SURVIVAL AND EFFICIENCY OF N_2-FIXING CYANOBACTERIA IN SOIL UNDER WATER STRESS

A. L. E. MAHMOUD*
A. A. ISSA*
M. H. ABD-ALLA*

SUMMARY: Survival of five genera of N_2-fixing cyanobacteria were studied under salt and drought stress in clay and sand soil. These conditions considerably decreased the survival of the tested organisms. Nitrogenase activity was also decreased and this could be attributed to the reduced of heterocyst frequency under the experimental conditions. Apparently, Nostoc microscopicum and Rivulara natans appeared to be water stress-tolerant species and remained for long period. There is a scope for selection of cyanobacterial species more tolerant to harsh conditions to prepare commercial inoculants for Agronomic practice.

Key Words: Cyanobacteria, nostoc microscopicum, rivulara natans.

INTRODUCTION

Microorganisms have frequently been introduced into soil to promote certain agriculturally-beneficial activities, e.g. nitrogen fixation, suppression of plant pathogens, and promotion of plant growth (1). However, inoculants may fail in field trails (2). Such inconsistency may be due to a number of factors but the most important of these likely to be differences in establishment and survival of the introduced inoculants (3). However, there is little information concerning the fate of inoculants in field soils.

Strains of cyanobacteria in inoculation must not only be effective in nitrogen fixation but should also be able to persist in the soil following inoculation. Drew and Anderson (4) studied the survival of algae under drought stress. They found that about 50-75% of added algae died, when soil moisture holding capacity reached 40 to 20%. Most of the available reports studied the growth and survival of different algae grown in liquid cultures under water-stress. In this respect, Ben-Amotz and Avron (5) found that the specific rate of Asteromonas gracilis, Dunaliella bardawil and Dunaliella salina is not affected by salt concentrations between 0.5 M and 2.0 M and only gradually decreased in salt concentrations above 2.0 M. Mohammed and Shaffa (6) stated that, 200 mM NaCl considerably decreased the cell numbers of *Scenedesmus obliquus* incubated for 7 days.

Few studies are available on the survival of cyanobacteria under drought and salinity stress. Our aim was to study survival of five genera of N_2-fixing cyanobacteria introduced into different soils under water stress, to select strain-resistant conditions for preparation of commercial inoculants.

MATERIALS AND METHODS

Organisms

The five genera experimented with, namely, Anabaena ascillaroides; *Calthorix wambaereasis, Nostoc microscopicum; Rivulara natans* and *Tolypothrix tenuis* were isolated from

* From Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.

grasses-cultivated soils in Assiut (Egypt) and then purified to be unialgal axenic cultures by applying a combination of Pringsheim (7) and Pinter, Provasoll (8) methods. The pure isolates were grown separately in Stewart medium (9) to obtain a mass productions.

Treatments

1gm of these organisms in ratio (1:1:1:1:1) was added to autoclaved clay and sand soils in pots containing 150 g soils in two group experiments. One salinized by three concentrations of NaCl (0.0, 0.5 and 1%) and another group was subjected to 100%, 25% and 12.5% of field capacity. The batch was incubated in light place at 25 ± 1°C for 40 days. The survival of algae were tested after 10, 20, 30 and 40 days by algal counts, heterocyst frequency and nitrogenase activity.

Algal counts

About 1 cm soil surface was moistened with Bold's basic mineral medium in petri dishes containing agar. The cultures were kept under 16 h-1 light day at 25 ± 1°C. After three weeks cultures were examined under a microscope to determine the numbers of colonies present for each organism.

Heterocyst frequency

Material for heterocyst observation was fixed in Lugol's iodine solution. Heterocysts were counted microscopically and their frequency was expressed as percentage of total cell population and was estimated by means of triplicate counts of at least a thousand cells in each sample (10).

Nitrogenase activity

Nitrogenase activity of soil samples was determined as previously described (11) using spectrophotometric method of LaRue and Kurz (12).

RESULTS AND DISCUSSION

Survival of five species related to 5 genera of N_2-fixing cyanobacteria incubated in clay and/or sand soil at different rates of drought is presented in Table 1. Populations of the five species decreased with increased the drought. There was a significant reduction in the populations of *Anabaena, Calthorix* and *Tolypothrix* however, survival of *Nostoc* and *Rivulara* remained throughout the period of this study under drought stress. Noticeable drop in heterocyst development occurred at 25% of field capacity. Nitrogenase activity was considerably decreased with a lower of soil moisture content. No reports available on the survival of cyanobacteria in soil. However, in reported cases; inoculants numbers of different microorganisms decreased soon after introduction into the soil with a low moisture content (13-17).

The effect of different levels of salt on the survival of different species of N_2-fixing cyanobacteria is shown in Table 2. Data of this study indicate that salinity at 0.5 and 1% reduced the survival of *Anabaena* and *Calthorix* up to

Table 1: Survival and efficiency of N_2-fixing cyanobacteria in soil under drought stress.

		Clay soil											Sand soil												
		Days ofter treatments											Days ofter treatments												
% field capacity		10			20			30			40			10			20			30			40		
		100	25	12.5	100	25	12.5	100	25	12.5	100	25	12.5	100	25	12.5	100	25	12.5	100	25	12.5	100	25	12.5
Anabaena	CN	16	8	3	12	-	-	10	-	-	2	-	-	9	-	-	-	-	-	-	-	-	-	-	-
ascillaroides	HF	22.1	7.8	3.9	13.1	-	-	9.6	-	-	6.2	-	-	8.2	-	-	-	-	-	-	-	-	-	-	-
Calthorix	CN	10	4	2	7	4	3	6	2	1	-	-	-	8	5	-	6	4	-	3	2	-	4	2	-
wambaereasis	HF	14.6	8	3.4	8.3	5.2	4.1	8.1	5.1	4.3	-	-	-	6.6	7.1	-	9.1	7.0	-	6.1	5.6	-	9.1	5.7	-
Nostoc	CN	15	11	11	8	9	10	9	7	8	11	10	8	7	5	4	6	5	5	6	6	6	5	3	3
microsopium	HF	13.2	13.6	14.2	12.5	11.6	11.8	10.1	11.1	9.6	10.2	9.6	10.1	12.8	11.0	7.4	7.8	8.6	8.1	8.5	10.3	6.9	11.0	10.6	9.1
Rivularia	CN	17	11	11	12	11	10	6	4	3	2	2	2	9	6	3	8	6	8	6	5	2	4	2	-
natans	HF	20.2	19.1	17.2	15.6	17.2	13.8	9	6.3	5.2	4.6	4.1	5.0	9.1	10	7.1	10.1	4.9	10.3	7.6	7.1	5.6	6.3	5.1	-
Tolyporthrix	CN	11	3	2	9	1	-	5	-	-	1	-	-	6	-	-	2	-	-	1-	-	-	-	-	-
tenuis	HF	13.2	6.0	4.2	10.3	5.1	-	7.2	-	-	5.3	-	-	4.9	-	-	4.0	-	-	4.0	-	-	-	-	-
	TCN	69	37	29	48	25	23	36	13	12	16	12	10	39	16	7	22	15	13	16	13	8	13	7	3
	HF	16.7	10.9	8.6	12	7.8	5.9	8.4	4.5	3.8	5.3	2.7	3	7.4	4.8	2.9	6.2	4.1	3.7	5.2	4.6	2.5	5.3	4.3	1.8
	Nase	11.3	6.8	4.1	8.3	4.2	2.6	3.2	1.8	0.6	2.2	0.6	0.0	6.2	4.3	1.9	4.0	3.2	1.5	1.8	0.6	0.0	0.0	0.0	0.0

CN: Colonies number HF: 9 heterocyst frequency

Nase: nitrogenase (nmol/g soil/h) TCN: Total colonies number

30 days. After this period, they completely disappeared. However, *Tolypothrix tenuis* survived for 20 days only. The data clearly indicate that *Nostoc microscopicum* and R*ivulara natans* appeared to be salinity-tolerant species and remained for 40 days. Under different salinity levels used, the nitrogenase activity decreased in comparison with the control. This may be due to the reduced in heterocyst frequency under these treatments.

It is worthily to mention that there was no colony of any tested species appeared after 50 days under the conditions of the present investigation.

NaCl is known to be toxic to cyanobacteria at high concentration. Shatta *et al.* (18) and Mahmoud *et al.* (19) found that nitrogenase activity of cyanobacterial strains was inhibited by NaCl concentrations more than 6000 ppm. The response of algae to salinity differs from one type to another. In this respect, the growth of *Chlorella emersonii* (20), *Anacystis nidulans* (21) and *Scenedesmus obliquus* (6) were variably reduced in response to salinization treatments.

Results reported here show differences in the ability of cyanobacterial species to colonize soil under severe stress and to survive for along time. *Nostoc microscopicum* and *Rivulara natans* persisted in soil subjected to water stress more than other tested species. Our results suggest that there is a scope for selection of cyanobacterial species more tolerant to harsh conditions to prepare commercial inoculants for promotion agriculturally-beneficial activities.

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Table 2: Survival and efficiency of N_2-fixing cyanobacteria in soil under drought stress.

		Clay soil											Sand soil												
		Days ofter treatments											Days ofter treatments												
Na Cl		10			20			30			40			10			20			30			40		
%		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
Anabaena	CN	15	11	5	13	10	3	11	7	-	-	-	-	7	3	1	5	2	1	2	-	-	-	-	-
ascillaroides	HF	20.1	13.1	6.9	16.2	15.6	4.2	16.3	10.1	-	-	-	-	8.2	4.1	1.3	4.9	3.0	1.3	1.9	-	-	2	-	-
Calthorix	CN	11	10	11	10	9	9	8	9	7	1	-	-	5	5	4	4	3	3	4	4	2	3	1	-
wambaereasis	HF	13.6	9.2	9.7	8.3	7.1	6.2	6.7	6.3	6.3	4.3	-	-	6.0	7.1	5.9	6.2	4.3	5.1	4.1	3.6	2.7	4.2	5.1	-
Nostoc	CN	14	11	11	10	8	9	9	8	7	6	7	6	8	8	8	6	6	4	6	4	2	4	1	1
microsopium	HF	11.5	10.1	10.1	8.3	7.1	8.1	7.3	6.6	6.8	3.9	5.1	3.8	5.8	9.1	8.2	7.3	6.6	5.2	7.4	5.3	2.8	5.3	4.2	4.3
Rivularia	CN	17	16	15	14	14	13	13	12	11	10	11	9	4	5	3	5	5	4	3	1	1	1	1	1
natans	HF	26.2	22.2	23.6	20.6	20.8	16.7	13.8	11.1	12.5	8.7	9.0	7.3	9.1	6.3	4.8	7	6.6	5.2	4.1	7.1	7.3	4.1	6	4.7
Tolyporthrix	CN	8	3	2	5	1	1	-	-	-	-	-	-	5	-	-	2	-	-	1	-	-	-	-	-
tenuis	HF	10.1	9.1	9.9	7.4	6.8	7.1	-	-	-	-	-	-	4.4	-	-	3.9	-	-	3.9	-	-	-	-	-
	TCN	62	51	42	52	32	35	41	36	25	17	18	15	29	21	16	22	16	12	16	9	5	8	3	2
	HF	16.3	12.7	11.9	12.2	11.5	8.5	8.8	6.8	5.1	3.4	2.8	2	6.7	5.3	4.1	5.9	4.1	3.4	4.3	3.3	2.6	2.7	3.1	1.8
	Nase	12.8	9.3	5.6	8.9	5.9	3.2	6.8	4.1	1.8	4.8	2.7	1	9.3	5.3	3.1	6.2	3.1	2.2	3.2	1.5	0.0	2.8	0.0	0.0

CN: Colonies number

HF: 9 heterocyst frequency

Nase: nitrogenase (nmol/g soil/h)

TCN: Total colonies number

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Correspondence:
A. L. E. Mahmoud
Botany Department,
Faculty of Science,
Assiut University,
Assiut, EGYPT.