ability to inhabit these locations. Distribution of the organism is fairly uniform over the study area. The sediment in this area was predominantly red clay and siliceous ooze[9]. The nodule coverage ranged from 0 to 17%. The organism was generally associated with faecal tubes of holothurians and acorn worms, and body casts of polychaetes, and with burrows and pellets for which they may themselves be responsible. The specific tracks of these animals could not be deciphered. A few pennatulids were also photographed along with them.

This report acquires significance because:

(i) It is the first report of genus *Freyella* from the Indian Ocean from depths greater than 3000 m. Since this species was observed even at 5400 m depth, it seems capable of surviving under extreme conditions (3–4°C temperature; 300×10^5 to 500×10^5 pascal pressure.

(ii) This is probably a new species.

(iii) The species can survive on sediment substrate as well as in regions of rocky exposures and thick nodule populations.

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Effect of culture filtrate on growth of *Spirulina platensis*

Tasneem Fatma

Department of Biosciences, Faculty of Natural Sciences, Jamia Millia Islamia, New Delhi 110 025, India

There is need for genetic improvement of the biotechnologically important cyanobacterium *Spirulina platensis*. Repeated unsuccessful efforts to get isolated colonies on agar plates after dilution of the cultures and synchronous growth, in contrast to thick mat-like growth, from bulk inoculum prompted an investigation of the effect of *Spirulina* culture filtrate on growth of fresh inoculum. The results show that sterilized *Spirulina* culture filtrate has extracellular growth-stimulatory factor(s).

DURING efforts to obtain synchronous growth of the cyanobacterium *Spirulina platensis* it was found that very dilute cultures fail to grow and tend to lyse both in liquid and on solid media. It appeared that a certain minimum cell population or inoculum size is necessary to initiate and sustain growth of *Spirulina* cultures. Furthermore, it was observed that when the inoculum size was large enough to initiate growth of the culture the growth rate was proportional to inoculum size. These observations indicated that the inoculum has a factor that is necessary for further growth and sustenance of the culture. If this factor falls below a certain critical level, the organism would not only fail to grow but the cells would also lyse eventually.

The present work was undertaken to ascertain, first of all, if this premise is true, and, if a growth-stimulating factor does exist, whether it is cell associated or is present extracellularly. *S. platensis* CFTRI cultures were raised in Zarrouk's[1] medium for six days under standard autotrophic culture conditions[2] on a shaker. The culture fluid was filtersterilized using a Millipore membrane filter (0.22 μm). Different volumes of culture filtrate were added to Zarrouk's medium (Figure 1). The total volume of the medium was 10 ml, to which 5 ml *S. platensis* inoculum was added, to give an absorbance at 560 nm of 0.1.

The cultures were allowed to grow and absorbance was read periodically for 9 days. Figure 1 shows that addition

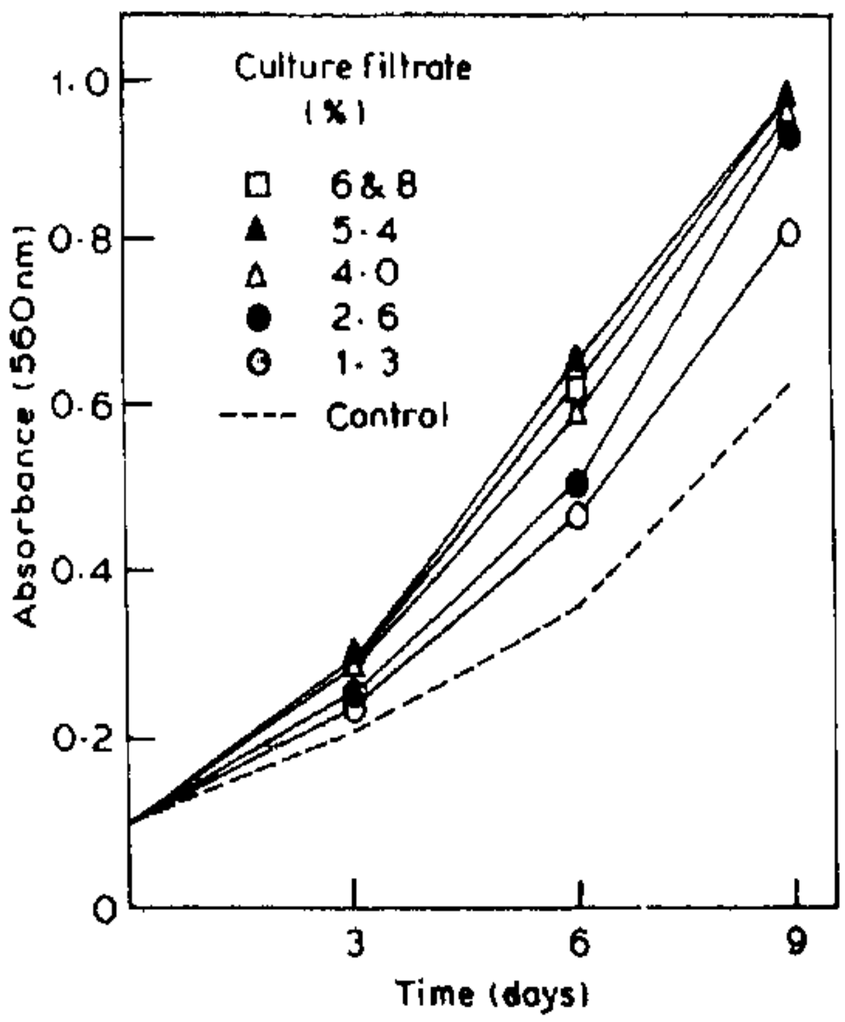

Figure 1. Growth of *Spirulina platensis* CFTRI with/without added sterilized culture filtrate.

of the culture filtrate has a pronounced and distinct stimulating effect on the growth of the cultures. The culture containing culture filtrate had an absorbance of 0.96 on day 9, as against 0.63 absorbance of the control. This amounts to more than 50% increase in growth at this point of time. Higher proportions of filtrate produced no further stimulatory effect. Possibly, at 4% itself, whatever factor is stimulatory to growth had attained saturation point for the culture under the conditions of the cultivation. Ciferri[3] observed lysis of *Spirulina* whenever the inoculum size was very small under mixotrophic conditions and had termed it 'mixotrophic lysis'. No tangible explanation for this phenomenon is available. The present results demonstrate that *S. platensis* CFTRI excretes some unknown factor(s) that is stimulatory to its own growth. It is understandable, therefore, that if the inoculum size is small the growth-stimulatory factor coming from it is inadequate to support the growth of the culture. This would also mean that a certain threshold concentration of this factor would be essential.

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Effect of fern extracts on growth and germination of fungi

Yasmeen and S. K. Saxena
Department of Botany, Aligarh Muslim University, Aligarh 202 002, India

The effect of three fern extracts on growth and germination of *Alternaria brassicicola* and *Aspergillus niger* was studied. Extracts of all three ferns had an adverse effect on the growth and germination of both fungi. Rhizome extracts were more toxic compared to leaf extracts.

ALTERNARIA BRASSICICOLA causes dark spot disease in the genus *Brassica*[1]. *Aspergillus niger* is a saprophytic fungus that attacks seeds/fruits of a large number of plants in storage[2]. Spray or treatment with chemical fungicides is the method recommended for their control. But the use of fungicides has some inherent problems of toxicity. In view of this many workers have suggested the use of plant extracts to reduce the incidence of various plant diseases[3,4].

Ferns are generally less prone to attack by fungal pathogens[5]. But nothing is known about the toxicity of fern extracts to fungi. Hence in the present study an attempt has been made to determine the effect of extracts of three ferns, viz. *Adiantum caudatum*, *Diplazium esculentum* and *Pteris vittata*, on growth, spore production and spore germination of *A. brassicicola* and A. niger.

The fern extracts were prepared by crushing 5 g green leaves and rhizome separately in 500 ml distilled water. The extract was steamed for 30 min, and strained through muslin cloth. Ten ml of extract was added to petri plates containing 20 ml of molten potato dextrose agar (PDA). The plates were then inoculated with the fungi. For control, plates with PDA alone were inoculated. The inoculated plates were incubated at 28°C for seven days. There were three replicates for each treatment and the experiment was repeated thrice.

Growth was determined by measuring the colony diameter after seven days of incubation (Table 1). For sporulation five agar discs, each of 5 mm diameter containing sporulated fungus, were selected at random from the fungus colony and transferred to 1 ml of distilled water. A suspension of 0.01 ml was placed on a clean slide and examined under the microscope. The number of spores per microscopic field was counted and average spore production was categorized as poor, fair, good or excellent (Table 1). For germination studies spores were taken in a test tube containing 5 ml distilled water and the suspension was mixed thoroughly. One drop was placed in a cavity slide containing 0.5 ml of fern extract. The cavity slides were incubated in a moist chamber at 30°C. Germination of the spores was observed after 15h and percentage of germination was calculated.

Table 1 shows that extracts of leaves and rhizomes of all three ferns had an adverse effect on germination and growth of both fungi. The highest toxicity was caused by

Table 1. Effect of fern extracts on germination, growth and sporulation in *Alternaria brassicicola* and *Aspergillus niger*.

Fern	Germination of spores (%) in extracts				Growth (mm)/sporulation in extracts			
	Leaf		Rhizome		Leaf		Rhizome	
	A. brassicicola	*A. niger*	*A. brassicicola*	*A. niger*	*A. brassicicola*	*A. niger*	*A. brassicicola*	*A. niger*
A. caudatum	60.3	58.6	5.1	7.2	36/(++)	32/(++)	27/(++)	23/(++)
D. esculentum	30.5	35.3	8.0	5.3	39/(++)	35/(++)	29/(++)	26/(++)
P. vittata	12.5	15.4	4.7	2.2	34/(+)	28/(+)	31/(+)	30/(+)
Control	97.2	95.3	97.2	95.3	41/(++++)	38/(++++)	41/(++++)	38/(++++)
LSD at 5%	1.73	1.38	3.84	2.80	2.38	2.87	2.26	2.58

+, Poor (1–10); ++, fair (11–30); +++, good (31–50); ++++, excellent (above 50).