

GENOTOXICITY TESTING OF SOME SPICES IN DIPLOID YEAST

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Abstract

A study was conducted to assess the mutagenic and recombinogenic potential of 10 commonly consumed spices and condiments viz., bay leaves, black pepper, cardamom small, caraway, red chilies, cinnamon, coriander, fennel, garlic and curry powder in the diploid yeast *Saccharomyces cerevisiae*. Aqueous extracts of these spices were tested for the induction of mitotic gene conversion and reverse mutation in the diploid strain D7 of the yeast. All except cinnamon failed to induce detectable recombination and mutations in the growing cells of the yeast without exogenous metabolic activation. Treatments with an aqueous extracts of cinnamon significantly increased the rate of gene conversion and reverse mutation. Cinnamon caused cells death and inhibition of cell division. Ethyl methane sulphonate (EMS) used as a positive control exhibited recombinogenic and mutagenic effects.

Introduction

Exposure to environmental chemicals is believed to induce most human cancers. The demonstration of mutagenic effects of most of the known carcinogens provided a method for identifying both environmental mutagens and carcinogens by using short-term tests for mutagenicity. The genotoxicity of industrial and agricultural chemicals has been widely studied and there has been increasing concern about the mutagenic potential of the chemicals present in our food products. A relationship between diet and certain cancers is known and many plant products are known mutagens and some are carcinogens.

Spices and condiments are an important group of natural compounds consumed as dietary constituents throughout the world. They are also used as ingredients of cosmetics and folk medicine. The present report describes the effect of 10 of the commonly consumed spices and condiments on induction of mitotic gene conversion and reverse mutation in growing cells of the diploid yeast *Saccharomyces cerevisiae*. This provides a very convenient and reliable test system for genotoxicity testing of environmental chemicals.

Materials and Methods

The names and the plant parts of the tested spices are given in Table 1. They were purchased from the local vegetable market. For preparing aqueous extracts, powdered

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Table 1. Names, family and plant part used of the spices tested for genotoxicity in diploid yeast.

English Name	Vernacular Name	Botanical Name	Family	Plant part used
Bay Leaf	Tazpat	<i>Lauris nobilis</i>	Lauraceae	leaf
Pepper black	Kali Mirch	<i>Piper nigrum</i>	Piperaceae	Berry
Cardamom (small)	Elachi Choti	<i>Ellettaria cardamomum</i>	Zingiberaceae	Seed
Caraway	Kala zeera	<i>Carum carvi</i>	Umbelliferae	Seed
Red Chilies	Surkh Mirch	<i>Capsicum annum</i>	Solanaceae	Fruit
Cinnamon	Darchini	<i>Cinnamom tamala</i>	Lauraceae	Bark
Coriander	Dhania	<i>Coriandrum sativum</i>	Umbelliferae	Fruit
Fennel	Saunf	<i>Foeniculum vulgare</i>	Umbelliferae	Fruit
Garlic	Lehson	<i>Allium sativum</i>	Liliaceae	Bulb
Curry Powder	Salan Musala	A mixture of 12 different spices including red chilies coriander, cinnamon, garlic, ginger, black pepper, turmeric, cloves, cardamom, cumin and bay leaves.	Product of National Foods Ltd. Karachi Pakistan	

spices were added to distilled water, left overnight at room temperature, passed through coarse cloth to remove debris and then centrifuged. Garlic was crushed to fine paste and distilled water added. The freshly prepared extracts were autoclaved prior to testing. Ethyl methane sulphonate (EMS), a product of Koch-Light Laboratories Ltd., was prepared by thoroughly mixing with sterile distilled water.

Induced mitotic gene conversion at the *trp5* locus and reverse mutation at the *ilv1* locus of strain D7 (Zimmermann *et al.*, 1975) was monitored by the appearance of tryptophan and iso-leucine non-requiring colonies, respectively from the originally tryptophan and iso-leucine requiring cells.

The compounds were tested by the simple rapid well-assay (Kunz *et al.*, 1980). Stationary phase cells (10^6 cells in 0.1 ml aliquot) were spread onto the surface of synthetic minimal media. For detection of convertants at the *trp5* and revertants at the *ilv1* locus, the synthetic minimal medium (Bacto-yeast Nitrogen base without amino acids 0.67%, Dextrose 2% and Bacto-agar 2%) was supplemented with isoleucine (30 mg/litre) and tryptophan (20 mg/litre), respectively. For survival estimations, 100-200 cells were plated onto the surface of each complete (YEPE) medium plate containing Yeast extract 1%, Peptone 2%, Dextrose 2% and Bacto-agar 2% which is a complex medium suitable for yeast growth. When the spreaded aliquot dried, a central well was made by pressing the mouth of a sterile 20-mm diameter test tube into the medium and removing the agar plug with a sterile spatula. The test solutions were then added to the

central well. Distilled water and EMS were added to the well as negative and positive controls, respectively. The yeast colonies were counted after 3 and 5 days of incubation at 30°C.

Results and Discussion

Of the 10 spices and condiments used, only cinnamon exhibited significant cytotoxic effects reducing cell survival to 66% of the negative control, while EMS (the positive control) caused slight cell killing (Table 2). Cytotoxic effects of cinnamon, cardamom small, garlic, curry powder, pepper black and coriander have been reported (Lafont *et al.*, 1984; Unikrishnan & Kuttan, 1988; Tasie & Jones, 1989; Islam *et al.*, 1990).

Cinnamon significantly increased the number of mitotic revertants at the *trp5* locus and revertants at the *ilv1* locus to about 2-folds of the respective negative control values (Table 2). EMS, the positive control increased the rate of revertants and revertants by 12- and 16-folds, respectively (Table 2). Cinnamon and EMS also induced mitotic crossing over at the *ade2* locus of D7 (data not shown). Thus, these results indicate relatively weak genotoxicity of aqueous extracts of cinnamon in the diploid yeast without metabolic activation. In *Salmonella*/mammalian microsome test system,

Table 2. Percent survival, and frequency of gene conversion at the *trp5* and reversion at the *ilv1* locus of diploid yeast following treatment with various spices.

Test compound	Concentration of Extract A	Survival (% of negative)	Frequency/10 ⁶ TRP+ (Converstants)	Survivors B TIV+ (Revertants)
Ethyl Methane-sulphonate (Positive control)	0.01 ml/plate	76**	570(45)**	550(34)**
Bay Leaf	1	102	32(38)	30(32)
Pepper black	1	104	29(31)	28(30)
Cardamom (small)	2	102	36(38)	34(32)
Caraway	1	95	19(18)	16(18)
Cinnamon	2	66**	54(27)**	44(24)**
Coriander	1	104	17(19)	17(18)
Fennel	1	104	18(19)	17(19)
Garlic	Extract of Fresh Bulbs	105	12(13)	12(14)
Curry Powder	1	96	14(18)	16(18)

A: Weight of powdered spices used for preparation of extract (gm/ml of distilled water):0.2 ml of this extract/plate was used for treatment of cells.

B: Frequencies are the means from 4 plates.

**Differ significantly (Student's test) from the respective negative control values given in parentheses

cinnamon has been reported as positive (Shashikanth & Hosono, 1987; Hosono *et al.*, 1989; Lafont *et al.*, 1984) and negative (Rockwell & Raw, 1979; Bersani *et al.*, 1981). It was also negative in *Drosophila* sex-linked recessive lethal test (Abraham & Kesavan, 1978, 1985; Abraham *et al.*, 1979). In *E. coli* rec-assay, cinnamon has been reported as negative (Morimoto *et al.*, 1982) and positive (Sekizawa & Shibamoto, 1982). Cinnamon was also active in *B. subtilis* rec-assay (Pavalo & Chularsiri, 1986; Ungsurungsie *et al.*, 1982). Cinnamaldehyde, the major component of cinnamon, did not induce reversions in *S. typhimurium* and *E. coli* but it induced DNA damage in rec-assay with *B. subtilis* without S9 mix (Sekizawa & Shibamoto, 1982).

Bay leaf, fennel, caraway, cardamom small, garlic and coriander gave negative response in Ames *Salmonella* mammalian microsome test (Rockwell & Raw, 1979; Bersani *et al.*, 1981), although coriander and caraway have been reported to be mutagenic in this test (Mahmoud *et al.*, 1992). The extracts of bay and coriander were also antimutagenic in Ames assay (Natake *et al.*, 1989). Cardamom and fennel were also negative in *Drosophila* sex-linked recessive lethal test (Abraham *et al.*, 1979). Chromosomal damaging activity for fennel and coriander has been noted (Abraham & Sudha, 1981; Sharma & Sharma, 1980). A weak mutagenic effect of garlic after larval feeding in *Drosophila* has been observed (S.K. Abraham, personal communication). Garlic has also been reported as genetically active in Ames test (Lafont *et al.*, 1984), and exhibit inhibitory effects on mutagenicity in *Salmonella* (Park *et al.*, 1991). The clastogenicity of curry powder in Chinese hamster ovary cells has been reported (Goodpasture & Arrighi, 1976).

The consumption of large quantities of red chilies has been associated with elevated risks of stomach cancers in India (S.K. Abraham, personal communication). Chilies were negative in *Drosophila* sex-linked recessive lethal (Abraham & Kesavan, 1985), and *Salmonella* tests but their nitrite treated product was mutagenic in this test (Namiki *et al.*, 1984). Red chilies have been reported as mutagenic in *Salmonella* strains (Shashikanth & Hosono, 1987).

The carcinogenicity of black pepper and its mutagenicity in Ames assay have been reported (Concon *et al.*, 1979, Lafont *et al.*, 1984; Namiki *et al.*, 1984; Shashikanth & Hosono, 1987). The extract of pepper has been reported to exhibit antimutagenic activity in *Salmonella* (Kim *et al.*, 1991) and to be inactive in this test (Rockwell & Raw, 1979; Bersani *et al.*, 1981).

It would suggest that condiments and spices mostly induced chromosomal damage in plants and to a certain extent in mammalian cells but they were inactive when tested in mice, rats, *Drosophila*, *Salmonella* and the diploid yeast cells.

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