Plant and Soil 101, 235–240 (1987)

PLSO 6891

Do cyanobacteria enhance germination and growth of rice?

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Received 28 May 1986. Revised October 1986

Key words: anabaena, axenic culture, cyanobacteria, germination, nostoc, plant growth regulators, rice

Abstract

A screening of 133 cyanobacterial strains in logarithmic growth phase was done to study their effects on rice germination and growth. In unialgal, non axenic culture 30% of the strains had no effect, while 70% of the strains had a negative effect on germination. In contrast, growth of rice was stimulated by 21% of the isolates and inhibited by 12%.

Although 57% of the unicellular strains had a positive effect and many Nostoc strains had a negative one, it was not possible to correlate specific effects with taxonomic groups. Among the eight strains showing a stimulatory effect on growth only Anabaena 77S19 remained effective in axenic culture. Partitioning Anabaena 77S19 exudates into three fractions revealed that the organic fraction was more inhibitory. From this work it is concluded that presoaking rice seeds in a cyanobacterial culture should be done with caution or avoided altogether.

Introduction

Cyanobacteria are abundant in rice fields where their enhancement of soil fertility by means of biological nitrogen fixation has often been studied (Roger and Kulasooriya, 1980), but their beneficial effect is not limited to that. Bentley (1958), in studying growth regulator production by phytoplankton showed that some strains of Anabaena and Oscillatoria exuded auxin-like substances.

Biochemical data and pot and field trials have implied the possible importance of cyanobacterial exudates, but there is a dearth of statistically evaluated experiments from which to judge the true significance of this in the field.

Cyanobacterial plant growth-regulator effects have been attributed to production of antibiotics and toxins (Metting and Pyne, 1986), organic acids (Hellebust, 1974), vitamins B (Brown *et al.*, 1956, Grieco and Desrochers, 1978), gibberellin-like substances (Gupta and Lata, 1964, Singh and Trehan, 1973), and cytokinin-like substances (Rodgers *et al.*, 1979).

In this paper we present results of screening cyanobacterial strains isolated from dry tropical Africa (mainly from rice fields) for effects on early development of rice. Strains which were shown in unialgal culture to have a marked positive effect on both germination and rice growth were purified and again tested. Additionally, both aqueous and organic exudate fractions were tested along a concentration gradient from 10 through 0.001 dilutions.

Materials and methods

Cyanobacteria

Isolation and growth. The 133 cyanobacterial strains involved in this study were isolated from habitats of tropical and Sahelian Africa by the serial-dilution method (Reynaud and Laloe, 1985) and were unialgalised by micromanipulation (Rippka *et al.*, 1981).

With the assignments of Rippka *et al.* (1979), there were 14 strains belonging to section 1 (3 Synechococcus, 5 Synechocystis, 3 Gloeothece, 3

Gloeocapsa), 19 to section 3 (8 Oscillatoria, 1 Pseudanabaena, 9 LPP group), 98 to section 4 (32 Anabaena, 6 Nodularia, 28 Nostoc, 11 Scytonema, 21 Calothrix) and 2 Fischerella belonging to section 5.

For the second part of the study, the cyanobacteria were axenised and their purity checked before each assay (Rippka *et al.*, 1979). The strains are deposited in the living collection at the Laboratoire de Biologie des Sols ORSTOM (Dakar, Senegal, West Africa).

Growth was in BG11 medium (Allen and Stanier, 1968) under continuous light (500–1000 lux) and a temperature of 20° to 28°C. The cyanobacterial suspensions were adjusted to 50% of optical transmission at 650 nm with sterile medium in order that different strains could be tested on a chlorophyll-equivalent basis. Two ml were added to petri dishes with twenty rice seeds.

Exudates. Exudates were collected from axenic cultures cultivated in 11 Fernbach flasks. At the end of the logarithmic phase, they were filtered through Millipore filters (0.45 μ). The filtrate was concentrated 10-fold under vacuum in a Rotavapor R-110 (Buchi) at 40°C in the dark under sterile conditions. This was the crude extract. One fraction of the crude extract was acidified to pH 5 with 0.1 *N* HCl, then filtered (0.45 μ). This was the aqueous fraction. A second fraction was extracted three times with n-butanol (organic fraction), then dried and washed 3 times with sterile water. Aqueous and organic fractions were again concentrated then resuspended in BG11 medium to the same concentration as the crude extract, acidified to pH 5, and filtered (0.45 μ).

Rice

Rice seeds (variety KN 1H 300) were surface-sterilized by agitation under partial vacuum in H_2O_2 (5%) and Tween 80 (1 ppt) for 30 minutes, rinsed 8 times in sterile demineralised water, soaked for 24 hours, then washed 5 more times before 20 each were added to petri dishes with two layers of filter paper and 5 ml fo BG11 medium. The procedure resulted in 94% of the seeds remaining free of contamination seven days after germination in the dark. Germination was determined at 24, 36, and 48 hours. In the cyanobacteria experiments, all dishes received sterile water every two days and were incubated under continuous 800 lux light at 24°. Percent germination, leaf length, and root length were measured after terminating rice growth by cooling the dishes to 4°C.

Results and discussion

Cyanobacterial screening

Effect of cyanobacterial cultures on rice germination. The 133 unialgal strains were checked in four batches, including a control for each (40 rice seeds). The mean rate of germination, observed after seven days of incubation, was lower for rice treated with cyanobacteria (83 $\pm$ 10%) than to the control (94 $\pm$ 5%). This negative effect was observed for 70% of the cyanobacteria tested (Fig. 1). Fifteen strains decreased the rate of germination by 75% or more, including half of the Scytonema strains but only 15% of the Nostoc and 10% of the Anabaena strains tested.

Effect of cyanobacterial cultures on rice shoot elongation. The length of the rice stems measured after seven days showed three responses (Fig. 2):

- no significant difference between the control and 90 of the cyanobacterial strains tested.
- a significant stimulation of the growth for 21% of the strains. Among these were 57% of the section 1, 21% of the section 3, 23% of Anabaena strains and 14% of the Calothrix strains.
- a significant inhibition of the growth for 12% of the strains. It occurred mainly among the Nostoc species (33%). The proportion of Anabaena strains inhibiting rice was 50%. There was only one section 1, 2 Scytonema and no Calothrix strains conferring inhibition.

If Scytonema and other strains inhibited rice germination, one might consider that it was mainly a physical effect because most of them produced large sheaths which smothered the rice seeds. But the seeds which germinated showed normal development. It was, however, impossible to make a correlation between the effect on rice growth and inclusion in a taxonomic group. For instance, some Nostoc strains had a marked positive effect. Also the fact that the cultures were non-axenic could

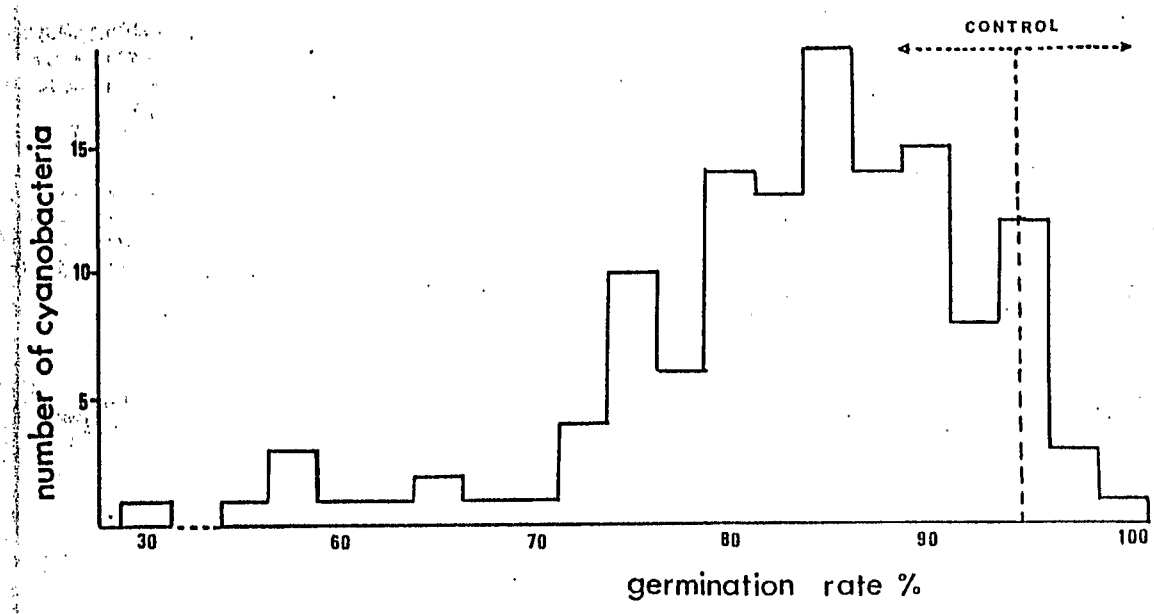

Fig. 1. Rice seed germination rate (variety KN 1H 300) after one week of soaking with one of the 133 cyanobacterial strains tested. The germination rate is determined on 40 seeds. Standard error on the control is illustrated by the *dotted arrows*.

have influenced the results although a second series of experiments with axenic cultures proved similar.

The nature of the positive or negative effect can be judged from the results to be due to the action of one or a combination of contaminants in unialgal cultures, a physical artifact due to coverage by the cyanobacterial culture of the germinating seed, and/or the exudation of growth-regulating substances.

Axenic cyanobacterial cultures

Among the strains with a positive effect on rice

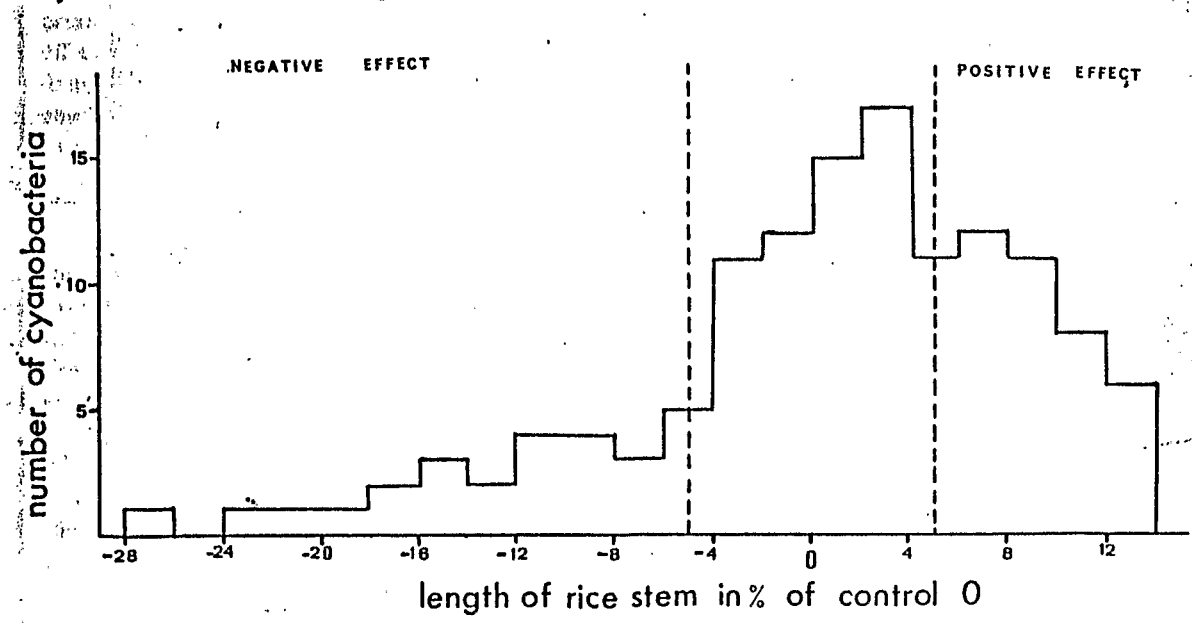

Fig. 2. Length of rice stems (variety KN 1H 300) after one week of incubation with the 133 cyanobacterial strains tested. A positive or a negative effect is mentioned when the average on 40 stems is significantly different from control.

Table 1. Effect of the axenic culture (ax) and the crude exudates (ce) of eight cyanobacteria on germination rate, stem and root size of rice (variety KN 1H 300). Level of significance in regard to the control: a-none; b-5%; c-2.5%; d-0.1%. The asterisk represents a significant difference between the axenic culture and the crude exudates. Results are the means of 60 seeds for the control and for each of the strains

Strains	Trt	Percentage of control		
		Germination	Stem size	Root size
Anabaena sphaerica 2	ax	91.7 a	−3.2 a	−23.3 d*
	ce	98.3 a	−0.5 a	−0.3 a
Chroococcus minor	ax	83.0 b	+5.6 c	+2.2 a*
	ce	91.7 a	+0.8 a	−22.7 d
Chroococcus minutus	ax	83.0 b	−3.2 a	−7.8 a
	ce	86.7 b	+1.5 a	+1.0 a
Microcoleus sp.	ax	90.0 a	+2.9 a	−17.7 d
	ce	93.3 a	+1.0 a	−16.5 d
Anabaena 77S19	ax	95.0 a	+1.3 a	+11.5 c
	ce	93.3 a	+2.9 a	+10.3 b
Synechococcus cedrorum	ax	96.7 a	0 a	−30.3 d*
	ce	86.7 b	−1.0 a	−8.9 a
Synechocystis aquatilis	ax	95.0 a	−4.6 a	−9.6 a
	ce	86.7 b	−0.5 a	−8.3 a
Tolypothrix nodosa	ax	86.7 b	−3.5 a	−15.1 d
	ce	93.3 a	−3.5 a	−18.2 d

growth eight were axenised and the experiment repeated. The effects of both the axenic strain and its crude exudate were measured on stem growth and on the main rice root seven days after the inoculation (Table 1).

Except for *Chroococcus minor* there was no significant stimulation of stems such as was observed for non-axenic cultures. Root response was more pronounced with an increase of 11.5% for Anabaena 77SS19, an inhibition of 15.1% for *Tolypothrix nodosa*, of 17.7% for *Microcoleus* spp., of 23.3% for *Anabaena sphaerica* 2, and of 30.3% for *Synechococcus cedrorum*.

Treatments with crude extract showed the same results as with axenic cultures for 5 of the 8 strains: no significant effect on rice germination and on rice stems, and a pronounced effect on the growth of the roots.

The fact that seven of these eight strains, tested for their positive effect in unialgal culture showed a negative or neutral effect in axenic culture can be explained in two ways. First the association in unialgal culture between cyanobacteria and other bacteria might stimulate rice growth. Second, the concentration of growth promoting substances and/or their ratio might have been different, thus altering the effect. Anabaena 77S19, the sole strain showing a positive effect in all conditions was used to determine the effect of the exudate at various concentrations on rice growth.

Effect of extracts of Anabaena 77S19 on rice germination

Characterisation of the strain. Anabaena 77S19 was described by Franche and Reynaud (1986). It has barrel-shaped vegetative cells, spherical terminal heterocysts, ellipsoid intercallary heterocysts and spherical akinetes. The doubling time during logarithmic growth in BG11 medium without nitrogen is 30 hours. The strain is not able to perform facultative photoheterotrophic growth, does not synthesize phycoerythrin or phycoerythrocyanin, and is resistant to gentamycin and mitomycin C. Three plasmids of 32, 25 and 14 million daltons were observed.

Rice seed germination. The extracts at the 10-fold concentration delayed germination (Table 2). This effect was important in the first 24 h, but decreased afterwards. Three days after soaking the seeds, the percent germination was always significantly lower in high concentrations of exudates. It was in the organic fraction that the inhibition was greatest.

Table 2. Effect of extracts of axenic cultures of Anabaena 77S19 on germination at 24 h, 36 h, and 48 h and on the average length of stems and roots of 4 day old seedlings (variety KN 1H 300). Treatments marked with the same letters in the same column are not significantly different at the 1% level

Treatment	Dilution	No seed germinated			Length after 4 days	
		24 h	36 h	48 h	Root	Stem
Control		18 a	18 a	20 a	88 a	55 a
Crude extract	10	0 d	12 c	18 abc	63 abc	40 de
	1	10 c	20 a	20 a	77 ab	47 bcd
	0.1	18 a	19 a	20 a	81 a	56 a
	0.01	16 ab	19 a	20 a	87 a	57 a
Aqueous fraction	10	2 d	14 bc	17 cd	54 bcd	43 cd
	1	13 abc	19 a	19 ab	84 a	50 abc
	0.1	14 abc	18 ab	18 abc	80 a	53 ab
	0.01	12 bc	16 b	16 d	77 ab	57 a
Organic fraction	10	1 d	11 c	17 cd	34 d	32 e
	1	16 ab	19 a	19 ab	83 a	53 ab
	0.1	16 ab	18 ab	20 a	87 a	55 a
	0.01	18 a	20 a	20 a	86 a	55 a

Size of rice stems and roots. Four days after soaking, the size of rice stems and roots was significantly lower in the 10-fold concentrated treatment compared to the control (Table 2), with the organic fraction being most inhibitory.

With the living culture of Anabaena 77S19, there was a significant positive effect on rice growth: with crude extracts, there was no effect although the effect was positive in Table 1. The separation of the exudates of Anabaena 77S19 into three fractions showed that the organic fraction was most inhibitory, corresponding to the possible presence of gibberellic acid-like constituents (Singh and Trehan, 1973).

The internal balance among growth regulators in the seed is the chief cause of dormancy or germination (Khan, 1975). This balance is affected by external factors such as growth-promoting or germination regulators produced by cyanobacteria. In an earlier study (Gupta and Shukla, 1969) the increase in growth of rice associated with a unialgal culture of *Phormidium* sp. varied as a function of the fraction, the concentration, and the culture from which the extract was obtained. For Anabaena 77S19, it is possible that during the preparation of the extracts one or more growth-promoting constituents was denaturated or changed in concentration causing an inverse effect. Another possibility is that exudation of growth-promoting substances resulted from synergism between the living Anabaena and the rice seed: obviously impossible with extracts. The extremely fragile constitution of such a positive balance among all the growth regulators of both the rice seed and the cyanobacteria probably explains the low rate of positive effects among the cyanobacteria tested.

Conclusion

In the paddy field presoaking rice seeds in a cyanobacterial non-axenic and often non-unialgal culture should be done with caution or avoided altogether because the effects on germination are uncertain but mostly negative, In dry tropical Africa the ecological succession in paddy fields is such that the best time to inoculate with cyanobacteria is at the beginning of tillering (Reynaud, 1987) thus limiting possible problems from growth-promoting substances at the young stages of rice growth.

Acknowledgements

This work was supported by the French Institute for Cooperative Research and Development (ORSTOM). The authors thank Blaine Metting for

reviewing the manuscript and R & A Plant-Soil, Inc. for use of facilities.

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