

**MUTAGENIC ACTIVITY OF *Achyrocline satureioides* (Lam.) DC.
(COMPOSITAE) DETECTED BY THE *bimeth* SYSTEM IN
*Aspergillus nidulans***

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ABSTRACT

Water infusions of *Achyrocline satureioides* (Lam.) DC., common name marcela, were tested for presence of mutagenic action on two strains of *Aspergillus nidulans*. Marcela is widely used in Southern Brazil for medicinal purposes. The infusions made with its inflorescences showed a direct mutagenic action on the *bimeth* system and on the strain A system.

INTRODUCTION

Though the exploitation of the therapeutic potentialities of plants can be highly beneficial, it is also cause for concern as undesirable effects of natural products are not rare. For instance, mutagenic activity of several different plants has been observed (Ramos, 1977; Ramos and Marques, 1978; Kuhara *et al.*, 1980; da Rocha, 1983; Poser *et al.*, 1988).

Achyrocline satureioides (Lam.) DC., Compositae, commonly called marcela or macela, is widely used in Southern Brazil as a medicinal beverage. Its natural habitat comprises Uruguay, Paraguay and Southern Brazil (Simões, 1988). The infusion made with its inflorescences is known in folk medicine to have digestive and antispasmodic properties, an antiinflammatory and analgesic activity being reported by Simões (1988).

Quercetin, a flavonoid found mainly in marcela inflorescences has been studied by several authors. Nagao *et al.* (1981) and Elliger *et al.* (1984) detected a mutagenic

effect on *Salmonella*. Brown and Dietrich (1979) observed its genotoxic activity after metabolic activation with S-9 mix (rat liver, microsomal fraction).

The study of individual compounds is useful for the identification of the active components present in medicinal plants. Yet, because it fails to detect possible interactions which might modify genotoxic activity, testing crude extracts or infusions can be of importance.

The collecting sites and the stage of development of the plant should be considered in the interpretation of results, for climatic conditions and stages of flowering influence the chemical composition of *A. satureioides* (Lam.) DC. (Sonaglio, 1987; Sonaglio, 1989).

We tested the mutagenic activity of *A. satureioides* (Lam.) DC. on *A. nidulans*, by means of the *bimeth* and strain *A* systems.

The *bimeth* system is based on the suppression of the mutation meth (methionine requirement) and was developed by Lilly (1965). A high number of loci are involved in this suppression and the revertants show different morphological aspects which can be classified among five types, according to da Rocha (1986). Type A mutant is essentially of normal phenotype; type B produces colonies with brown pigment and sparse conidiation; type C has densely conidiating green colonies with hyaline edges; type D produces small white colonies and type E are the unidentified mutants.

The *A. nidulans* strain *A* has a duplication of part of linkage group I, translocated to linkage group II. It is highly unstable at mitosis and produces frequent sectors in two broad classes: improved and deteriorated. The normal frequency and pattern of this instability are altered by environmental changes, such as the presence of certain drugs in the culture medium (Cooke *et al.*, 1970; Roper *et al.*, 1978; Bonatelli Jr. and Azevedo, 1977; Majerfeld and Roper, 1978).

MATERIAL AND METHODS

Strains

The *A. nidulans* strains *biA1 methG1* and *A* were kindly provided by J.L. Azevedo, ESALQ-USP, Piracicaba, SP (Brazil).

Sample collection and preparation

Plants were collected at a farm in Piçarras, SC, botanically classified and kept in closed glass flasks in the dark. The infusion of the inflorescences was prepared at a concentration of 20% (W/V) in water. Samples were sterilized using a millipore filter with 0.22 μm pore size.

Culture media

Minimum medium supplemented with biotin - MM; minimum medium supplemented with biotin and methionine - MMS and complete medium - MC were prepared according to Pontecorvo *et al.* (1953). Both vitamins were from Merck.

Controls

Diethyl sulphate - DES (Sigma) and warfarin (Glaxo) were used as positive controls in the systems *bimeth* and strain *A*, respectively.

Bimeth assay

Conidial suspensions in phosphate buffer 0.2M, pH 7, containing Tween 80, were prepared from colonies grown for five days on MMS. Concentrations in the range of 10^5 conidia/ml were plated onto MM in order to obtain the revertants and 10^{-3} dilutions in buffer were plated on MMS in order to count the viable conidia. Control (blank) and treated suspensions were incubated for 24 h at 37°C. A positive control (DES 0.06M) was incubated for 30 min at 37°C. After treatment, samples of conidia were plated and incubated for five days at 37°C before counting and classification of colonies. Twenty MM plates and 10 MMS plates were used for each treatment in each experiment. Two concentrations of marcela were tested: 5.5 and 11 mg per ml of suspension, chosen according to the viability of treated conidia, 65 and 36%, respectively. Three experiments were performed for each concentration of marcela infusion and for DES.

Strain A assay

Conidia from seven day cultures were inoculated in the center of Petri dishes containing MC, MC with 0.06M warfarin and MC with 80 µg/ml marcela. After incubation at 37°C for seven days, the sectors were counted and classified.

RESULTS AND DISCUSSION

The number of viable conidia $\times 10^5$ /ml and the number of each mutant/ml are shown in Table I. The total number of mutants/ 10^5 viable conidia and the relative number of each mutant type are shown in Table II. Types A and D were the most frequent spontaneous mutants. After treatment with marcela, an increase of all types of mutants was observed. The Student test (Beiguelman, 1988) was employed in the statistical treatment of data. The samples treated with marcela at 5.5 mg/ml and at 11 mg/ml both

Table I - Effect of *A. saturoioides* on reversion of *A. nidulans bimeth* strain to methionine independence.

Treatment (mg/ml)		No. of viable conidia* (x 10 ⁵ /ml)	No. and type of mutants/ml				
			A	B	C	D	E
Control (5.5)	1	38	8	3	0	7	10
	2	24	7	1	0	18	3
	3	14	2	2	7	33	1
	\bar{x}	25	6	2	2	19	5
Marcela (5.5)	1	9	6	5	0	25	2
	2	8	1	3	0	45	0
	3	7	2	2	10	34	3
	\bar{x}	8	3	3	3	35	2
Control (11)	1	22	3	3	5	8	5
	2	43	6	1	0	9	1
	3	24	7	1	0	18	3
	\bar{x}	30	5	2	2	12	3
Marcela (11)	1	3	3	7	5	23	5
	2	6	7	7	1	29	5
	3	4	3	2	1	42	1
	\bar{x}	4	4	5	2	31	4
Control (DES)	1	11	1	1	0	3	0
	2	11	3	3	5	9	5
	3	64	9	12	0	29	12
	\bar{x}	29	4	5	2	14	6
DES (0.06M)	1	10	32	43	4	51	13
	2	13	42	52	6	73	5
	3	14	21	48	7	60	13
	\bar{x}	12	32	48	6	61	10

* Calculated from the number of mutants per plate (0.1 ml of conidial suspension).

Table II - Relative numbers of reversion from methionine requirement to methionine independence under the effect of *A. satureioides*.

Treatment (mg/ml)		Total no. of mutants (x 10 ⁵ viable conidia)	Relative no. of mutant types				
			A	B	C	D	E
Control (5.5)	1	0.74	0.21	0.08	0.00	0.18	0.26
	2	1.21	0.29	0.04	0.00	0.75	0.12
	3	3.21	0.14	0.14	0.50	2.36	0.07
	\bar{x}	1.72	0.21	0.09	0.17	1.10	0.15
Marcela (5.5)	1	4.22	0.67	0.55	0.00	2.78	0.22
	2	6.12	0.12	0.38	0.00	5.62	0.00
	3	7.28	0.28	0.29	1.43	4.86	0.43
	\bar{x}	5.87	0.36	0.40	0.48	4.42	0.22
Control (11)	1	1.09	0.14	0.14	0.23	0.36	0.23
	2	0.39	0.14	0.02	0.00	0.21	0.02
	3	1.21	0.29	0.04	0.00	0.75	0.12
	\bar{x}	0.90	0.19	0.07	0.08	0.44	0.12
Marcela (11)	1	14.33	1.00	2.33	1.67	7.67	1.67
	2	8.17	1.17	1.17	0.17	4.83	0.83
	3	12.25	0.75	0.50	0.25	10.50	0.25
	\bar{x}	11.58	0.97	1.33	0.70	7.67	0.92
Control (DES)	1	0.45	0.09	0.09	0.00	0.27	0.00
	2	2.27	0.27	0.27	0.45	0.82	0.45
	3	0.97	0.14	0.18	0.00	0.45	0.18
	\bar{x}	1.23	0.17	0.18	0.15	0.51	0.21
DES (0.06M)	1	14.30	3.20	4.30	0.40	5.10	1.30
	2	13.69	3.23	4.00	0.46	5.61	0.38
	3	10.64	1.50	3.43	0.50	4.28	0.93
	\bar{x}	12.88	2.64	3.91	0.45	5.00	0.87

showed a significantly higher number of total mutants than in control samples ($P < 0.05$). When individual mutants were considered, the number of Type A mutant was significantly higher in the samples treated with marcela at 11 mg/ml ($P < 0.05$). The number of Type D mutants was significantly higher in the samples treated with marcela at 5.5 and at 11 mg/ml ($P < 0.01$). Although the total number of mutants and the relative numbers of all types of mutants were higher for marcela at 11 mg/ml than at 5.5 mg/ml treated conidia, the differences were not statistically significant.

The number of different mutant types observed in the *A. nidulans bimeth* system was higher than that described by Lilly (1965) and the classification suggested by da Rocha (1986) was more in accordance with the types observed in the present work.

Table III shows the results obtained with the strain *A* system. The number of both improved and deteriorated sectors observed in colonies grown in medium containing 0.06M warfarin was significantly higher than that observed in the control colonies ($P < 0.01$ and $P < 0.05$, respectively). The colonies grown in medium containing 80 μ g/ml marcela infusion also showed significantly higher numbers of both improved and deteriorated sectors ($P < 0.05$).

Table III - Effect of *Achyrocline satureioides* on the instability of the duplication strain of *A. nidulans*.

Treatment	No. of colonies analyzed	Sectors/Colony		
		Improved	Deteriorated	Total
Control	19	1.63	0.21	1.84
	15	1.70	0.43	2.13
	10	1.60	0.22	1.82
	\bar{x}	1.64	0.29	1.93
Warfarin 0.06M	11	2.54	0.72	3.27
	11	2.54	0.66	3.20
	10	2.60	0.60	3.20
	\bar{x}	2.56	0.66	3.22
Marcela 80 μ g/ml	18	2.55	0.39	2.94
	11	2.36	0.64	3.00
	17	2.88	0.76	3.64
	\bar{x}	2.60	0.60	3.19

OBS: No alteration was observed in the diameter of colonies treated with warfarin or marcela, in the concentrations tested, in comparison with the control.

A mutagenic effect of the infusion of the inflorescences of *A. satureioides* (Lam.) DC., marcela has been described by Vargas *et al.* (1990). They used the Ames Test with metabolic activation and the Chromotest SOS.

Our results show a direct mutagenic effect of marcela on *A. nidulans*. This effect does not seem to be gene specific. Although it was only statistically significant for types A and D, these were also the most frequent types among the spontaneous mutants. We failed to detect a dosage effect.

The significant increase in both improved and deteriorated sectors of *A. nidulans* strain A indicates that marcela increases the instability of the chromosomal duplication in this strain.

In conclusion, the tests using *A. nidulans* strains employed in the present paper allowed the confirmation of the results obtained in prokariotic organisms by Vargas *et al.* (1990). Further investigations on mammals are necessary for the evaluation of the effects after persistent use of this medicinal plant.

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RESUMO

A atividade mutagênica de infusões aquosas de *Achyrocline satureioides* (Lam.) DC. (marcela) foi investigada através dos sistemas *bimeth* e linhagem A de *Aspergillus nidulans*. A marcela é uma planta popularmente utilizada no sul do Brasil, na forma de chá, com propósitos medicinais. As infusões mostraram um efeito mutagênico direto, nas condições em que foram testadas.

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