SHORT COMMUNICATION

Mutagenic Effects of Saccharin on *Aspergillus niger*

By ARATI DAS

Department of Microbiology, Bose Institute, 93/1 Acharya Prafulla Chandra Road,
Calcutta 700009, India

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Some mutagenic effects of commercial saccharin on conidial germination, colonial morphology and biochemical characteristics of *Aspergillus niger* strain 350 are described.

INTRODUCTION

There is a large volume of literature on the mutagenic activity of saccharin, and the subject has been reviewed recently by Kramer (1975). Both positive and negative evidence for mutagenicity have been reported (Sax & Sax, 1968; Sanjeeva Rao *et al.*, 1971; Kristoferson, 1972; Wu *et al.*, 1974), emphasizing the need for further investigations. We have therefore tested the mutagenic activity of commercial saccharin on *Aspergillus niger*.

METHODS

Strains and media. *Aspergillus niger* strain 350, used as the test organism, was maintained on Czapek Dox (CD) slants. CD agar was used as minimal medium (MM) while complete medium (CM) was 5% (w/v) malt extract (ME) agar. Auxotrophs were isolated by replica plating on CM and MM, and identified by Holliday's method (1956). Complementation tests were done by the method of Hannan (1975). All incubations were at 30 °C.

Saccharin treatment. Commercial saccharin (sodium salt) was used in all tests. Conidial suspensions (2×10^6 to 6×10^6 ml⁻¹) were prepared in test tubes in 5% ME medium containing different concentrations of saccharin (1 to 7 mg ml⁻¹), and the tubes were incubated with shaking to determine the extent of germination. In addition, CM plates containing different concentrations of saccharin (1 to 7 mg ml⁻¹) were inoculated with conidia and incubated for 10 d for morphological and biochemical studies of the colonies.

RESULTS AND DISCUSSION

Effect of saccharin on conidial germination. In the absence of saccharin, 10% of the conidia germinated during the first 15 h incubation and 61% had germinated by 55 h. The extent of germination after longer incubation periods could not be determined because of excessive mycelial growth. With 3 mg saccharin ml⁻¹, 8% of the conidia germinated during the first 25 h and 63.3% had germinated after 75 h. No counting was possible after longer periods. With 7 mg saccharin ml⁻¹, no germination occurred during the first 45 h incubation, but it had reached 19% by 55 h and 48.7% by 95 h. Thus, saccharin at between 3 and 7 mg ml⁻¹ affected cellular metabolism causing a delay in the initiation of germination.

Effect of saccharin on morphological and biochemical characters of colonies. Some colonies on plates containing 3 mg saccharin ml⁻¹ or more had altered morphological or biochemical characters (Table 1). Morphological variants were dwarf and petite, whereas parental colonies were rapidly spreading with abundant conidia. The dwarf colonies were very slow

Table 1. *Analysis of A. niger colonies growing on saccharin plates*

Saccharin concn (mg ml ⁻¹)	No. of colonies tested	Percentage frequency of colonies			
		Prototrophs			Auxotrophs
		Normal	Dwarf	Petite	
0	131	100	0	0	0
1	150	100	0	0	0
3	270	82.3	11.8	0	5.9
5	205	66.8	15.1	9.8	8.3
7	256	47.8	17.9	11.7	22.6

growing and mostly sparsely conidiating, often with rudimentary conidiophores and heads. Some dwarf and all the petite colonies were totally non-conidiating or aconidial. Complementation tests revealed that in at least some of the dwarf colonies the changed character was under nuclear control.

Of the 91 auxotrophic mutants found, one required thiamin for growth and the rest required lysine. All these auxotrophs retained their specific growth requirements even after a series of subcultures over a period of 10 months, indicating that their auxotrophy was due to stable nuclear mutations induced by saccharin.

The results suggest that under the experimental conditions tested, saccharin had a mutagenic effect on cells of *A. niger*, often causing genetic alterations. Hence, it may be inferred that when living cells are fed with saccharin, there is a risk of genetic damage.

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