## Primary and secondary structure of a new viroid 'species' (CbVd 1) present in the *Coleus blumei* cultivar 'Bienvenue'

Reiner L.Spieker, Bernd Haas, Yuh-Chyang Charng, Klaus Freimüller and Heinz L.Sänger Max-Planck-Institute für Biochemie, D-8033 Martinsried bei München, FRG

Submitted May 29, 1990

EMBL accession no. X52960

The detection of a viroid in *Coleus blumei* species in Brazil (1) has prompted us to search for viroids in C. blumei cultivars (cvs) commercially propagated by cuttings for more than 50 years in Germany. 'Return' PAGE (2) of nucleic acid extracts from the cv 'Bienvenue' revealed a band of circular viroid-like RNA molecules. Northern blot analysis (3) with radioactive probes specific for viroids representative for the currently known viroid groups (4) did not exhibit any signals indicating that this circular RNA might represent a new viroid 'species'. This also precluded the use of known viroid-specific DNA primers for the reverse transcription and sequence analysis of this RNA. Consequently, the sequence of parts of it was established by direct RNA sequencing (5), which allowed the synthesis of DNA primers specific for the reverse transcription of the new viroid. Its isolation, purification and sequence analysis by primer elongation were performed as described (6). The sequence and the secondary structure model of the 248 nt long new viroid, tentatively named Coleus blumei viroid 1 (CbVd 1), are shown in the figure.

Sequence comparison revealed that it has only a few and rather short sequence motifs in common with the hitherto sequenced viroids. Despite this remarkable difference in sequence, the structural features of the rod-shaped molecule and of the central domain of CbVd 1 have nevertheless been conserved in a viroid-specific way. This renders CbVd 1 the prototype of a new class of viroids. Sequence analysis of the authentic Brazilian Coleus viroid (1) and of two additional viroid-like circular RNA molecules present in various *Coleus blumei* cvs in Germany is in progress.

## REFERENCES

- 1. Fonseca, M.E.N., Boiteux, L.S., Shing, R.P. and Kitajima, E.W. (1989) Fitopatol. Bras. 14, 94-96.
- Schumacher, J., Meyer, N., Weidemann, H.L. and Riesner, D. (1986) J. Phytopathol. 115, 332-343.
- 3. Spieβmacher, E., Mühlbach, H.P., Tabler, M. and Sänger, H.L. (1985) *Biosc. Rep.* 5, 251–265.
- 4. Sänger, H.L. (1988) Acta Horticulturae 234, 78-88.
- 5. Haas, B., Klanner, A., Ramm, K. and Sänger, H.L. (1988) EMBO J. 7, 4063-4074.
- Schnölzer, M., Haas, B., Ramm, K., Hofmann, H. and Sänger, H.L. (1985) EMBO J. 4, 2181–2190.

1	UGGCUCGAAC	UGACUAGAAC	GGUUUUAAGA	AAAAGUUCUU	ACCUGGAGUU	50
51	GCUUACCUGG	GUUCCCUGGC	AGCGCUGCAA	CGGAAUCCAG	UGCCCACCGG	100
101	AGCGCCAGUU	CGUGCGAGAG	UUGCUUCGGC	UUCUUCUUCG	UGGAACGCUC	150
151	CGCGUGUACG	CCUGGUGACC	CGCGAGCGAU	UGCAAAGGUC	CUGGUUCGCU	200
201	GACCCGGUUC	GAACCUCUUU	UCUAAACCCU	AGUUCAGCUU	AACGAGCU	248

