structure[2], reduction in porin level or from the presence of plasmid-specified major envelope components. The possibility that LPS structure is altered in ColV$^+$ strains has not been studied so far, but the other 2 possibilities have been considered with respect to sensitivity to deoxycholate and erythromycin. Firstly it seems unlikely that the increased sensitivity of ColV$^+$ strains to these 2 hydrophobic agents results from a reduction in porin levels because membrane analysis (fig. 1a), studies of ampicillin sensitivity (table 1) and tests of sensitivity to phages T4 and Tula (table 1) suggest that porin levels are normal in the tested ColV$^+$ strains. It seems likely that sensitivity to deoxycholate and erythromycin is not dependent on the presence of the ColV-specified colicin or VmpA protein because some mutants derived from P678-54 ColV form reduced levels of colicin and VmpA protein but are still sensitive to inhibition by the 2 agents (mutant 2, table 2). The presence of ColV-encoded transfer components may be responsible for sensitivity to deoxycholate because repression of such transfer properties (by the *fi*$^+$ plasmid ColB-K98) led to complete DOC resistance.

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The role of zinc in regulating tabtoxin production[1]

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Summary. The phytotoxin, tabtoxinine-β-lactam, is produced by several *Pseudomonas syringae* pathovars if adequate Zn is available, otherwise its biologically inactive form, tabtoxin, is produced. The Zn is required for the action of a peptidase which cleaves tabtoxin, releasing the toxic β-lactam.
Key words. Pseudomonas syringae; phytotoxin; zinc, regulating tabtoxin; tabtoxin; tabtoxin-β-lactam.

The phytopathogenic bacterium *Pseudomonas syringae* pv. *tabaci* and several closely related pathovars are known to produce tabtoxin, a dipeptide which is responsible for the chlorotic leaf symptom associated with infection by these pathogens[2]. Tabtoxin per se is not toxic but when hydrolyzed by either plant or bacterial enzymes produces serine, threonine and the toxic entity, tabtoxinine-β-lactam[3]. This compound irreversibly inactivates glutamine synthetase[4].

It has been known for many years that log-phase cultures of pv. *tabaci* synthesize only tabtoxin. Recently, we found that the addition of 20% intercellular fluid from leaves would induce tabtoxinine-β-lactam, rather than tabtoxin, production[5]. At lower levels of fluid ($< 5\%$) a mixture of tabtoxin and tabtoxinine-β-lactam was produced. This communication reports on an examination of the fluid to determine what factor, or factors, is responsible for regulating which compound is produced.

Materials and methods. Initially, all experiments were done using *P. syringae* pv. *tabaci,* isolate 113. Once conclusive results had been obtained, additional isolates were tested to ascertain if they behaved similarly. The bacteria were grown with agitation in Woolley's medium at 23 °C for three days. The medium was supplemented with 20% filter-sterilized intercellular fluid (v/v), or various treatments of the fluid equivalent to the same concentration. The fluid was obtained by Klement's method[6] from mature leaves of tobacco, *Nicotiana tabacum* 'Havana 38'.

The culture filtrates were quantitatively assayed for tabtoxin and/or tabtoxinine-β-lactam content by running 0.2 ml aliquots of the crude filtrates on an amino acid analyzer (Beckman 118 BL containing W-2 resin).

Results and discussion. Results from the first test showed that the regulating factor in the fluid was heat stable (121 °C for 30 min), thus eliminating the presence of hydrolytic enzymes in the fluid as a possible explanation. This was further substantiated when ultra filtration (YCO5, Amicon Corp.) showed that all the activity was associated with a fraction of mol.wt $\lesssim 500$. Absorption of this fraction onto Chelex-100 (BioRad) showed that the active component(s) was cationic. When ashed (870 °C for 4 h), the low-molecular-weight fraction still retained complete activity.

Next, the medium was supplemented singly with various metal salts at 25 µmolar (i.e., Ca, Mg, Zn. Cu, Co, Ni, Sr, Fe, Mn and Cd). Of these compounds only the one containing Zn was active. Atomic absorption spectrometry of the intercellular fluid showed that its Zn concentration was 2.5 µmolar (analysis was done on a Perkin Elmer 603 with a D_2 arc background corrector, using an air-acetylene flame at 213.9 nm and zinc detection limits of 18 ppb[7]). Supplementation of the medium to this Zn concentration induced tabtoxinine-β-lactam produc-

tion similar to that obtained with intercellular fluid. Tests with 15 additional isolates of six tabtoxin-producing pathovars of *P. syringae* gave the same result.

The effect of Zn is rapidly evident. If it is added to a 3-day-old culture of pv. *tabaci,* all the tabtoxin present is converted to tabtoxinine-β-lactam within 1 h. If however the culture is maintained at 4 °C for 2 h after the Zn addition, there is only a 66% conversion. These effects strongly suggest that the Zn effect is associated with enzymatic activity in the bacterium.

This supposition was confirmed when a hydrolase for tabtoxin, located in the periplasmic space, was shown to require Zn (personal communication, C. Levi). Woolley's medium contains Zn only as a contaminant, and during bacterial growth the pH rises, commonly to eight or more, under which conditions Zn rapidly becomes unavailable. Thus, there normally is not sufficient Zn in the culture to satisfy the hydrolase's requirement, however the addition of either intercellular fluid or Zn will satisfy it. There is though sufficient Zn in the plant, because the extracted intercellular fluid is more dilute than that found *in planta,* and it has an acidic reaction (pH 6.1) which favors Zn solubility. In support of this, the bacterial isolates responding to Zn were found to produce only tabtoxinine-β-lactam in infected plants[5].

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Growth of *Nostoc muscorum* mutants in the presence of diuron (DCMU) and L-methionine-DL-sulfoximine[1]

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Summary. Diuron (DCMU) is inhibitory to the photoautotrophic and photoheterotrophic growth of the N_2-fixing blue-green alga *Nostoc muscorum* at concentrations of 1.0×10^{-5} M and 2.0×10^{-5} M, respectively. A mutant of this organism resistant to 5.0×10^{-5} M DCMU under its photoheterotrophic growth conditions, with the ability to utilize DCMU as a carbon and nitrogen source for growth, and complete inability to grow photoautotrophically has been isolated. With the apparent defect in its photosynthetic ability, it is suggested that the *DCMU*^{r} mutant lacks the step inhibited by 1.0×10^{-5} M DCMU, and metabolizes DCMU by an existing enzyme system in the absence of such inhibition. That this enzyme may be glutamine synthetase (GS) is explained with the help of a L-methionine-DL-sulfoximine (MSO)-resistant mutant of *N. muscorum* which is able to grow faster with 2.0×10^{-5} M DCMU and is known to contain an altered GS.

Key words. Blue-green algae; *Nostoc muscorum;* diuron; L-methionine-DL-sulfoximine; photoautotrophic growth; photoheterotrophic growth.

Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), is known to cause the abolition of photochemically generated reducing power ($NADPH_2$), thus blocking CO_2 assimilation without causing any adverse effect on the generation of ATP through cyclic photophosphorylation in oxygenic photosynthetic organisms[2]. Accordingly, while obligate photoautotrophs fail to recover for DCMU inhibition of growth in the presence of an organic carbon supplement, heterotrophs, including the blue-green algae *Anacystis nidulans*[3] and *Nostoc muscorum*[4], show rapid recovery from DCMU inhibition of growth under similar conditions. DCMU contains methylurea groups, and recent genetic studies have shown that organic sources like methylamine (an inhibitor of photophosphorylation in *Nostoc muscorum*)[5] is utilized by the methylamine-resistant mutant of *N. muscorum* like a metabolizable source of carbon[6] and nitrogen[7]. Therefore, in the present investigation an attempt was made to isolate a diuron-resistant (*DCMU*^{r}) mutant of this heterotroph and to study the metabolic fate of DCMU in such a mutant. Studies on the apparent metabolic utilization of DCMU by the *DCMU*^{r} strain of *N. muscorum* have been helped by the use of a previously-isolated *MSO*^{r} mutant of this organism[8].

Methods. Organisms. The axenic clonal cultures of the parent *Nostoc muscorum* and its *MSO*^{r} mutant strain, obtained from Professor H.N. Singh's personal culture collections, Central University of Hyderabad, India, were grown routinely in modified Chu 10 medium[9] under the culture conditions described previously[8]. While N_2 (nitrogen-free, i.e., at the expense of elemental nitrogen)-growing cultures of these two strains form 5–6 and 12–13% heterocysts, respectively, NH_4^+-growing similar cultures remain non-herocystous (heterocysts are specialized cells which are non-photosynthetic, but are sites of elemental nitrogen fixation)[4].

Physiological assessments. The NH_4^+-growing non-heterocystous parent organism was harvested, washed and pre-suspended in N_2 medium to an OD of 2.5. 2 ml aliquots were then inoculated into fresh sterile N_2 or 1 mM NH_4^+ medium containing or lacking 3 mM glucose, at different concentrations (0 or 1.0 to 2.0×10^{-5} M) of DCMU. All samples were incubated in the growth chamber and their growth (by optical density determinations) on the 10th day, and heterocyst frequency (by microscopic examination) on the 3rd day were assessed. Phycocyanin content of the samples was estimated on the 3rd day of inoculation by the method used ealier[10].

Mutant isolation and characterization. A mutant of the parent *Nostoc muscorum* resistant to 5.0×10^{-5} M DCMU was sought by plating a heavy suspension of the parental culture on solid (1.2% difco bacto agar) N_2 medium containing 5.0×10^{-5} M DCMU, and incubating the set for two weeks in the growth chamber, according to the general method of mutagenesis studies used in *N. muscorum*[11]. A dozen putative *DCMU*^{r} colonies, appearing after a fortnight's incubation, were raised separately into clonal cultures and examined for their growth in N_2 or 1 mM NH_4^+ medium containing or lacking 3 mM glucose, 5.5×10^{-5} M $NADPH_2$ or 5.0×10^{-5} M DCMU. The newly-isolated *DCMU*^{r} and the previously-isolated *MSO*^{r}