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The Mutagenicity of Some Spanish Edible Mushrooms in the Ames Test

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ABSTRACT

The mutagenic activity of the aqueous extracts of nine wild and two cultivated, fresh and frozen species, of Spanish edible mushrooms was studied in the Ames Salmonella/microsome test system. All the mushrooms were mutagenic to TA100 and TA98 strains which are sensitive to base-pair substitution and frameshift mutagens, respectively. In some cases, metabolic activation with the rat liver microsomal fraction enhanced the mutagenic response. The mutagenic activity detected in frozen mushrooms was generally slightly higher than in the fresh mushrooms.

INTRODUCTION

A number of reports have described the mutagenic activity of some edible, wild and cultivated, mushroom species when tested by the Salmonella/microsome assay (Knuutinen & Von Wright, 1982; Sterner *et al.*, 1982; Von Wright *et al.*, 1982). Because of the relatively large and potentially increasing use of mushrooms as human food and as a valuable source of nourishment, it was considered desirable to test the possible mutagenic effects of some of the most popular and extensively consumed mushroom species in Spain. Because fungi have a proven ability to produce compounds with unusual

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chemical characteristics and largely unexplored physiological properties, it is reasonable to be somewhat apprehensive about some allegedly edible mushrooms (Sterner *et al.*, 1982).

The heightened concern over the role of diet has led to a rapid increase in the number of investigations to detect the presence of mutagens and carcinogens in foods. Epidemiological and laboratory data indicate that diet is an important factor in the aetiology of various forms of cancer (Lijinsky, 1983; Sugimura & Sato, 1983). Mutagenic substances have been found in a wide variety of foods: in pickled vegetables (Cheng *et al.*, 1980), fish (Ichinotsubo & Mower, 1982), fried ground beef, beef steak, ham, pork chops and bacon (Bjeldanes *et al.*, 1983) and in a variety of Norwegian food products (Herikstad, 1984). Mutagens are formed during pan frying of salmon, sole, snapper and turbot fillets (Krone & Iwaoka, 1981). Mutagenic substances must be considered as being potentially carcinogenic (Sugimura & Sato, 1983).

Here, we report a search for mutagens in eleven wild and cultivated, fresh and frozen, species of edible mushrooms consumed in Spain.

MATERIALS AND METHODS

Test materials

Samples of the nine wild species of mushrooms under investigation, *Lactarius deliciosus*, *Pleurotus eryngii*, *Coprinus comatus*, *Agaricus campester*, *Boletus luteus*, *Agrocybe cylindracea*, *Marasmius oreades*. *Lepista personata* and *Meripilus giganteus*, were collected around Madrid during the autumn and winter of 1986 and 1987. Champignon (*Agaricus bisporus*) and *Pleurotus ostreatus*, the two most important cultivated mushroom species, were purchased from local food markets. After gathering, the mushrooms were cleaned, and aliquots were used to prepare the fresh mushroom aqueous extracts or distributed in plastic packs, frozen and maintained at −20°C for 3 months.

Preparation of the mushroom aqueous extracts

Standard amounts of 100 g of mushrooms were sliced and further homogenized at 4°C in an homogenizer (Sorvall, Norwalk, CO, USA). The resulting homogenate was filtered with suction, and the filtrate was centrifuged at 10 000 × *g* for 30 min to remove any remaining mushroom debris. The supernatant was sterilized by serial filtration through two Millipore filters (0·45 μm and 0·22 μm). The aqueous extracts were kept refrigerated at 4°C until use, usually no longer than 8–12 h after extraction.

The yields from the aqueous extractions varied slightly from species to species. To facilitate comparisons, the volume of mushroom extract (ml) has been substituted by the corresponding value of wet matter (mg). This means a recalculation of the millilitres of the aqueous extract obtained from each mushroom species to give the milligrams of wet matter of sample as it is. No appreciable water losses were observed during freezing of the mushroom species for 3 months.

Mutagenicity test

Salmonella typhimurium frameshift mutant strain TA98 and the base-pair substitution mutant TA100 were kindly provided by Professor Bruce Ames, University of California, Berkeley, USA. These strains, which contain different types of histidine mutations, are described by Maron and Ames (1983), and were chosen so as to cover the widest possible range of mutagens. Overnight cultures (0·1 ml/plate) were used throughout.

The rat liver homogenate (S9 fraction) was kindly provided by the National Center for Research in Food and Nutrition (CENAN), Madrid, and it was obtained from phenobarbital-induced male Wistar rats of 200 g body weight. The S9 mix (4% by volume) was freshly prepared, according to Maron and Ames (1983), for each mutagenicity assay. Four dose levels of mushroom extract (20, 50, 100 and 150 μl/plate) were used, these volumes being substituted by the corresponding wet matter concentrations in Tables 1 and 2. In all cases the sample solution was 0·1 ml/plate and each dose was tested in duplicate plates with and without metabolic activation. All experiments were repeated once.

The mutagenic response is expressed as the number of revertant colonies per plate at the given concentration of mushroom extract. Ames *et al.* (1975) recommended that results in mutagenicity testing should be evaluated as positive when the number of revertants exceeds twice the number of spontaneous revertants.

Positive and negative controls

Sensitivity of strains was verified with daunomycin for TA98 and sodium azide for TA100, both reagents from Sigma Chemical Co., St Louis, MO, USA. Daunomycin (6 μg/plate) gave 100 colonies for TA98 and sodium azide (10 μg/plate) gave 1000–1500 colonies for TA100. As a positive control of the S9 activation, 2-aminofluorene (Sigma) was tested in strains TA98 and TA100; 10 μg/plate gave approximately 200–500 colonies in strain TA98 and 500 in strain TA100. Negative controls were run without mushroom extract.

RESULTS AND DISCUSSION

The mutagenic activity of the aqueous extracts of freshly harvested mushrooms is presented in Table 1. The mutagenic response ($>2 \times$ blank) is indicated with an asterisk, facilitating the observation that the frameshift tester strain TA98 was reverted by the aqueous extracts of four out of the 11 mushroom species tested, whereas strain TA100 was sensitive to the mutagens of two mushroom species. The extracts of most of the mushroom species examined showed some mutagenic activity in both tester strains. The S9 mix enhanced the mutagenicity of *Pleurotus ostreatus* and *Marasmius oreades* to TA98 and *Pleurotus eryngii*, *Agrocybe cylindracea* and *Marasmius oreades* to TA100.

The mutagenic activity of extracts of mushrooms that had been frozen at $-20°C$ for 3 months was, in some cases, slightly higher than in the fresh

TABLE 1
Mutagenic Activities of the Extracts of Eleven Species of Fresh Mushrooms, Determined by the Ames Test

Mushroom species	*Correspondence of mushroom extract to wet matter (mg/plate)*[a]	*Number of revertants*[b]*/plate*			
		TA98		*TA100*	
		−S9	*+S9*	*−S9*	*+S9*
Agaricus bisporus	0	16	16	109	88
	50	22	22	109	96
	140	27	25	131	118
	290	32	31	175	128
	440	35*	33	141	150
Pleurotus ostreatus	0	22	21	167	142
	100	21	33	195	194
	250	25	45*	253	226
	500	35	46*	298	238
	750	32	61*	300	258
Lactarius deliciosus	0	17	24	119	140
	90	22	39	144	173
	250	29	38	140	179
	470	24	46	163	192
	710	34	43	181	172
Pleurotus eryngii	0	15	26	146	120
	50	17	30	183	143
	120	18	31	205	184
	250	35	41	228	221
	380	30	48	252	271*

TABLE 1—*contd.*

Mushroom species	*Correspondence of mushroom extract to wet matter (mg/plate)*[a]	*Number of revertants*[b]*/plate*			
		TA98		*TA100*	
Coprinus comatus	0	23	17	127	140
	60	25	29	117	127
	150	24	26	248	284
	300	38	35	142	162
	450	35	30	196	157
Agaricus campester	0	26	28	200	203
	50	31	20	278	220
	120	32	49	267	238
	250	50	55	274	227
	380	43	40	248	259
Boletus luteus	0	22	32	208	200
	150	21	35	253	237
	380	29	31	288	233
	760	27	33	293	279
	1 150	30	25	332	293
Agrocybe cylindracea	0	29	32	83	126
	80	29	37	82	230
	200	39	35	161	249
	400	34	39	128	256*
	600	44	46	169*	318*
Marasmius oreades	0	19	22	166	143
	110	29	30	209	210
	270	35	32	225	252
	550	44*	48*	261	234
	830	42*	67*	288	325*
Lepista personata	0	20	24	170	105
	50	31	33	271	144
	130	34	41	357	157
	260	48*	36	322	153
	390	40*	45	391*	150
Meripilus giganteus	0	20	21	182	175
	50	23	24	146	220
	125	33	26	209	232
	250	31	30	263	260
	375	44*	40	301	311

[a] The volume of the aqueous mushroom extract has been substituted by the corresponding value of wet matter (mg).

[b] Values are the means from two experiments.

* Asterisks indicate a mutagenic response ($>2 \times$ blank).

mushrooms. The results are shown in Table 2, in which the only tabulated results are from those extracts showing a higher mutagenic activity than non-frozen. Strain TA98 was reverted by the aqueous extracts of six out of the 11 mushroom species examined, whereas three species showed some mutagenic activity to TA100. The remaining mushroom species showed some mutagenic activity in both tester strains. The S9 mix slightly enhanced the mutagenicity of *Lactarius deliciosus* to TA98 strain.

It should be noted that, although all the tests were performed with the same dose levels of aqueous extracts, this resulted in different final concentrations of the mushroom wet matter, due to differences in yields in the aqueous contents of the mushroom species. Also, results corresponding to a maximum dose level of 150 μl/plate are shown in both tables, since above this dose we observed either no more increase in the number of revertants or a variable cytotoxic effect in most mushroom species analysed. This cytotoxic effect remains unexplained.

Mutagenic activity was found in the aqueous extracts of the fresh mushroom species under investigation. Our results reveal that most large fungal species tested show a significant, although rather weak, mutagenicity. These findings are in agreement with reports of mutagenic activity found in other mushroom species. Knuutinen and Von Wright (1982) evaluated the mutagenic activity of edible wild mushrooms of the genus *Lactarius* and Von Wright *et al.* (1982) screened the mutagenicity of *Lactarius* sp. and other mushroom species such as *Boletus edulis*, champignon (*Agaricus bisporus*) and shiitake (*Lentinus edodes*). Sterner *et al.* (1982) screened a large number of mushroom species for mutagenic activity in the Salmonella/microsome assay.

Results from this work indicate that both tester strains are sensitive to the mutagens of *Agaricus bisporus*, strain TA98 being more sensitive than TA100, whereas Von Wright *et al.* (1982) reported a higher sensitivity of TA100. Results from other mushrooms show that *Lepista personata* had mutagenic activity in both tester strains, while Sterner *et al.* (1982) only found mutagenicity in strain TA100; moreover, the aqueous extract from *Marasmius oreades* showed mutagenic activity in both tester strains while Sterner *et al.* (1982) found no activity in this mushroom species. The results obtained with *Lactarius deliciosus* and *Coprinus comatus* are in agreement with those of Sterner *et al.* (1982). Possibly, geographical, seasonal and intraspecific variations in the chemistry of these fungi may account for some of the differences observed. As far as we know, this constitutes the first report on the mutagenic activity of some mushroom species such as *Pleurotus eryngii*, *Agaricus campester*, *Boletus luteus*, *Agrocybe cylindraceae* and *Meripilus giganteus*.

These results indicate that mutagenic compounds occur widely even in

TABLE 2

Mutagenic Activities of the Extracts of a Number of Frozen Mushrooms,[a] Determined by the Ames Test

Mushroom species	*Correspondence of mushroom extract to wet matter (mg/plate)*[b]	*Number of revertants/plate*[c]			
		TA98		*TA100*	
		−S9	*+S9*	*−S9*	*+S9*
Pleurotus ostreatus	0	18	20	156	139
	100	25	36	218	184
	250	37*	46	218	211
	500	41*	44	237	221
	750	47*	46	254	220
Lactarius deliciosus	0	20	19	117	125
	90	32	32	191	162
	250	43*	47*	207	223
	470	55*	62*	249*	211
	710	61*	73*	292*	243
Pleurotus eryngii	0	10	15	135	143
	50	16	15	159	170
	120	12	24	276	195
	250	13	23	414*	465
	380	20	18	519*	562
Agaricus campester	0	14	17	200	189
	60	20	33	226	229
	150	33*	30	250	237
	310	35*	41	242	267
	460	39*	53	235	258
Boletus luteus	0	19	25	166	155
	200	23	32	190	180
	500	25	27	214	205
	1 000	39*	44	225	180
	1 500	40*	69	185	194
Agrocybe cylindracea	0	13	13	130	147
	60	21	24	147	144
	160	23	30	146	179
	320	29*	38	134	103
	480	38*	42	130	125
Meripilus giganteus	0	18	15	170	122
	50	29	23	222	156
	125	48*	25	246	148
	250	68*	27	317	191
	375	60*	33	352*	236

[a] Only tabulated are results from those extracts showing a higher mutagenic response than non-frozen.

[b] The volume of the aqueous mushroom extract has been substituted by the corresponding value of wet matter (mg).

[c] Values are the means from two experiments.

* Asterisks indicate a mutagenic response ($> 2 \times$ blank).

taxonomically unrelated mushroom species. The chemical nature of these compounds is at present unknown. Their different tester-strain specificities strongly suggest that they differ structurally.

Agaricus bisporus (cultivated specimen) was weakly mutagenic towards strains TA98 and TA100 of *S. typhimurium*. This widely eaten mushroom species contains relatively large amounts of agaritine and further hydrazine derivatives in trace amounts (Levenberg, 1964; Ross *et al.*, 1982). It has been found that agaritine and the 4-(hydroxymethyl)benzenediazonium ion are mutagenic to TA1535 and TA1537 strains of *S. typhimurium* (Rogan *et al.*, 1982), whereas the *N'*-acetyl derivative of 4-(hydroxymethyl)phenylhydrazine and the tetrafluoroborate and sulphate forms of 4-(hydroxymethyl)benzenediazonium ion are carcinogenic to Swiss mice (Toth *et al.*, 1978, 1982; Toth, 1979). More importantly yet, a recent study has shown that oral administration to Swiss mice of the fresh, uncooked, cultivated mushroom *Agaricus bisporus* induced tumours of bone, the forestomach, liver and lungs (Toth & Erickson, 1986).

The aqueous extracts of fresh *Lactarius deliciosus* also showed some mutagenic activity in both tester strains, but not so high as that induced by *Lactarius necator* (Knuutinen & Von Wright, 1982; Von Wright *et al.*, 1982); this is fortunate, since the latter mushroom species contains a compound termed necatorin, which is one of the strongest mutagenic compounds found in nature (Suorti & Von Wright, 1983; Von Wright & Suorti, 1983). Other *Lactarius* spp. such as *L. vellerus* contain sesquiterpene dialdehydes with apparent importance in chemical defence systems against parasites and with antifeedant, antimicrobial and antitumour activities, but from which isovelleral is a potent base-pair substitution mutagen (Sterner *et al.*, 1982, 1987).

The mutagenic activity in the aqueous extracts of frozen mushrooms maintained at $-20°C$ for 3 months was generally slightly higher than in the fresh mushrooms. This may suggest that chemical changes during freezing probably affect the structure and activity of the many compounds present in the mushroom flesh. Further studies should be continued to evaluate the mutagenic activity of frozen mushrooms since some mushroom species are actually commercially sold in frozen packs with a longer shelf life, and since many collectors will maintain (frozen) some wild mushroom species picked during the autumn for a variable period of time.

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REFERENCES

Ames, B. N., McCann, J. & Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/microsome mutagenicity test. *Mut. Res.*, **31**, 347–64.

Bjeldanes, L. F., Morris, M. M., Timourian, H. & Hatch, F. T. (1983). Effects of temperature and time on mutagen formation in pan fried hamburger. *J. Agric. Food Chem.*, **31**, 18–21.

Cheng, S. J., Sala, M., Li, M. H., Wang, M. Y., Pot-Deprun, J. & Chouroulinkov, I. (1980). Mutagenic, transforming and promoting effect of pickled vegetables from Linxian County, China. *Carcinogenesis*, **1**, 685–92.

Herikstad, H. (1984). Screening for mutagens in Norwegian foods by the Ames test. *J. Sci. Food Agric.*, **35**, 900–8.

Ichinotsubo, D. Y. & Mower, H. F. (1982). Mutagens in dried/salted Hawaiian fish. *J. Agric. Food Chem.*, **30**, 937–9.

Knuutinen, J. & Von Wright, A. (1982). The mutagenicity of *Lactarius* mushrooms. *Mut. Res.*, **103**, 115–18.

Krone, C. A. & Iwaoka, W. T. (1981). Mutagen formation during the cooking of fish. *Cancer Letters*, **14**, 93–9.

Levenberg, B. (1964). Isolation and structure of agaritine, a δ-glutamyl-substituted arylhydrazine derivative from Agaricaceae. *J. Biol. Chem.*, **239**, 2267–73.

Lijinski, W. (1983). Summation and new approaches to diet and cancer. *Cancer Res.*, **43**, 2441–3.

Maron, D. M. & Ames, B. N. (1983). Revised methods for the Salmonella mutagenicity test. *Mut. Res.*, **113**, 173–215.

Rogan, E. G., Walker, B. A., Gingell, R., Nagel, D. & Toth, B. (1982). Microbial mutagenicity of selected hydrazynes. *Mut. Res.*, **102**, 413–24.

Ross, A. E., Nagel, D. & Toth, B. (1982). Evidence for the occurrence and formation of diazonium ions in the *Agaricus bisporus* mushroom and its extracts. *J. Agric. Food Chem.*, **30**, 521–5.

Sterner, O., Bergman, R., Kesler, E., Magnusson, G., Nilsson, L., Wickberg, B., Zetterberg, G. & Zimersson, E. (1982). Mutagens in larger fungi, I. Forty-eight species screened for mutagenic activity in the Salmonella/microsome assay. *Mut. Res.*, **101**, 269–81.

Sterner, O., Carter, R. E. & Nilsson, L. M. (1987). Structure-activity relationships for unsaturated dialdehydes 1. The mutagenic activity of 18 compounds in the Salmonella/microsome assay. *Mut. Res.*, **188**, 169–74.

Sugimura, T. & Sato, S. (1983). Mutagens–carcinogens in foods. *Cancer Res.*, **43**, 2415–21.

Suorti, T. & Von Wright, A. (1983). Isolation of a mutagenic fraction from the wild edible mushroom *Lactarius necator* (a preliminary note). *J. Chromatograph.*, **255**, 529–32.

Toth, B. (1979). Hepatocarcinogenesis by hydrazine mycotoxins of edible mushrooms. *J. Toxicol. Environ. Hlth*, **5**, 193.

Toth, B. & Erickson, J. (1986). Cancer induction in mice by feeding of the uncooked cultivated mushroom of commerce *Agaricus bisporus*. *Cancer Res.*, **46**, 4007–11.

Toth, B., Nagel, D., Patil, K., Erickson, J. & Antonson, K. (1978). Tumor induction with the *N'*-acetyl derivative of 4-hydroxymethylphenylhydrazine, a metabolite of agaritine of *Agaricus bisporus*. *Cancer Res.*, **38**, 177–80.

Toth, B., Nagel, D. & Ross, A. (1982). Gastric tumorigenesis by a single dose of 4-(hydroxymethyl)benzenediazonium ion of *Agaricus bisporus*. *Brit. J. Cancer*, **46**, 417–24.

Von Wright, A. & Suorti, T. (1983). Preliminary characterisation of the mutagenic properties of 'necatorin', a strongly mutagenic compound of the mushroom *Lactarius necator*. *Mut. Res.*, **121**, 103–6.

Von Wright, A., Knuutinen, J., Lindroth, S., Pellinen, M., Widden, K. G. & Seppa, E. L. (1982). The mutagenicity of some edible mushrooms in the Ames test. *Food Chem. Toxicol.*, **20**, 265–7.