implications relevant to solar energy conversion.

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Effect of Fungal Staling Growth Products on Growth Behaviour of Rhizosphere Microfungi

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Summary. Strong mycostatic property of *Trichoderma harzianum*, *Aspergillus flavus* and *Penicillium rubrum*, which have very high competitive ability in staled agar disc from rhizosphere soil inocula of Lentil (*Lens esculantum* Moench.), was observed corresponding to similar growth-behaviour in staled culture filtrates of dominant microfungi.

Effect of staling growth products of microfungi on growth behaviour of rhizosphere mycoflora has received little attention. DWIVEDI and GARRETT[2] reported that tolerance of microfungi to mycostatic substances was an important factor in their colonization on nutrient agar plates. PARK[3] demonstrated that mycostatic substances of microbial origin might be analogous with staling products of fungal cultures. In the present investigation, fungal flora from rhizosphere of Lentil (*Lens esculantum* Moench) was studied in relation to competitive colonization on staled agar plates at different time intervals. This study was supplemented with the effect of staled culture filtrates on growth behaviour of microfungi.

Materials and methods. Rhizosphere soil samples were collected and thoroughly mixed in aseptic conditions. 15 ml sterilized nutrient Czapek agar (acidified to pH 4.5 by orthophosphoric acid) were poured into Petri dishes and soil impression was given over the whole agar surface by using a flat bottomed glass beaker. 5 series of plates, each with 5 replicates were incubated at 25 ± 1 °C for 24, 48, 72, 96 and 120 h and thereafter the entire circle of agar in each series of plates was placed upside down and

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the same soil was inoculated over the reverse of agar discs and the fungi grown were recorded. The percentage colonization of fungi on nutrient virgin agar was determined by the following formula:

$$\% \text{ colonization} = \frac{\text{Total number of the respective fungal colonies}}{\text{Total number of all fungal colonies}} \times 100$$

In another set of experiments, growth behaviour of some microfungi was studied in liquid metabolites of certain fungi colonizing staled agar discs after 96 and 120 h of staling. For this purpose, these fungi were inoculated in liquid Czapek-Dox +0.5% yeast extract medium in flat medicine bottles and incubated at 25 °C. After 5 days of incubation, cultures were filtered through Seitz filter. 30 ml of fungal filtrate of individual fungus were transferred to flat medicine bottles, respective fungi inoculated and incubated at 25 °C for a fortnight. Hyphal mat of each fungus was filtered separately, that of replicate ones pooled and dried at 80 °C for 24 h.

Results and discussion. Fungi isolated from the rhizosphere of the test plant have been arranged in Table I. There was a decrease in the number of fungi colonizing the reverse of agar discs due to diffusion of staling growth products from composite rhizosphere mycoflora. According to the tolerant capacity of staling products, the fungi were classified in groups I to VI. Microfungi with the best competitive tolerance capacity of staling products were *Aspergillus flavus, A. niger, Curvularia lunata, Fusarium poae, Penicillium rubrum, Trichoderma harzianum, T. album* and white sterile mycelia. The failure of appearance of virgin agar fungi on the staled agar was possibly due to low growth rate and less inoculum potential, as success in the saprophytic colonization of a particular fungus depends upon its population level which is related to the inoculum potential[2]. Amongst the fungi listed in Table I, several might have colonized the staled agar because of high degree of competitive saprophytic ability, while this may not be true for the remaining ones having lower degree of competitive ability.

A perusal of Table II indicates that there were differences in growth-supporting values of the culture filtrates of different filtrate producers. The growth-supporting values of the filtrate of *P. rubrum, F. poae, C. lunata* and *S. rolfsii* showed a reducing effect on other fungi, greater than of their own. Higher the values of

Table I. Grouping of fungi based on their tolerance capacity of staling products in staled agar discs at different periods

Fungi isolated	Presence/absence on staled agar disc after different periods of staling (h)						Colonization on nutrient virgin agar (%)
	0	24	48	72	96	120	
Group I							
Alternaria tenuis	+	−	−	−	−	−	2.1
Aspergillus sulphureus	+	−	−	−	−	−	0.8
Humicola sp.	+	−	−	−	−	−	1.3
Mortierella subtilissima	+	−	−	−	−	−	0.4
Rhizopus nigricans	+	−	−	−	−	−	0.8
Syncephalastrum racemosum	+	−	−	−	−	−	1.3
Group II							
Aspergillus luchuensis	+	+	−	−	−	−	3.0
Cladosporium herbarum	+	+	−	−	−	−	2.6
Curvularia maculans	+	+	−	−	−	−	3.9
Helminthosporium tetramera	+	+	−	−	−	−	1.3
Trichophyton sp.	+	+	−	−	−	−	1.7
Group III							
Absidia spinosa	+	+	+	−	−	−	1.7
Aspergillus nidulans	+	+	+	−	−	−	3.9
Penicillium restrictum	+	+	+	−	−	−	2.1
Group IV							
Aspergillus fumigatus	+	+	+	+	−	−	3.4
A. sydowi	+	+	+	+	−	−	3.4
Mucor luteus	+	+	+	+	−	−	2.1
Brown septate sterile mycelium	+	+	+	+	−	−	4.7
Group V							
Hormiscium sp.	+	+	+	+	+	−	3.4
Penicillium nigricans	+	+	+	+	+	−	3.0
P. biforme	+	+	+	+	+	−	0.8
Phoma glomerata	+	+	+	+	+	−	1.7
Sclerotium rolfsii	+	+	+	+	+	−	4.3
Group VI							
Aspergillus flavus	+	+	+	+	+	+	9.1
A. niger	+	+	+	+	+	+	6.1
Curvularia lunata	+	+	+	+	−	+	3.4
Fusarium poae	+	+	+	+	+	+	5.6
Penicillium rubrum	+	+	+	+	+	+	6.5
Trichoderma harzianum	+	+	+	+	+	+	7.3
T. album	+	+	+	+	−	+	2.6
White septate sterile mycelium	+	−	−	+	+	+	4.3

+, Presence; −, absence.

Table II. Growth behaviour of 6 fungi in staled liquid culture filtrates (dry weight of hyphal mat in mg)[a]

Name of fungi	Control	*A. flavus*	*C. lunata*	*F. poae*	*P. rubrum*	*T. harzianum*	*S. rolfsii*	Mean growth in all filtrates
Aspergillus flavus	380	195.4	145.0	96.3	162.8	91.5	231.5	153.7
Curvularia lunata	298	62.5	173.0	109.3	71.6	52.2	102.8	95.2
Fusarium poae	270	78.9	106.4	170.6	88.9	70.0	142.4	109.5
Penicillium rubrum	320	139.5	141.6	143.0	200.5	89.3	213.0	154.5
Trichoderma harzianum	360	153.5	173.2	160.0	142.6	139.5	219.3	164.7
Sclerotium rolfsii	287	68.4	138.9	103.3	88.5	60.6	175.6	105.9
mean growth of all fungi	319	116.3	146.3	130.3	125.8	83.8	180.7	130.5[b]

[a] Average of 3 replicates. [b] Mean growth of all fungi in all filtrates

hyphal mat harvested, lower will be the toxic effect of filtrates. Growth-supporting values of *A. flavus* and *T. harzianum* were much less as compared to *C. lunata*, *F. poae*, *S. rolfsii* and *P. rubrum*. The growth-supporting values of *S. rolfsii* was very high in all cases showing the least toxicity of its culture filtrate. The comparative survival of *T. harzianum* was higher in all the fungal metabolites followed by *P. rubrum*, *A. flavus*, *F. poae*, *S. rolfsii* and *C. lunata*. DWIVEDI and GARRETT[2] reported that the growth-supporting values of different filtrates depended directly on the concentration of the residual nutrient unused by the filtrate-producing fungus, and inversely on the concentration of staled mycostatic products.

Nuclear Numbers in Encysted Dormant Embryos of Different *Artemia salina* Populations

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Summary. The number of nuclei in dormant cysts from world-wide populations of the brine shrimp, *Artemia salina* (L.) was determined. These nuclear numbers proved to be quite constant considering the diversity of geographical localities, ploidies, and modes of reproduction represented by these populations. We believe this constancy indicates a tight coupling between the development and dormancy of these embryos. Chromosome counts on *Artemia* from Jamnagar, India indicated this population to be triploid.

The brine shrimp *Artemia salina* (L.) is found in highly localized populations that are widely distributed around the world. Some of these populations differ in ploidy[2,3] as well as the amount of DNA per somatic cell[4]. As a normal part of its embryonic development this organism often produces encysted dormant embryos halted at the late blastula or early gastrula[5,6]. The encysted embryos, often called 'cysts', have been the object of an increasing number of studies in biochemistry and developmental biology, but little is known concerning the precise stage at which dormancy occurs, and the variation from population to population in this regard. Reasoning that the number of cells in the encysted dormant embryo should provide information on the foregoing considerations we made nuclear counts on cysts, from widely separated populations, and report the results here.

The cysts were treated with aniformin solution[7] to digest the outer shell (chorion), washed with saline, stained in acetic orcein or fixed in ethanol-acetic acid (3:1), run through the feulgen procedure, and squashed under a coverslip. The coverslip was sealed with 'Lubriseal' and the nuceli were counted at 320 × with the help of a lined grid. These nuclear counts presumably reflect the number of cells per cyst. Although some syncitial areas may exist[6] we have not seen them in electron photomicrographs[8].

The chromosome number for the Jamnagar (India) population has not been examined previously to our knowledge. We made this measurement on Feulgen stained squashes of nauplii at 1300×. 25 cells from 4 animals were measured and found to have a mean of 57 chromosomes. This was compared with measurements on cysts from the Great Salt Lake where 43 cells from 7 animals revealed a mean of 38 chromosomes. Although the data are not accurate enough for precise description of the Jamnagar population, it seems clear that the number is triploid with respect to the Great Salt Lake population. These findings are consistent with those of IWASAKI[9] who concluded that the Great Salt Lake population is diploid

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