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Relationships between cytokinin production, presence of plasmids, and fasciation caused by strains of *Corynebacterium fascians*

[tRNA/6-(3-methyl-2-butenylamino)purine/*cis*-zeatin]

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ABSTRACT **Cytokinin activity in the culture medium of four pathogenic strains of *Corynebacterium fascians* varied from 168 to 0.4 μg of kinetin (6-furfurylaminopurine) equivalents per liter, as compared to 0.2 in an avirulent control. N^6-Isopentenyladenine was the predominant cytokinin in the medium of all five strains, and its increased production was correlated with the degree of pathogenicity; however, the virulent strains also produced 8–13 times more *cis*-zeatin [6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)purine] than the avirulent strain. The three most virulent strains (with the higher cytokinin contents in the medium) contained a large ($M_r \approx 10^8$) plasmid. The barely virulent strain contained a smaller plasmid. No plasmid was detected in the avirulent control. The total cytokinin content (mole %) and biological activity (μg of kinetin equivalent per mg of tRNA) in tRNA were about the same in all three virulent strains and in the avirulent control. Five cytokinins were isolated from each strain. Four were rigorously characterized as 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)-9-β-D-ribofuranosylpurine, 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine, and their 2-methylthio derivatives. The fifth cytokinin was tentatively identified as 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)-9-β-D-ribofuranosylpurine. Ribosyl-*cis*-zeatin was 3-fold higher and N^6-isopentenyladenosine was correspondingly lower in the plasmid-containing strains than in the plasmidless control. Because the entire syndrome of fasciation caused by infection can be induced with synthetic cytokinins, the disease would appear to be caused by plasmid-induced high rates of cytokinin production by the bacteria.**

Corynebacterium fascians causes fasciation disease or witches' broom in dicotyledonous plants. The disease is characterized by release of apical dominance and outgrowth of lateral buds, which gives rise to the characteristic witches' broom syndrome (1). The symptoms of the disease can be duplicated by treatment of seeds or seedlings with cytokinins, indicating that it may be caused by cytokinins produced by the microbe (2). From *C. fascians* culture medium four cytokinins, 6-(3-methyl-2-butenylamino)purine (i^6Ade), 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)purine (*c*-io^{6}Ade), 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)-2-methylthiopurine (ms^2-*c*-io^{6}Ade), and 6-methylaminopurine (m^6Ade), have been characterized, and three additional cytokinins, 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)purine (*t*-io^{6}Ade), 6-(4-hydroxy-3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (io^{6}A), and 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (i^6A), have been tentatively identified (3–6). The tRNA of the bacterium has been reported to contain several cytokinins (7–9), but only *c*-io^{6}A has been rigorously identified (10).

Biosynthesis of the i^6A moiety in tRNA involves the transfer of isopentenyl from Δ^2-isopentenyl pyrophosphate to specific adenosine residues in preformed tRNA (11, 12), and it has been proposed that i^6Ade and other free cytokinins in the culture medium derive from tRNA degradation. The existence of alternative pathways is probable, as suggested by the *in vitro* synthesis of free i^6Ade from AMP and Δ^2-isopentenyl pyrophosphate recently achieved with an enzyme preparation from *Dictyostelium* (13).

This report deals with the kinds and quantities of cytokinins released into culture media and present in tRNA of five strains of *C. fascians*, ranging from highly virulent to avirulent, and examines relationships of the presence of plasmids to cytokinin production and to pathogenicity.

MATERIALS AND METHODS

Origin and Growth of *C. fascians* Strains. Strain MW2 was isolated by and obtained from M. A. Rahman (University of Alexandria, Egypt). Cultures of Cf2 and C616, originating from the Burkholder Collection at Cornell University, were supplied by J. P. Helgeson (University of Wisconsin). Strain Cf1, also originating from the Burkholder Collection, was obtained from K. V. Thimann in 1965, and has been subcultured in our laboratory since that time. Strain Cf15 was provided by M. Starr (University of California-Davis). Cultures were grown routinely at 25°C on Bacto nutrient broth and nutrient agar or on minimal salts/thiamine medium with 0.5% (wt/vol) glucose. For isolation of cytokinin and tRNA, the bacteria were cultured in defined synthetic medium as described (3), and as modified (5) in 1-liter or 10-liter batches at 25°C for 4 days, at which time they reached early stationary phase.

Virulence Tests. Virulence was determined essentially as described by Lacey (14). Seeds of *Pisum sativum* L. var. Dwarf Grey Sugar were soaked for 2 days in sterile water, then immersed in a few milliliters of a 25-hr culture of bacteria for 5 min and planted in sterile vermiculite. Within a week, seedlings treated with virulent strains developed lateral shoots, whereas untreated seeds and those exposed to avirulent strains developed single main shoots

Determination of Number of Plasmids. Cultures (36 hr) grown in minimal salts/thiamine plus glucose, 0.05% (wt/vol) yeast extract, and 0.1% (wt/vol) peptone were diluted with the same medium to 0.1 A_{540} unit and incubated for an additional hour with 0.25% (wt/vol) glycine. [^{3}H]Adenosine (10 μCi/ml; 1 Ci = 3.7×10^{10} becquerels) was added in three portions at 1-hr intervals. The cells were incubated for one more hour and harvested by centrifugation at 10,000 × *g* for 20 min. Lysates were prepared by the method of Sharp *et al.* (15); 0.1–0.2 ml of lysate was layered on 5–20% (wt/vol) sucrose gradients prepared in 300 mM NaOH/500 mM NaCl/20 mM EDTA.

Abbreviations: kinetin, 6-furfurylaminopurine; KE, kinetin equivalent; TMS, trimethylsilyl; zeatin, io^{6}Ade; *c*- or *t*-io^{6}A, 6-(4-hydroxy-3-methyl-*cis* or *trans*-2-butenylamino)-9-β-D-ribofuranosylpurine; *c*- or *t*-io^{6}Ade, 6-(4-hydroxy-3-methyl-*cis* or *trans*-2-butenylamino)-purine; i^6A, 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine; i^6Ade, 6-(3-methyl-2-butenylamino)purine; ms^2-*c*-io^{6}A, 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine; ms^2-*c*-io^{6}Ade, 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)-2-methylthiopurine; ms^2i^6A, 6-(3-methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine; ms^2i^6Ade, 6-(3-methyl-2-butenyl-amino)-2-methylthiopurine; m^6Ade, 6-methylaminopurine.

The gradients were centrifuged at 115,000 × *g* for 55 min in an SW41 rotor. The number of plasmids was determined from the number of radioactive peaks below the major peak of radioactivity in the alkaline sucrose gradients and confirmed by electrophoresis of purified plasmids in agarose gels by the method of Meyers *et al.* (16). Plasmid DNA was isolated by the technique of Currier (17) modified as suggested by E. W. Nester.

Purification of Cytokinins from Culture Medium. The cells were separated from the culture medium by centrifugation at 5700 × *g* for 10 min. Cytokinins were purified from the supernatant fraction as described (5). Cytokinin activity was determined by the tobacco callus bioassay (18, 19) and is expressed as μg of kinetin equivalents (KE) [μg of kinetin (6-furfurylaminopurine) required to give the same growth response as the test sample under the specified bioassay conditions].

Isolation and Identification of Cytokinins from tRNA. The harvested cells were suspended in 5 vol of 0.1 M Tris·HCl (pH 7.3), incubated with 0.02% (wt/vol) lysozyme for 60 min at 22°C, mixed with 0.5% (wt/vol) sodium lauryl sulfate, and ruptured by two passages through a French press (840–1260 kg/cm^2). tRNA was purified as described (20) and hydrolyzed to ribonucleosides (21). Cytokinins were separated and their activities were determined as described (22). The UV spectra of cytokinin-active ribonucleosides in ethanol were determined on a Beckman model 25 spectrophotometer. The quantities of cytokinin were calculated from the λ_{max} absorbances and the known extinction coefficients of the ribonucleosides (23). Purified cytokinin-active ribonucleosides were trimethylsilylated as described (18). Gas/liquid chromatophy–mass spectrometry of trimethylsilylated cytokinins was performed on a Varian aerograph series 2700 gas chromatograph coupled via a glass jet separator to a model 21-491B DuPont Instruments mass spectrometer equipped with oscillograph. Two microliters of trimethylsilylated samples were chromatographed on a coil Pyrex column (180 × 0.2 cm) packed with 1% (vol/wt) OV-17 on Gas Chrom Q with He at a flow rate of 30 ml/min. The stereoisomers of trimethylsilylated 6-(4-hydroxy-3-methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine (ms^{2}io^{6}A) were separated by isothermal chromatography at 260°C. The trimethylsilylated *c*-io^{6}A, i^{6}A, and 6-(3-methyl-2-butenylamino)-methylthio-9-β-D-ribofuranosylpurine (ms^{2}i^{6}A) were separated from each other by chromatography with the temperature programmed from 200 to 280°C at a rate of 6°C/min.

Materials. Synthetic cytokinins were kindly provided by N. J. Leonard (University of Illinois). Lysozyme (from egg white), Tris, sodium lauryl sulfate, and Dowex 50W-X4, H$^+$ (50–100 mesh) were purchased from Sigma; Sephadex LH-20 from Pharmacia; and Porapak Q from Waters Associates (Milford, MA).

RESULTS

Cytokinin Activity in Culture Medium. From a series of 17 strains tested for pathogenicity (E. Doyle, unpublished observation), five strains, ranging from highly virulent to avirulent, were tested for cytokinin activity. Cytokinin activity in the medium of 4-day-old cultures varied greatly from strain to strain and was closely correlated with virulence (Table 1).

i^{6}Ade was the predominant cytokinin in the culture medium of all four tested strains (Fig. 1 and Table 2) and also in Cf1 cultures (3, 5). The quantity of i^{6}Ade increased with the degree of pathogenicity, from 2 μg of KE per liter in the avirulent cultures without a plasmid to 24 μg of KE per liter in the highly virulent strain MW2, as measured on purified preparations freed from inhibitors. As shown in Table 1, culture media of Cf15 and Cf16 tested prior to the removal of inhibitors by chromatography on Sephadex LH-20 had less total cytokinin activity, and the difference between pathogenic and nonpathogenic strains, therefore, was still more extreme. io^{6}Ade

Table 1. Comparisons of virulence, number of plasmids, growth, and cytokinin activity of culture media in five strains of *C. fascians*

Strain	Virulence*	No. of plasmids	Growth, g/liter	Cytokinin activity†
MW2	High	2	12.0	168
Cf2	Mod.	3	10.0	8.3
Cf1	Mod.	3	9.0	2.3
Cf15	Weak‡	1	11.4	0.4
Cf16	None	0	9.0	0.2

* Pathogenicity was tested on *P. sativum* seedlings. Mod., moderate.
† Cytokinin activity in tobacco callus bioassay expressed as μg of KE per liter of culture medium.
‡ Only 1 out of 10 seedlings showed fasciation symptoms.

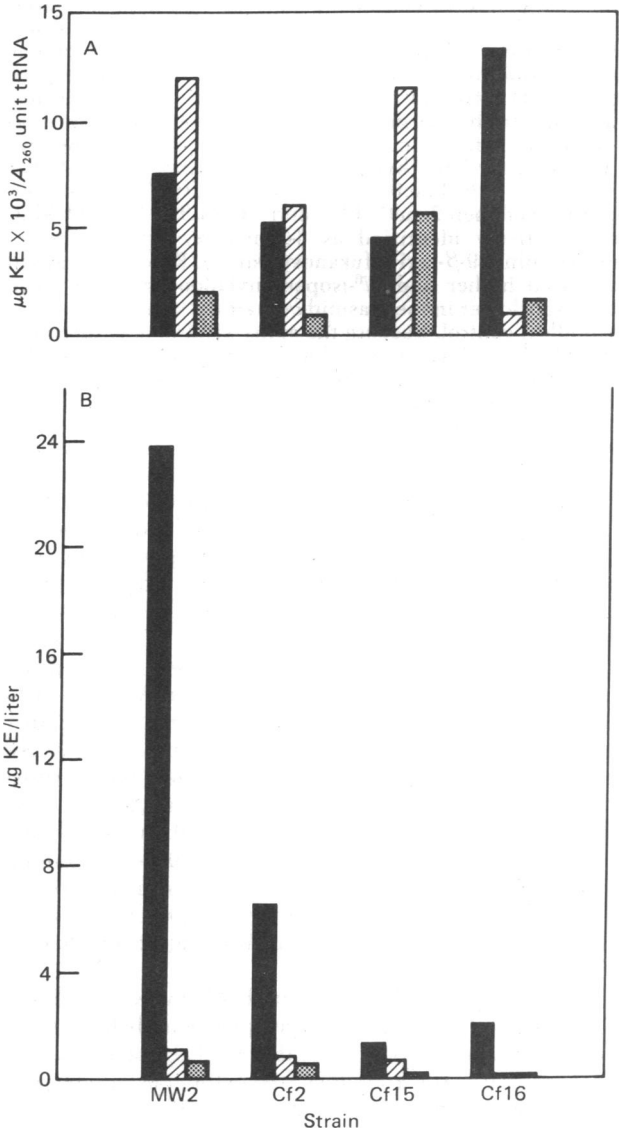

FIG. 1. Comparison of activities of different cytokinins in the tRNA hydrolysates (*A*) and the culture medium (*B*) of 4-day-old cultures of three virulent and one avirulent strain of *C. fascians*. (*A*) ■, i^{6}A; ▨, io^{6}A; ▦, ms^{2}is^{6}A. (*B*) ■, i^{6}Ade; ▨, io^{6}Ade; ▦, ms^{2}io^{6}Ade.

Table 2. Comparison of biological activities of individual cytokinins* in the media of four strains of *C. fascians*

Cytokinins	Strains MW2	Cf2	Cf15	Cf16
io^{6}A	0.8	1.4	2.9	0.8
io^{6}Ade	13.3	10.0	8.3	1.0
i^{6}A	0.4	0	0	0
i^{6}Ade	237.4	66.3	15.8	20.0
ms^{2}io^{6}Ade	8.0	5.8	5.0	0.5
ms^{2}i^{6}A†	0.1	0	0	0

* Fractions corresponding to the elution positions of the specified authentic cytokinins on Sephadex LH-20 columns (60 g, 2.6 × 44.4 cm) chromatographed with 35% ethanol were tested by the tobacco callus bioassay. Values are given as μg of KE per 10 liters of medium.

† 6-(3-Methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine.

was ≈10 times more abundant in media of the virulent strains than in that of the avirulent Cf16 strain. In cultures of the weakly virulent Cf15, io^{6}Ade was about half as active as i^{6}Ade, and, therefore, allowing for differences in specific activity, the more abundant of the two, but in culture media of the more virulent strains (Cf1 and MW2) the io^{6}Ade accounted for only a minor part of the total cytokinin activity. The other four detected cytokinins (Table 2) contributed even less to the total biological activity. ms^2-*c*-io^{6}Ade activity was 10- to 16-fold higher in the strains with plasmids.

Cytokinin Activity in tRNA Hydrolysates. The kinds and quantities of cytokinin-active ribonucleosides in tRNA from each of these four strains were also determined (Table 3). The total yields of cells in the 4-day-old cultures used for preparations of tRNA and their yields of tRNA did not differ significantly for the four strains. In each case, bioassays of ethyl acetate-soluble ribonucleosides from the tRNA, chromatographed on Sephadex LH-20 columns (30 g, 1.9 × 42.0 cm) in 35% (vol/vol) ethanol, revealed three peaks of cytokinin activity, fractions 3, 5, and 8, corresponding to the elution positions of io^{6}A, i^{6}A plus ms^{2}io^{6}A, and ms^{2}i^{6}A, respectively.

Table 3. Comparison of content and biological activity of cytokinin-active ribonucleosides from tRNAs of four strains of *C. fascians**

	MW2	Cf2	Cf15	Cf16
Cells, g	71	66	66	65
tRNA, mg	49	49	49	45
Cytokinin content, μg (mole %)†				
t-io^{6}A	Trace	Trace	Trace	Trace
c-io^{6}A	5.9 (0.8)	4.4 (0.7)	3.9 (0.6)	1.5 (0.2)
i^{6}A	3.2 (0.5)	2.6 (0.4)	2.1 (0.3)	7.8 (1.3)
ms^2-*c*-io^{6}A	9.8 (1.3)	6.4 (0.8)	16.0 (2.1)	8.1 (1.1)
ms^{2}i^{6}A	3.2 (0.4)	2.6 (0.3)	1.7 (0.9)	9.3 (1.4)
Cytokinin activity, μg of KE				
t-io^{6}A	3.8	2.1	3.0	0.2
c-io^{6}A	7.8	3.8	8.4	0.8
i^{6}A	7.3	5.1	4.4	12.0
ms^2-*c*-io^{6}A	2.0	1.2	5.6	1.5
ms^{2}i^{6}A	0.4	<0.1	<0.1	2.1
Total	21.3	12.4	21.4	16.6

* Values are total per sample.

† Cytokinin-active ribonucleosides were identified by comparison of their UV and gas/liquid chromatography-mass spectra with those of authentic, synthetic compounds. The quantities were calculated from UV absorption measurements. Trace, detectable amount but <0.1 μg.

Cytokinins in these three active fractions for each of the four strains were further purified and identified. Fraction 3 was purified on a Sephadex LH-20 column (8 g, 1.0 × 45.4 cm) in H_2O and fractionated on a Porapak Q column (5 g, 1.0 × 20.5 cm) in 16% (vol/vol) ethanol to yield active compounds I and II, with elution volumes corresponding to those of *trans*- and *cis*-io^{6}A, respectively. On this basis I was tentatively identified as *t*-io^{6}A, but the amount was not enough for rigorous identification. Compound II exhibited a UV maximum at 269–270 nm, indicative of N^6-alkyl adenosine. A peak obtained with trimethylsilylated (TMS) II by gas/liquid chromatography had the same retention time (8.6 min) as authentic *c*-io^{6}A(TMS)$_4$. The mass spectrum of this peak compared with that of authentic *c*-io^{6}A(TMS)$_4$ had the same characteristic abundant ions at *m/e* values 639 (M$^+$), 624, 549, 536, 483, 406, 320, and 290 (Fig. 2*A*), thus proving the structure of II to be 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)-9-β-D-ribofuranosylpurine.

Fraction 5, chromatographed on a Sephadex LH-20 column (12 g, 1.2 × 40.0 cm) in H_2O, gave two active peaks (III and IV) with elution volumes corresponding to those of i^{6}A and ms^{2}io^{6}A, respectively. The UV spectrum of III was typical of N^6-alkyl adenosines with λ_{max} at 268 nm. Gas/liquid chromatography of trimethylsilylated III gave a peak with the same retention time (6.9 min) as synthetic i^{6}A(TMS)$_3$. The mass spectrum of this peak exhibited the prominent *m/e* values 551 (M$^+$), 536, 318, 232, and 203, which are characteristic of i^{6}A(TMS)$_3$ (Fig. 2*B*). From these data, III was assigned the structure 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine.

Compound IV had UV absorption maxima at 244 and 283 nm, characteristic of N^6,2-disubstituted adenosines. The mass spectrum of a prominent peak of trimethylsilylated IV in gas/liquid chromatography gave abundant ions at *m/e* 685 (M$^+$), 670, 595, 554, 529, 452, 366, and 336, typical of ms^{2}io^{6}A(TMS)$_4$ (Fig. 2*C*). The 4.2-min retention time of this peak was identical with that of authentic *cis* isomer of ms^{2}io^{6}A(TMS)$_4$ and was distinctly less than the 4.7-min retention time of the *trans* isomer. From these data, IV was assigned the structure, 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine.

Fraction 8 (V) exhibited only weak cytokinin activity. Its UV spectrum resembled that of ms^{2}i^{6}A, with λ_{max} at 282–283 nm and a shoulder at 245 nm. Gas/liquid chromatography of trimethylsilylated V gave a number of prominent peaks, the retention time of one of which was 10.6 min and identical with that of ms^{2}i^{6}A(TMS)$_3$. The mass spectrum of this peak was characteristic of ms^{2}i^{6}A(TMS)$_3$, with peaks at *m/e* values 597 (M$^+$), 582, 554, 529, 364, 278, and 249 (Fig. 2*D*). These data show that V, the cytokinin-active component of fraction 8, was 6-(3-methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine.

The tRNA of each of the four examined *C. fascians* strains contained all five identified cytokinins (Table 3). The total cytokinin content in mole % did not differ markedly between the strains, but the cytokinin composition was very different in the presence and absence of plasmids. In each of the plasmid-containing strains the abundance of the different cytokinins decreased in the order ms^2-*c*-io^{6}A, *c*-io^{6}A, i^{6}A, ms^{2}i^{6}A, and *t*-io^{6}A, but in the plasmidless strain Cf16, ms^{2}i^{6}A was predominant and was followed in order by i^{6}A, ms^2-*c*-io^{6}A, *c*-io^{6}A, and *t*-io^{6}A. In each of the strains with a plasmid the cytokinins with a 4-hydroxylated isopentenyl side chain were more than twice as abundant as cytokinins with an unsubstituted isopentenyl side chain, whereas in the strain without a plasmid this ratio was the reverse. In terms of total cytokinin activity in tRNA hydrolysates as measured in the tobacco bioassay, the observed differences between plasmid and plasmidless strains were less than between strains which contained plasmids.

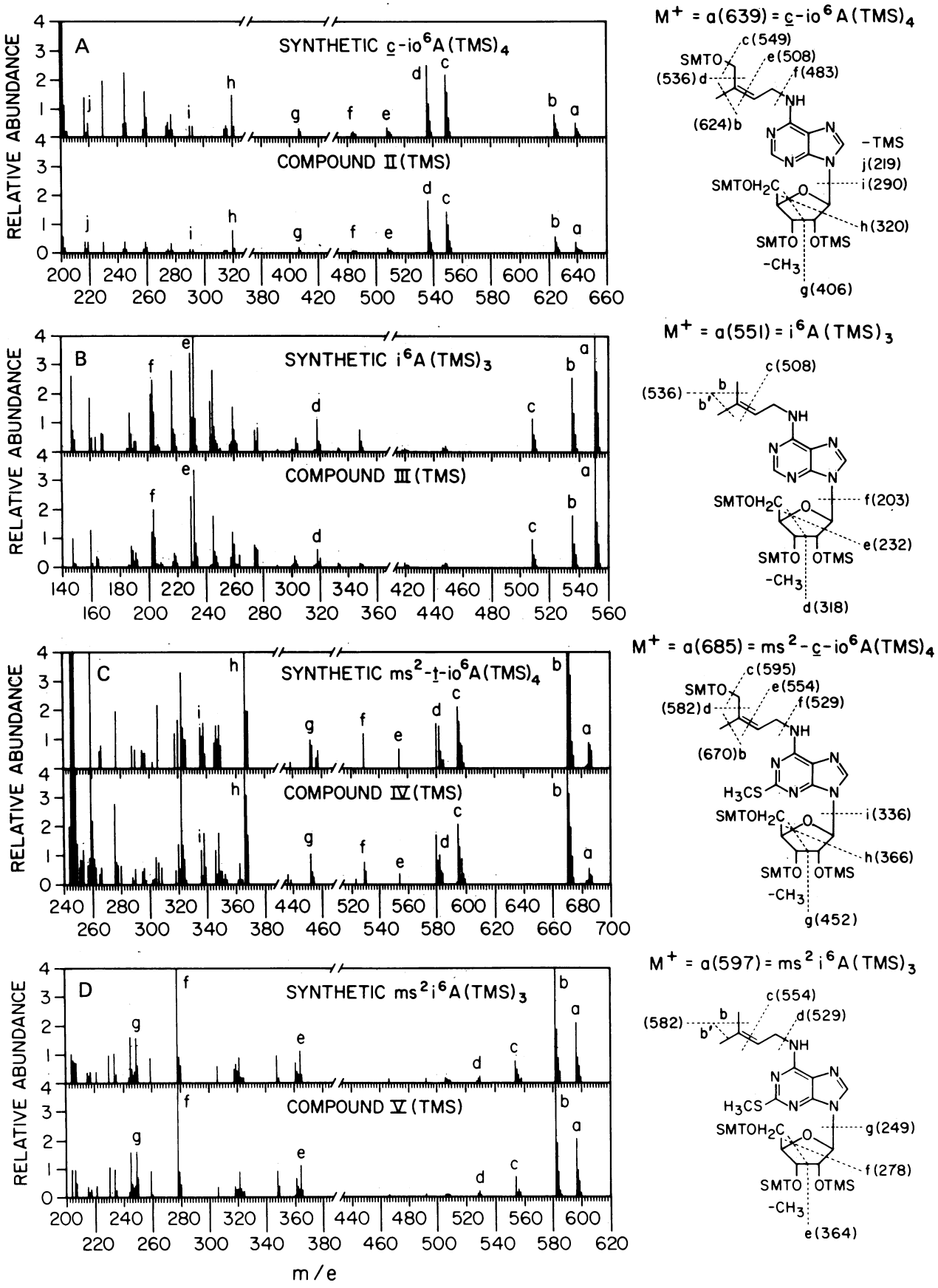

FIG. 2. Mass spectra of trimethylsilylated preparations of (*Upper*) synthetic and (*Lower*) extracted cytokinins isolated from *C. fascians* strain MW2 tRNA hydrolyzed with ribonucleases. (*A*) *c*-io⁶A; (*B*) i⁶A; (*C*) ms²-*c*-io⁶A; (*D*) ms²i⁶A.

Plasmids. All four pathogenic strains contained one or more plasmids; the first three (MW2, Cf1, and Cf2) contained a large ($M_r \approx 10^8$) plasmid, but the fourth, very weakly pathogenic strain (Cf15), contained only a smaller plasmid. Small plasmids also have been found in Cf1 and may be present in MW2. The avirulent strain (Cf16) contained no detectable plasmid but still may contain some extrachromosomal DNA. It appears from these data that pathogenicity is associated with the presence of plasmids, and in strains tested so far a high degree of virulence was associated with the presence of a large plasmid. In MW2, loss of virulence was associated with loss of its large plasmid.

DISCUSSION

All cytokinin-active bases and ribonucleosides expected from degradation of tRNA can be recovered from the culture medium of all strains of *C. fascians* examined. The very low or undetectable content of ms^2i^6A and ms^2i^6Ade can be attributed to loss of these substances in the purification by Dowex chromatography. Improved yields of ms^2i^6A and a detectable amount of ms^2i^6Ade were obtained from culture medium of MW2 when BioRex-70, a weak cation exchange resin, was used instead of the usual Dowex-50 resin.

The clear precursor–product relationship between cytokinin ribonucleosides in tRNA and the presence of free cytokinin-active bases and ribonucleosides in the culture medium suggests that tRNA may serve as one primary source of cytokinins released by *C. fascians*, as proposed earlier (5, 6, 8). The relatively constant relationship between quantities of io^6A and ms^2io^6A in tRNA and the quantities of these ribonucleosides and derived bases in the culture medium of the virulent strains is consistent with this view. However, the extreme increase in free i^6Ade associated with pathogenicity points to an alternative biosynthetic pathway for this cytokinin. An alternative pathway of i^6Ade from AMP and Δ^2-isopentenyl pyrophosphate occurs in *Dictyostelium* (13). Both the marked increase in i^6Ade content in the culture medium and the presence of high contents of hydroxylated isopentenyladenine derivatives (*cis*- and *trans*-zeatin and their derivatives) in the culture medium or in tRNA (or in both) of *C. fascians* appear to be dependent on the presence of plasmids. Similarly, high cytokinin activity in the culture medium and the presence of zeatin ribonucleotides in the tRNA have been correlated with pathogenicity and with the presence of plasmids in *Agrobacterium tumefaciens* (24, 25). High contents of zeatin or its derivatives are also present in other organisms that cause overgrowths in plants, such as mycorrhizal fungi (26), gall-forming insects (27–29), etc. (29), even though hydroxylated isopentenyladenines normally are found neither in fungi nor in animals (29, 30). These results raise the question of whether DNA that codes for high cytokinin production and zeatin biosynthesis in prokaryotic plant pathogens, and in the case of *A. tumefaciens* may be transmitted to serve this function in host tissue, is of prokaryotic origin or has been acquired by the pathogens from plants during evolution. They also raise the question of whether gall-forming insects and other eukaryotic pathogens which cause overgrowths in plants may possess DNA originally derived from plants.

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1. Roussaux, M. J. (1965) *Rev. Gen. Bot.* **72,** 21–53.
2. Thimann, K. V. & Sachs, T. (1966) *Am. J. Bot.* **53,** 731–739.
3. Klämbt, D., Thies, G. & Skoog, F. (1966) *Proc. Natl. Acad. Sci. USA* **56,** 52–59.
4. Helgeson, J. P. & Leonard, N. J. (1966) *Proc. Natl. Acad. Sci. USA* **56,** 60–63.
5. Scarbrough, E., Armstrong, D. J., Skoog, F., Frihart, C. R. & Leonard, N. J. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3825–3829.
6. Armstrong, D. J., Scarbrough, E., Skoog, F., Cole, D. L. & Leonard, N. J. (1976) *Plant Physiol.* **58,** 749–752.
7. Matsubara, S., Armstrong, D. J. & Skoog, F. (1968) *Plant Physiol.* **43,** 451–453.
8. Rathbone, M. P. & Hall, R. H. (1972) *Planta* **103,** 93–102.
9. Cherayil, J. D. & Lipsett, M. N. (1977) *J. Bacteriol.* **131,** 741–744.
10. Einset, J. W. & Skoog, F. (1977) *Biochem. Biophys. Res. Commun.* **79,** 1117–1121.
11. Skoog, F. & Armstrong, D. J. (1970) *Annu. Rev. Plant Physiol.* **21,** 359–384.
12. Kline, L. K., Fittler, F. A. & Hall, R. H. (1969) *Biochemistry* **8,** 4361–4371.
13. Taga, Y., Tanaka, Y. & Nishimura, S. (1978) *Nature (London)* **271,** 545–547.
14. Lacey, M. S. (1955) *Trans. Br. Mycol. Soc. Jpn.* **38,** 48–58.
15. Sharp, P. A., Hsu, M., Ohtsubo, E. & Davidson, N. (1972) *J. Mol. Biol.* **71,** 471–479.
16. Meyers, J. A., Sauchex, D., Elevell, L. P. & Falkow, S. (1976) *J. Bacteriol.* **127,** 1529–1537.
17. Currier, T. C. & Nester, E. W. (1976) *Anal. Biochem.* **76,** 431–441.
18. Armstrong, D. J., Skoog, F., Kirkegaard, L. H., Hampel, A. E., Bock, R. M., Gillam, L. & Tener, G. M. (1969) *Proc. Natl. Acad. Sci. USA* **63,** 504–511.
19. Linsmaier, E. M. & Skoog, F. (1965) *Physiol. Plant.* **18,** 100–127.
20. Murai, N., Taller, B. J., Armstrong, D. J., Skoog, F., Micke, M. A. & Schnoes, H. K. (1977) *Plant Physiol.* **60,** 197–202.
21. Hall, R. H. (1964) *Biochemistry* **3,** 769–773.
22. Armstrong, D. J., Burrows, W. J., Evans, P. K. & Skoog, F. (1969) *Biochem. Biophys. Res. Commun.* **37,** 451–456.
23. Dunn, D. B. & Hall, R. H. (1970) in *Handbook of Biochemistry. Selected Data for Molecular Biology,* ed. Sober, H. A. (The Chemical Rubber Co., Cleveland, OH), pp. G-3–G-98.
24. Chapman, R. W., Morris, R. O. & Zaerr, J. B. (1976) *Nature (London)* **262,** 153–154.
25. Kaiss-Chapman, R. W. & Morris, R. O. (1977) *Biochem. Biophys. Res. Commun.* **76,** 453–459.
26. Miller, C. O. (1967) *Science* **157,** 1055–1056.
27. Engelbrecht, L. (1971) *Biochem. Physiol. Pflanz.* **162,** 9–27.
28. Van Staden, J. (1975) *Plant. Sci. Lett.* **5,** 227–230.
29. Greene, E. M. (1980) *Bot. Rev.*, in press.
30. Skoog, F. & Schmitz, R. Y. (1979) in *Biochemical Actions of Hormones,* ed. Litwack, G. (Academic, New York), Vol. 6, pp. 335–413.