Early Communication in the *Gunnera-Nostoc* Symbiosis: Plant-Induced Cell Differentiation and Protein Synthesis in the Cyanobacterium

Ulla Rasmussen, Christina Johansson, and Birgitta Bergman

Department of Botany, Stockholm University, S-10691 Stockholm, Sweden
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Establishment of the symbiosis between the angiosperm *Gunnera* L. and the N_2-fixing cyanobacterium *Nostoc* is achieved by infection of specialized plant stem glands by the cyanobacterium. Here we report the finding that the acidic mucilage secreted by the *Gunnera* glands carries signal molecules that specifically 1) induced the differentiation of *Nostoc* filaments into motile so-called hormogonia, essential for infection. This effect was seen in compatible as well as incompatible strains. Proteinase K treatment of the mucilage abolished hormogonium induction, indicating that the inducing compound was a protein. Neither extracts from different *Gunnera* plant parts, nor seed rinse induced hormogonium formation. In addition, red light as well as darkness induced, while low pH *per se* inhibited hormogonium differentiation. The mucilage also 2) stimulated growth, and 3) rapidly induced a new polypeptide of approximately 40 kDa and an increased synthesis of a 65-kDa polypeptide exclusively in compatible *Nostoc* strains. Hence, we conclude that mucilage secreted by the *Gunnera* glands is a major component in a signaling system between the plant and the cyanobacterium, and that establishment depends on both symbiosis-specific and unspecific events.

Additional keywords: gland, hormogonium differentiation, mucilage, plant-cyanobacterial signaling, symbiosis-specific proteins.

Cyanobacteria form symbiosis with a variety of organisms, ranging from protists and fungi to plants and animals (Rai 1990; Bergman *et al.* 1992b). In many cases, cyanobacteria provide a source of photosynthetic products, while in others their prime role is to fix atmospheric nitrogen (N_2). *Gunnera* L. is the only angiosperm that enters into symbiosis with a cyanobacterium. In nature, this plant is invariably infected by members of the N_2-fixing cyanobacterial genus *Nostoc* (see Bonnett 1990; Osborne *et al.* 1991; Bergman *et al.* 1992a). *Nostoc* resides within the host cells, fixing dinitrogen and exporting a nitrogenous compound to the plant (Silvester and Smith 1969). In return, the plant supplies the cyanobiont with fixed carbon (Söderbäck and Bergman 1993). Infection is achieved through a process involving several steps (Johansson and Bergman 1992). Specialized mucilage-secreting glands are formed by the *Gunnera* seedling independent of the presence of cyanobacteria. On these, *Nostoc* assembles and differentiates a motile life stage, the hormogonium. Hormogonia move through intercellular channels into the glands. Subsequently, *Nostoc* enters the host cells and undergoes various additional modifications, such as the differentiation of a disproportionally high frequency of N_2-fixing cells, heterocytes (also termed heterocysts; see Komárek and Anagnostidis 1989). New glands are subsequently produced on the stem as the plant grows, and each gland is separately infected by *Nostoc*.

Even though the formation of hormogonia is not the only prerequisite for establishment of cyanobacterial symbioses (Campbell and Meeks 1989; Johansson and Bergman 1994), it is of crucial importance (Bergman *et al.* 1992b). For instance, strains unable to form hormogonia are incapable of infection, while among the hormogonium-producing *Nostoc* strains, a correlation was found between the extent of hormogonium production and the number of infected glands (Bonnett and Silvester 1981). Furthermore, cocultivation of *Nostoc* with the hornwort *Anthoceros punctatus* L. results in the induction of hormogonium differentiation in compatible (Campbell and Meeks 1989) and, as in the *Gunnera* symbiosis (Johansson and Bergman 1994), in some incompatible strains. Likewise, cocultivation of *Nostoc* and wheat also leads to differentiation of hormogonia (Gantar *et al.* 1993).

The hormogonium is a part of the normal life cycle of many heterocyteous cyanobacteria, including *Nostoc*, and differentiates under adverse conditions. Hormogonium differentiation is preceded by rapid cell divisions without concomitant expansion in volume, which also precede other types of differentiation (Campbell *et al.* 1993). Hormogonia are short filaments that differ from the "parental trichome" by 1) cell size or shape, 2) the lack of heterocytes even in media without combined nitrogen, 3) the presence of gas vacuoles, or 4) gliding motility over solid substrates (Rippka *et al.* 1979). In some cases, they may serve for survival under harsh conditions (Damerval *et al.* 1991), and those that exhibit gliding motility or form gas vacuoles function as an efficient means of dispersal in nature. Hormogonium differentiation can be induced by a variety of treatments, such as dilution or transfer of a culture to fresh medium or from a medium with nitrate to one without (Herdman and Rippka 1988), transfer to a solid substrate (Hernández-Muniz and Stevens 1987), incubation under red light (Robinson and Miller 1970; Tandeau de Marsac *et al.* 1988; Damerval *et al.* 1991; Campbell *et al.* 1993) and auxin treatment (Bunt 1961). Any specific genes or gene products involved in hormogonium differentiation still awaits identification.

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Exchange of chemical signals, and the responses to these, are important events in all plant-microbe interactions, and have received considerable attention during recent years. In particular, signal molecules and specific genes important during establishment of legume-rhizobial symbioses have been studied extensively (e.g., Fisher and Long 1992; Verma 1992; Denarié and Cullimore 1993). Although there are a number of indications that communication also occurs in plant-cyanobacterial symbioses (Bonnett and Silvester 1981; Bergman *et al.* 1992a; Johansson and Bergman 1992), the molecular basis of such a communication has so far not been investigated.

Being itself unable to move in search of a suitable cyanobacterial partner, it lies in the plant's interest to make the cyanobiont move to the plant and ultimately to the susceptible plant organ. We have therefore examined the ability of *Gunnera* to induce the differentiation of motile hormogonia. In addition, the possible role of *Gunnera* mucilage in communication between the plant and the cyanobacterium was investigated. Evidence for the presence in *Gunnera* mucilage of signal molecules that induce hormogonium differentiation and protein synthesis in *Nostoc* is presented.

RESULTS

Hormogonium induction.

The capacity of various plant solutes to induce hormogonia in the compatible strain *Nostoc* PCC 9229 was studied using

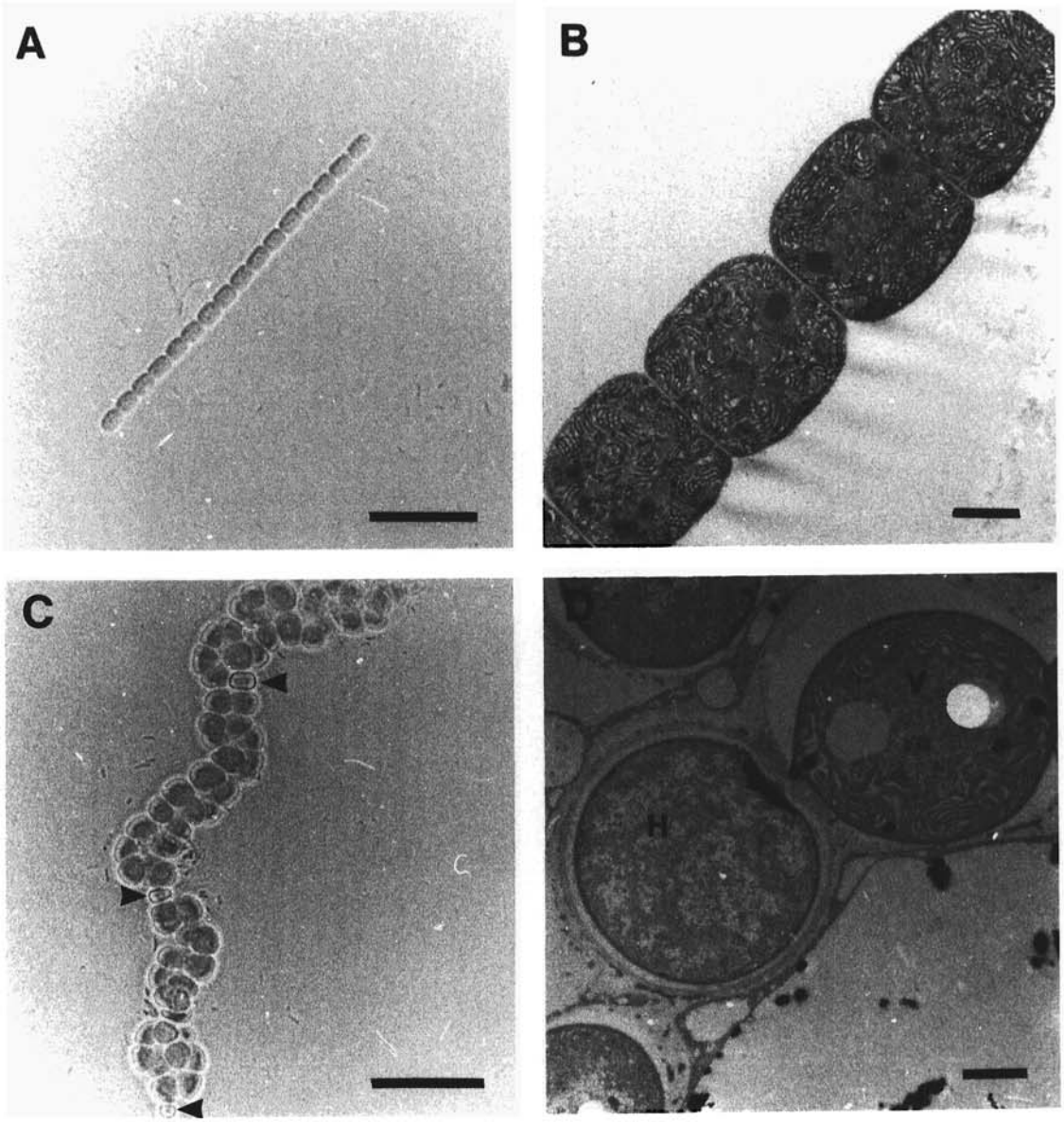

Fig. 1. Two important stages during the life cycle of *Nostoc*. **A,** A light micrograph of a motile *Nostoc* hormogonium. The filament is composed of narrow, uniform cells and is tapering at the ends. **B,** A transmission electron micrograph of a *Nostoc* hormogonium. In contrast to hormogonia in e.g. *Calothrix*, *Nostoc* hormogonia do not have gas vacuoles. **C,** A light micrograph of a heterocyteous *Nostoc* filament (aseriate stage). This is composed of round vegetative cells interspersed with heterocytes (arrowheads). **D,** A transmission electron micrograph of a heterocyteous *Nostoc* filament, with vegetative cells (V) and thick-walled heterocytes (H). Bars represent 20 μm in A and C, 1 μm in B and D.

extracts of sterile *Gunnera* seedlings, mucilage, and seed rinse. Heterocyteous filaments (Fig. 1) were incubated 15–20 hr in the presence of the putative inducer. Among the plant solutes only the mucilage possessed a strong inducing activity (Fig. 2). Induced hormogonia were short, motile filaments composed of uniform, cylindrical cells without gas vacuoles, and had tapered end cells (Fig. 1). Suspending the cells in the growth medium BG-11_0 did not result in hormogonium induction, and BG-11_0 with incubation under white light was therefore included as a negative control. Mucilage from fully mature infected and fertilized *Gunnera* plants, as well as that from noninfected seedlings grown in the absence of combined nitrogen gave similar results (data not shown). Also mucilage from different *Gunnera* species (Table 1) gave similar results (data not shown).

Induction of hormogonia by *Gunnera* mucilage was compared using different *Nostoc* strains previously identified (Table 2; Johansson and Bergman 1994) as compatible (PCC 9229 and PCC 9230) or incompatible (PCC 6310 and PCC 7422). As can be seen in Figure 3, mucilage induced abundant hormogonia in all strains tested. No statistically certified differences in numbers of induced hormogonia were found between the different strains (Median test, $P = 0.01$).

Next, the inducing capacity of the mucilage was examined in more detail using the compatible strain *Nostoc* PCC 9229 (Figs. 4–6). Red light induced hormogonia to the same extent as did *Gunnera* mucilage in white light (Fig. 4; Mann-Whitney test, $P = 0.001$), and was therefore used as an abiotic positive control in some experiments. Since the *Gunnera* mucilage has a pH between 4 and 5 (Söderbäck 1992), it was of interest to determine whether the induction of hormogonia was an effect of low pH. For this purpose, BG-11_0 medium (pH 9) was buffered to pH 5 and used for incubation with

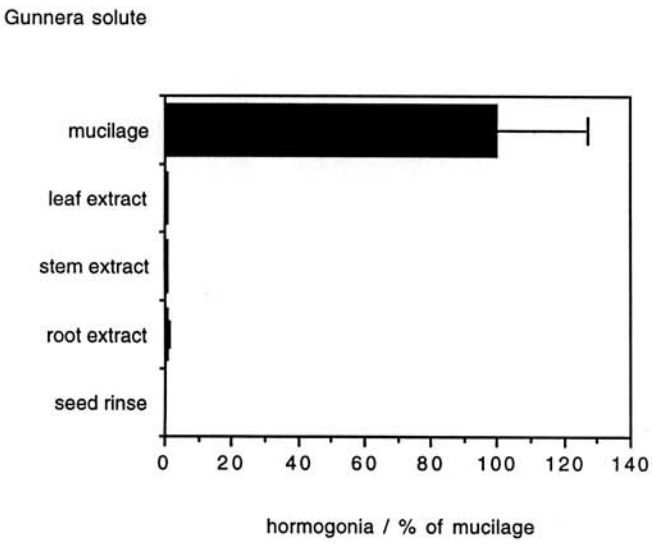

Fig. 2. Induction of hormogonium differentiation in the compatible strain *Nostoc* PCC 9229 by different *Gunnera* solutes. The amount of hormogonia induced by each solute is given as a percentage of the number obtained in mucilage. Horizontal lines show SE of the mean.

Table 1. *Gunnera* plant material used in the study

Species	Growth conditions	Locality
Gunnera sp.	Greenhouse	Stockholm, Sweden
G. chilensis	Garden	Gothenburg, Sweden
G. chilensis	Garden	Kew, Richmond, UK
G. chilensis	Garden	Waikato, New Zealand
G. manicata	Axenic seedlings in the lab.	Stockholm, Sweden

Table 2. Source and compatibility of the *Nostoc* strains used

Strain	Source	Compatibility[a]
Nostoc PCC 9229	*Gunnera monoika*	+
Nostoc PCC 9230	*G. chilensis*	+
Nostoc PCC 73102	*Macrozamia* sp.	+
Nostoc PCC 7422	*Cycas* sp.	–
Nostoc PCC 6310	Pond	–

[a] Compatible strains infecting *G. manicata* Linden seedlings are denoted by (+), incompatible by (–) (Johansson and Bergman 1994).

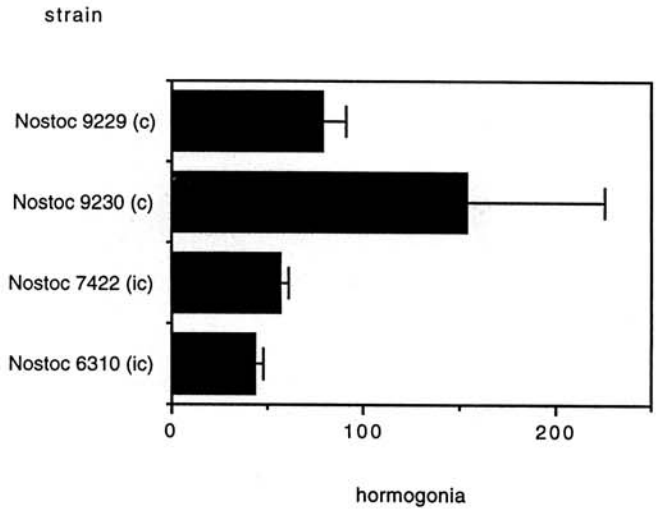

Fig. 3. The number of hormogonia induced by *Gunnera* mucilage in different *Nostoc* strains; the compatible (c) *Nostoc* PCC 9229 and *Nostoc* PCC 9230, and the incompatible (ic) *Nostoc* PCC 7422 and *Nostoc* PCC 6310. Horizontal lines show SE of the mean.

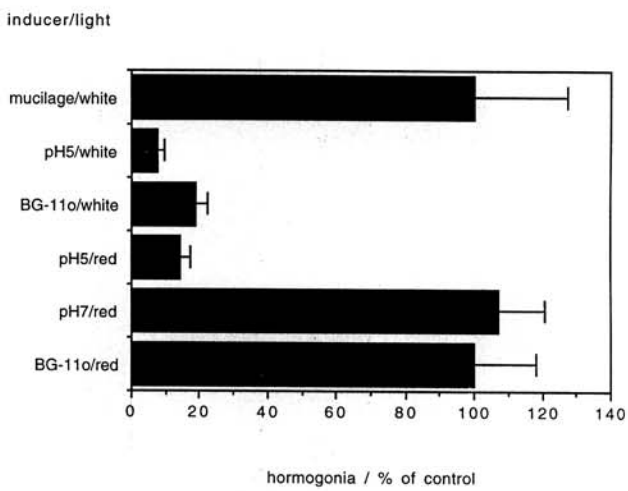

Fig. 4. Effects of pH on the induction of hormogonium differentiation in the compatible *Nostoc* PCC 9229. The amount of hormogonia induced is given as a percentage of the number obtained in mucilage under white light or in unbuffered BG-11_0 under red light. Horizontal lines show SE of the mean.

Nostoc cells. However, hormogonia were not induced (Fig. 4; Mann-Whitney test; $P = 0.001$). The same result was obtained when the induction was performed under red light, while a medium buffered to pH 7 allowed red light-induction to the same extent as did unbuffered BG-11_0. These data indicate that low pH had an inhibitory effect on hormogonium formation in this strain.

In search of a hormogonium-inducing factor, *Gunnera* mucilage was dialyzed at different molecular weight cutoffs. Dialysis at M_r cutoff 12–14 kDa led to a loss of the inducing capacity (Fig. 5; Mann-Whitney test, $P = 0.001$), whereas inductive activity was retained after dialysis at M_r cutoff 0.5 kDa (Mann-Whitney test, $P = 0.001$). Furthermore, when mucilage was treated with proteinase K, hormogonium induction was abolished (Mann-Whitney test, $P = 0.001$). Autoclaving the mucilage also led to the loss of inductive capacity. Thus it appears that the mucilage contained a hormogonium-inducing, heat-labile protein between 0.5 and 12 kDa.

Since *Nostoc* enters the dark gland channels, the ability of the mucilage to induce hormogonia in darkness was tested (Fig. 6). Darkness itself induced hormogonia in BG-11_0 to some extent (65% of the value in mucilage/white light). Mucilage added in the dark, however, induced twice as many hormogonia as did mucilage in white light.

Growth responses to *Gunnera* mucilage.

A simple experiment designed to demonstrate a chemotactic response by cyanobacteria to *Gunnera* mucilage was performed. A cyanobacterial suspension was placed on agar plates at a distance of 1 cm from drops of mucilage or BG-11_0. The compatible strain PCC 9229 and the incompatible PCC 6310 were used. Hormogonia present in the suspension and formed on contact with the solid substrate moved randomly in all directions from the inoculation site, and no chemotactic response was observed. Nevertheless, after a prolonged incubation time (about 2 wk), the compatible strain adjacent to the mucilage had grown more than when adjacent to BG-11_0 (Fig. 7A, C). No such stimulation of growth was observed with the incompatible strain (Fig. 7B, D).

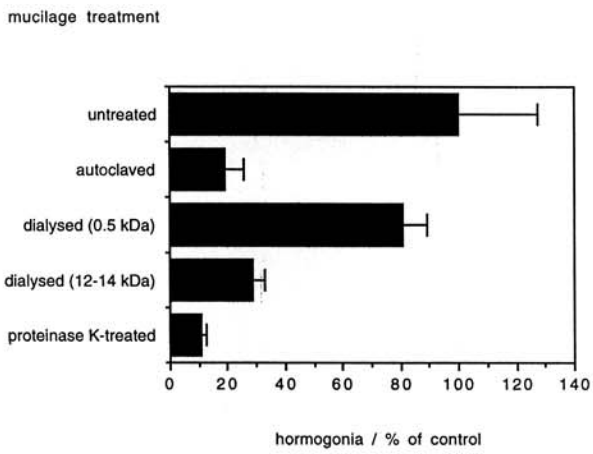

Fig. 5. Effects of different treatments of *Gunnera* mucilage on its ability to induce hormogonia. The amount of hormogonia induced is given as a percentage of the number obtained in untreated mucilage. Horizontal lines show SE of the mean.

***In vivo* labeling of *Nostoc* proteins.**

To determine whether *Gunnera* mucilage was capable of activating any specific genes in *Nostoc,* protein synthesis was investigated by *in vivo* labeling with ^{35}S-methionine (^{35}S-Met) and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The protein banding patterns from non-induced cells, cells induced with mucilage, and cells induced with the abiotic factor red light were compared. Interestingly, in the compatible strains *Nostoc* PCC 9229 (Fig. 8A) and *Nostoc* PCC 73102 (data not shown), a new polypeptide with a molecular weight of approximately 40 kDa was specifically induced in the mucilage-treated cells. In addition, a polypeptide with a molecular weight of 65 kDa, was found to be more abundant after mucilage treatment than in the non-induced or red light-induced controls. Occasionally, an additional polypeptide of 25 kDa was also detected in mucilage-treated cells. Nonetheless, no proteins were induced, irrespective of treatment, in the incompatible *Nostoc* PCC 6310 (Fig. 8B) and *Nostoc* PCC 7422 (data not shown). Because the mucilage used was unsterile, proteins synthesized by possible microorganisms other than *Nostoc* were analyzed. No polypeptides at 40 or 65 kDa were detected (data not shown).

DISCUSSION

Establishment of the *Gunnera-Nostoc* symbiosis depends on a cascade of events, some of which are symbiosis-specific in the sense that only compatible *Nostoc* strains are involved, while at least one is unspecific (hormogonium induction). Here we demonstrate that the acidic mucilage secreted by the *Gunnera* stem glands has a most important role during the early phases of infection, when the cyanobacterium is still extracellular (stages I–III; Johansson and Bergman 1992). It contains at least one, probably several, signal(s) that act(s) on the cyanobacterium.

Infection of *Gunnera* relies on the ability of a compatible cyanobacterium to reach the infection-susceptible *Gunnera* cells. Since the plant is sessile, cyanobacterial motility may serve a purpose to reach 1) the plant, 2) the gland, and 3) the interior of the gland. Indeed, induction of motility in the microsymbiont must be a major early event in any plant symbiosis. The fact that neither seed rinse nor extracts of seedling

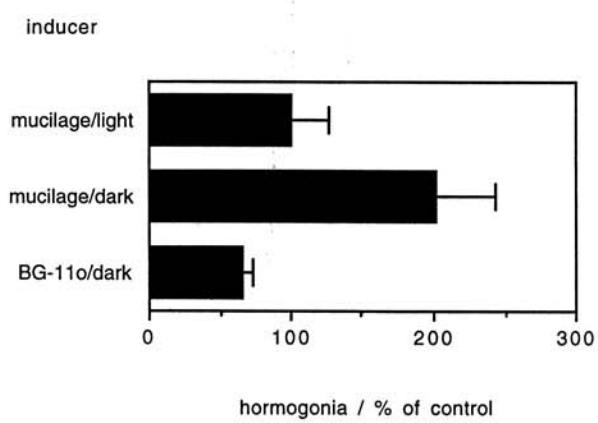

Fig. 6. Effects of darkness on hormogonium induction. The amount of hormogonia induced is given as a percentage of the number obtained in mucilage under white light. Horizontal lines show SE of the mean.

tissue induced hormogonia implies that the cyanobacterium reaches the gland by chance. This contrasts to the situation in legume-*Rhizobium* symbioses, where compounds (e.g., flavonoids) released by seeds and roots serve as attractants (Gaworzewska and Carlile 1982; Munoz Aguilar *et al.* 1988). Abundant hormogonia were previously observed on the surface of glands of inoculated *Gunnera* seedlings (Bonnett 1990; Johansson and Bergman 1992). In view of the present study, the differentiation of these was undoubtedly induced by the *Gunnera* mucilage, which obviously carries an inducer of hormogonium differentiation and plays an important role for the two latter of the steps given above. We also confirm that the capacity of hormogonium formation is not sufficient for successful establishment of the symbiosis (Bonnett and Silvester 1981; Johansson and Bergman 1994), as hormogonia are induced in both compatible and incompatible strains.

Since cyanobacteria generally prefer neutral or alkaline habitats (Fogg 1973), the acidity of the mucilage might in itself be a "hostile" factor leading to the formation of hormogonia. Notwithstanding, our data show that the differentiation of hormogonia is not a consequence of the low pH, which in fact inhibited hormogonium formation under red light, but is due to a biotic factor in the mucilage. This factor is sufficiently potent to override any negative effects of the low pH, possibly also those seen on hormogonial motility. In *Nostoc cycadae* the gliding rate at pH 4–5 is only about half the rate at pH 7–9 (Hirose 1987). Nevertheless, since both acidic growth conditions and acidic polysaccharides alter gene expression in *Agrobacterium* (Winans *et al.* 1988; Rong *et al.* 1990), the acidity of the mucilage may be of significance for the interaction.

The hormogonium-inducing factor present in *Gunnera* mucilage is a heat-labile protein between 0.5 and 12 kDa. It may be constitutively produced since it is present in mucilage from axenic, non-infected seedlings grown in the absence of combined nitrogen, as well as in mucilage collected from infected and fertilized plants, but it cannot be excluded that the factor is synthesized only by non-infected glands. Extracellular products from the hornwort *Anthoceros* (Campbell and Meeks 1989) and wheat roots (Gantar *et al.* 1993) also induce hormogonia in *Nostoc*. Infected and non-infected *Anthoceros* thalli produce the heat-labile factor, less than 12 kDa in size, in the absence of exogenous combined nitrogen. In contrast, wheat produces a hormogonium-inducing factor irrespective of the source of nitrogen.

Similar to the *Anthoceros* hormogonium-inducing factor (Campbell and Meeks 1989), that of *Gunnera* induced hormogonia both in the light and in the dark. In fact, mucilage and darkness acted synergistically in *Nostoc* PCC 9229. In contrast, hormogonium differentiation in *Calothrix*, another heterocyteous cyanobacterium, is a light-dependent process, regulated by the redox state (Campbell *et al.* 1993). This, as well as the inhibition of red light-induction by low pH, may indicate that two different pathways leading to hormogonium differentiation exist in cyanobacteria.

Considering that hormogonia normally show positive phototaxis, compatible strains supposedly are chemotactically attracted towards the dark interior of the gland. *Gunnera* mucilage is a would-be candidate as an attractant or a carrier of such a compound. Nevertheless, *Nostoc* hormogonia were not attracted towards *Gunnera* mucilage under the circumstances tested. Instead, the mucilage apparently contained a growth-

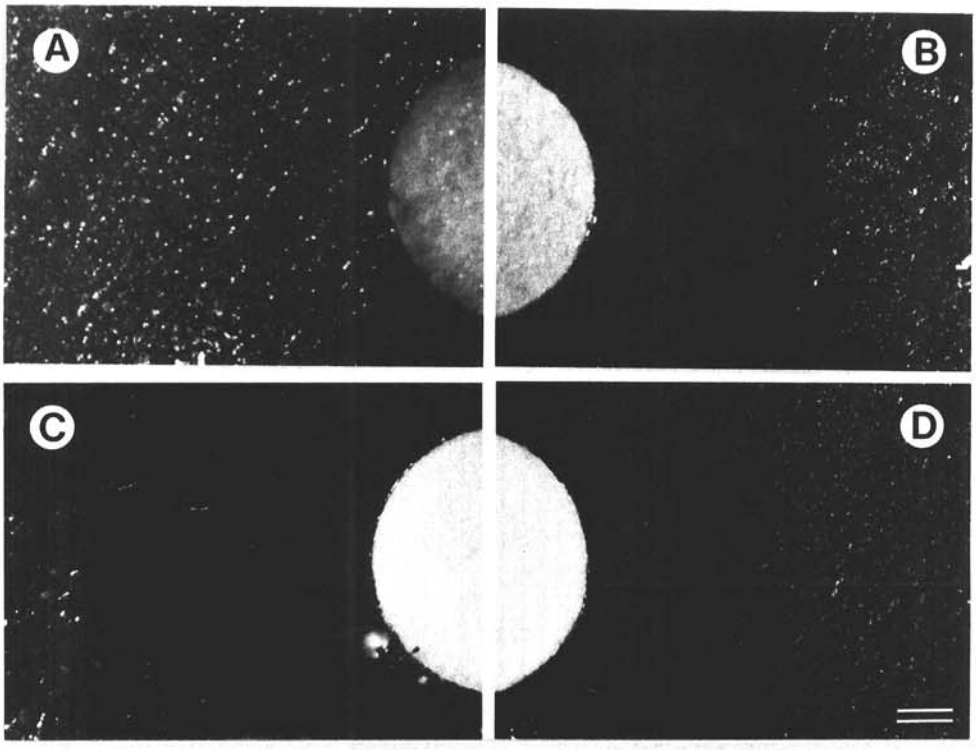

Fig. 7. Growth on agar plates in response to the presence of *Gunnera* mucilage **(A, B)** or BG-11_0 **(C, D)** by the compatible *Nostoc* PCC 9229 (A, C) and the incompatible *Nostoc* PCC 6310 (B, D).

promoting factor, which exclusively affected compatible strains. The remarkable growth stimulation is most likely important for keeping a viable, extracellular population of compatible cyanobacteria for infection of new glands (see Bonnett and Silvester 1981; Bonnett 1990), and might also "force" cyanobacteria into the gland channels.

A third phenomenon caused by the mucilage is the rapid synthesis of at least two specific *Nostoc* proteins (40 and 65 kDa), occurring in compatible strains only. Since these proteins are not detected in red light-induced cells, they are not involved in hormogonium differentiation. Instead, they are probably symbiosis specific, and may have vital functions during the early phases of establishment. Such a symbiosis-specific response has previously not been shown in any symbiotic cyanobacterium, and these proteins also represent the first molecular selectable markers for compatible strains. Further studies will now be carried out at the molecular level to clarify their function and importance.

MATERIALS AND METHODS

Cultivation of cyanobacteria.

Axenic suspensions of five different *Nostoc* strains (Table 2) were cultivated under sterile conditions in BG-11_0 medium (Stanier *et al.* 1971) with continuous light and shaking as described in Johansson and Bergman (1992). The cyanobacteria were obtained from the Collection Nationale de Cultures de Microorganismes, Institut Pasteur, Paris, France.

Plant material.

Seeds of *G. manicata* Linden were kindly provided by H. Zetterlind, Gothenburg Botanic Garden, Sweden. The seeds were surface-sterilized and germinated under sterile conditions, and the seedlings were cultivated as described previously (Johansson and Bergman 1994). The sterile seedlings were subsequently subdivided into leaves, stems, and roots and were mashed separately in small volumes of sterile water. The suspensions obtained were subsequently centrifuged for 5 min at 14,000 rpm, and the supernatant was collected and stored at 4° C until use.

Seed rinse was obtained by soaking 1g of sterile seeds of *G. manicata* in 5 ml of sterile water for 5 days.

Mucilage was collected from *Gunnera* sp. plants (Table 1) grown in the greenhouse of the Department of Botany, Stockholm University and *G. chilensis* Lam. grown outdoors in the Royal Botanic Gardens, Kew, Richmond, UK. *Gunnera chilensis* mucilage was also kindly provided by W. B. Silvester, Department of Biological Sciences, University of Waikato, Hamilton, New Zealand, and H. Wallin, Gothenburg Botanic Garden, Sweden. The mucilage was diluted with water to at least double the original volume and stored at 4° C until use.

Treatment of *Gunnera* mucilage.

The diluted *Gunnera* mucilage was treated as follows:

1) Dialysis (M_r cutoff 0.5 kDa or M_r cutoff 12–14 kDa, respectively [Spectra/Por, Spectrum]) against 10 mM phosphate-buffered saline (PBS), pH 7.4, at 4° C for 1wk.

2) Treatment with 3 mg/ml Proteinase K (Boehringer-Mannheim) overnight at 37° C. The Proteinase K in the treated mucilage was inhibited with 1 mM phenylmethylsulfonyl fluoride (PMSF; Boehringer-Mannheim) for 6 hr, after which the treated mucilage was dialyzed (M_r cutoff 0.5 kDa) against PBS at room temperature until PMSF had been removed (about 1 wk).

Induction of hormogonia.

Cyanobacterial cells of an exponentially growing suspension culture were washed three times with BG-11_0, the fresh weight was determined, and the pellet was resuspended in BG-11_0 at a concentration of 20 mg/ml. For hormogonium induction this suspension was diluted with the putative inducer at a ratio of 1:5. The inducers used were: unbuffered BG-11_0 (pH 9); BG-11_0 buffered to pH 5 and 7, respectively, with 5 mM MES buffer; *Gunnera* seed rinse; *Gunnera* seedling extracts; or untreated or treated mucilage. The cyanobacteria were incubated for 15–20 hr at room temperature in white light, red light, or darkness. After the incubation, the contents of the tubes was mixed and spun briefly, 5-µl samples were taken out, and the number of hormogonia was counted in a Bürkner counting chamber. The results are presented as means of at least three experiments with five replicas per experiment, except for induction by mucilage in darkness, which was only performed once with three replicas.

Cyanobacterial growth responses to *Gunnera* mucilage.

A 5-µl drop of a 50 mg/ml cyanobacterial suspension was laid on the surface of a 1% Bacto-Agar-BG-11_0 plate, 1 cm from a piece of filter paper, which had been allowed to absorb 2 µl of *Gunnera* mucilage or BG-11_0. The compatible strain *Nostoc* PCC 9229 and the incompatible PCC 6310 were used. The agar plates were kept under continuous light at room temperature for approximately 2 wk, during which time the

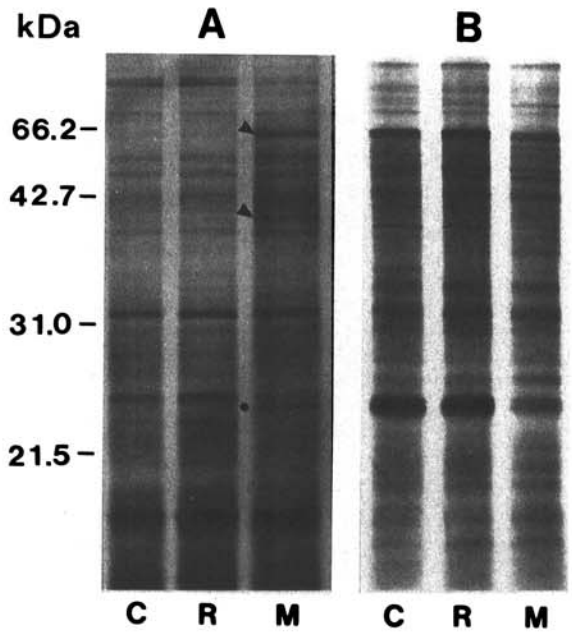

Fig. 8. Protein banding patterns obtained after ^{35}S-Met labeling and SDS-PAGE in non-induced cells (C), cells incubated under red light (R) and cells incubated with *Gunnera* mucilage (M) in the compatible *Nostoc* PCC 9229 (A) and the incompatible *Nostoc* PCC 6310 (B). Arrows indicate polypeptides induced by mucilage. The dot indicates a polypeptide detected occasionally in mucilage-treated cells.

growth of the cyanobacterial inocula was recorded visually and photographically.

In vivo labeling of *Nostoc* proteins.

Suspension cultures of *Nostoc* PCC 9229, PCC 73102, PCC 6310, and PCC 7422 were washed three times in BG-11_0 and centrifuged for 10 min at 14,000 rpm, after which the fresh weight was determined. The pellet was resuspended in BG-11_0 at a concentration of 50 mg/ml. Mucilage or BG-11_0 was added to the cyanobacterial suspension at a ratio of 1:4 (v/v). At the same time, 50 μCi of ^{35}S-Met (specific activity >1,000 Ci/mmol, Amersham Corp.) was added to 1.5 ml of the cultures, and the samples were incubated for 30 min at room temperature. Cell suspensions in BG-11_0 and white light served as non-induced controls, while suspensions in BG-11_0 and red light served as controls of proteins related to hormogonium differentiation. Mucilage-treated suspensions were incubated in white light. The *Nostoc* cells were then pelleted by centrifugation, washed three times with water, and suspended in lysis buffer (62.5 mM Tris/HCl [pH 6.8], 2% sodium dodecyl sulphate [SDS], 10% glycerol). The samples were boiled for 5 min, and the supernatant was collected by centrifugation. Equal amounts of radioactivity were loaded onto 12.5% polyacrylamide gels, and analyzed by SDS-polyacrylamide gel electrophoresis (Laemmli 1970). The gels were dried and subjected to autoradiography with Kodak XAR 5 x-ray film.

Statistical analyses.

The significance of differences between treatments in the ability to induce hormogonium differentiation was assessed statistically using the Median test or the Mann-Whitney test according to Conover (1980).

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