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Short Communication

Cytokinins in tRNA of Corynebacterium fascians[1]

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The plant pathogen *Corynebacterium fascians* contains high cytokinin activity (1, 2). One main active factor has been identified as 6-(γ,γ-dimethylallylamino)purine (2iP) (2, 3). This cytokinin also has been found to be a minor base in yeast seryl-tRNA I and II (4, 5) and tyrosyl-tRNA (6) and is present in tRNA of some other organisms (7, 8). In fact, cytokinin activity seems to be a general property of tRNA hydrolysates (7, 8, 9). It was of interest, therefore, to determine whether 2iP was a constituent of *C. fascians* tRNA, as well as to investigate further the possible presence of other cytokinins in this organism.

The methods of growing *Corynebacterium fascians* were the same as reported by Klambt *et al.* (2). tRNA was extracted with 0.025 M tris-HCl buffer in the presence of buffer-saturated phenol, and purified by methoxyethanol partition (10), CTA precipitation (11), and DEAE-cellulose chromatography (12). From 480 g cell paste (48 l liquid culture), 106 mg tRNA (*ca.* 2330 OD_{260} units) were obtained.

Cytokinin activity was assayed by the tobacco callus test as described by Linsmaier and Skoog (13) with the exception that 5 replicate 50 ml flasks were used, each with 20 ml medium and 3 callus explants.

To release cytokinins, samples of tRNA (1.5 mg) were heated in 5 ml 0.1 N HCl at 100° for 30 minutes (9). The hydrolysates were tested for cytokinin activity, directly and after fractionation by chromatography on Whatman No. 1 filter paper with several solvent systems. Chromatograms were cut crosswise into 10 equal sections and these were extracted twice with a total of 50 ml water on a steam bath. The extracts were added to the basal medium for bioassay.

As shown in table I, acid hydrolyzed tRNA had detectable cytokinin activity at 1.5 mg tRNA equivalents/l, and reached about 10 μg/l kinetin equivalents at 15 mg tRNA/l.

Table I. *Cytokinin Activity of* C. fascians *tRNA Hydrolysate in the Tobacco Test*

Additives	Conc	Fr wt per flask
		g
	0	0.33
	0.6	0.33
sRNA	1.2	0.45
hydrolysate	3	0.46
(mg/l)	6	1.01
	15	1.74
	30	2.82
Kinetin	1.2	0.62
(μg/l)	6	1.23
	30	4.74

When the hydrolysate was chromatographed in 5 solvent systems, as shown in figure 1, most of the activity always moved with 2iP. An additional minor peak was observed on chromatograms developed with solvents A and B (fig 1). This activity coincided with the zone of zeatin and 6-(3-hydroxy-3-methylbutylamino)purine in solvent A, but not in solvent B.

Enzymatically hydrolyzed tRNA (according to Robins *et al.* 14), further degraded by periodate oxidation (Yu and Zamecnik, 15) and fractionated by silver precipitation, *n*-butanol extraction (16) and paper chromatography with solvent A yielded cytokinin activity at R_F 0.8 to 0.9, corresponding to 2iP, and R_F 0.6 to 0.8, corresponding to 6-(γ,γ-dimethylallylamino)-9-β-D-furanosylpurine (2iPA). The minor peak in the zone of zeatin *etc.*, which was found in the acid hydrolysate, was not detected in the enzymatic hydrolysate.

When whole cells were extracted in the cold with 75 % ethanol and then with *n*-butanol and the pooled extracts were purified by silver precipitation and *n*-butanol extraction (16), an active product was obtained with R_F peaks in 4 solvent systems corresponding to the R_F's of 2iP. Only in solvent B was there a minor peak corresponding to the R_F of

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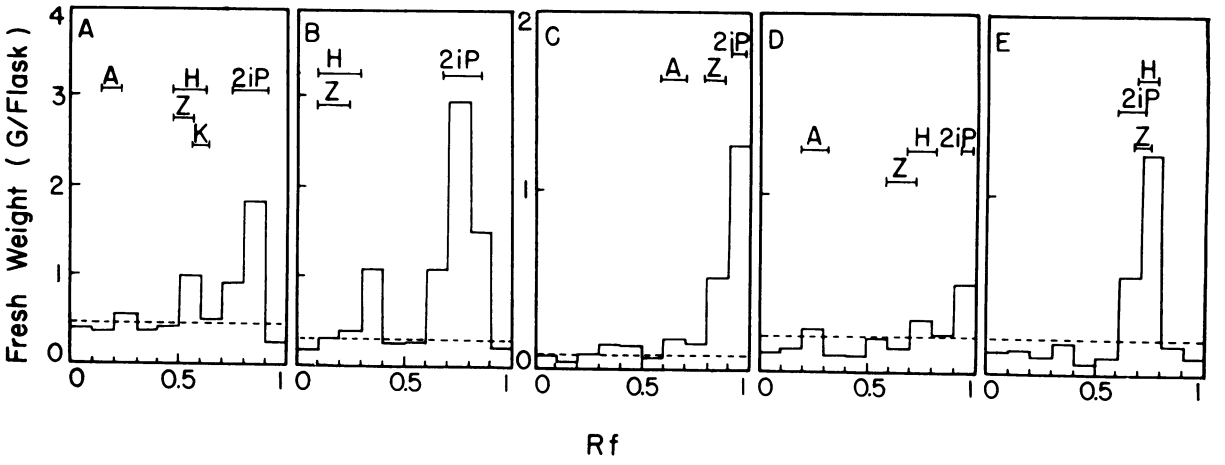

FIG. 1. Location of cytokinin activity on paper-chromatograms of tRNA hydrolysate developed with 5 solvent systems: A) *iso*-propanol:conc HCl:water (16:1:3); B) ethylacetate:formic acid:water (60:5:35); C) *n*-butanol:acetic acid:water (12:3:5); D) ethylacetate:*n*-propanol:water (4:1:2); and E) 2 N NH_4OH. Lines at the top of the histograms indicate the loci of adenine (*A*), 6-(3-hydroxy-3-methylbutylamino)purine (*H*); kinetin (*K*); 6-(γ,γ-dimethylallylamino)purine (*2iP*) and zeatin (*Z*). The broken lines represent the fresh weights of the controls without cytokinin.

zeatin and 6-(3-hydroxy-3-methylbutylamino)purine. When the cells had been heated in 0.5 N HCl for 30 minutes at 100° prior to the extraction, the same general distribution pattern was obtained and with an expected, definite increase in activity in the zone corresponding to 6-(3-hydroxy-3-methylbutylamino) purine.

The above results show conclusively that cytokinin activity is present in *Corynebacterium fascians* tRNA. The chromatographic behavior together with the earlier established presence of 2iP are strong evidence that this substance is the main cytokinin in the tRNA of this bacterium. As 6-(3-hydroxy-3-methylbutylamino)purine is known to be formed on heating 2iP in acid, the minor peak at the R_F's of this compound was to be expected. Whether or not this substance occurs in the intact cells, it evidently was produced in part in the isolation process. Although no additional active fractions were found, their presence is not excluded. This is especially true of zeatin which could not be distinguished from 6-(3-hydroxy-3-methylbutylamino)purine in the solvent systems used. Unfortunately, the presence of inhibitors in the medium and in the crude cell extracts, and other difficulties in making quantitative estimates of cytokinin activity precluded reliable data on the relative amounts of free bases, ribosides, and bound forms or on the respective roles of these substances as sources of the cytokinin activity in the cultures.

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