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Mutation Induction in Aspergillus terrus Using N-Methyl-N'-Nitro-N-Nitrosoguanidine (NTG) and Gamma Rays

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Abstract: Chemically and physically induced mutations of *Aspergillus terrus* strain isolated and identified from Saudi Arabia were studied by exposing the conidia of this fungus to N-methyl-N'-nitro-N-Nitrosoguanidine (NTG) at a concentration of 0.0075g/10ml and different doses of gamma rays at 20, 30, 40 and 50 KRad. The mutagens resulted in the induction of autotrophic and color mutants of conidia of *A. terrus*. The percentage of survival conidia and the percent of indicated mutants linearly decreased when exposing time to NTG increased. On the other hand, the survival percent of *A. terrus* linearly decreased when the dose of gamma rays increased in the second experiment. It was found that the majority of obtained mutants in two experiments were amino acid auxotrophs.

Key words: Mutation induction, *Aspergillus terrus*, N-methyl-N'-nitro-N-Nitrosoguanidine, Gamma rays.

INTRODUCTION

N-Methyl-N'-nitro-N-nitrosoguanidine (NTG) has been widely used to induce mutations in bacteria. It has proved highly effective, so much so that it has been suggested to be the most potent chemical mutagen yet discovered (Adelberg, *et al.,* 1965). They found that mutations to valine resistance and to autotrophy occurred at high frequency (up to 42-5 % auxotrophs) after exposure of *E. coli* to NTG under conditions such that about 5 % of the treated bacteria remained viable. NTG has also proved a very effective mutagen for yeast, though the results are less dramatic than with *E. coli*. (Megnet, 1965) with *Schizosaccharomyces pombe* found that, without selection, NTG-induced auxotrophs increased to a maximum frequency of about 8% at 20% survival. On the other hand Nordstrom (1967) obtained up to 50 % petite mutants among survivors of *Saccharomyces cerevisiae* after NTG treatment. Pal *et al*. (2005) isolated two cadmium resistant mutants (Cd1 and Cd2) of *Aspergillus niger*, among the six isolated by mutagenization with N-methyl N-nitro-N-nitrosoguanidine (MNNG). Selection and mapping of mutations affecting cysteine synthesis in *A. nidulans* was carried out. A new locus, *cysE*, is described, the mutants of which are deficient in *in vivo* conversion of O-acetylserine to cysteine, a step mediated by cysteine synthase. Three loci (*cysB, C* and *E*) were thus found to control this step *in vivo*, apparently without affecting the enzyme activity *in vitro*. By scoring for propargyl glycine sensitivity of *cys* mutants, chromosomal map positions were obtained for all five cysteine loci (*A, B, C, D* and *E*) (Cybis *et al*., 1988). The mutagenic effect of (N.T.G) on *A. fumigates* and *A. nidulans* were studied by Levadoux *et al*., (1981), Das *et al*., (1983), Baeshen and Sabir (1987) who found that NTG has proved a very effective mutagen and produce a great number of autotrophic mutants in these fungus.

A. niger C was treated with UV and gamma irradiation (^{60}Co), nitrogen mustard and colchicines. Mutagenic treatments resulted in substrains with either increased or decreased citric acid production, while some were unchanged in this respect. Low-yielding strains predominated in all the experiments. Analysis of the superior sub-strains showed gamma irradiation to be most effective in producing the greatest number of superior mutants (Das and Nandi, 1972). Gamma-ray exposure was used as a mutagenic agent for mutant induction from a strain of *Humicola lutea* 72 producing acid proteases. The second stage of irradiation resulted in a double active mutant *(H. lutea* 120-8). Further treatment gave rise to a triple active mutant (*H. lutea* 120-5) with a maximal proteolytic activity of 1,800~g tyrosine liberated from 2% casein/m1 culture filtrate/min at pH 3,0 and 40 ~ C. A change in the composition of proteolytic enzymes caused by gamma-ray exposure was established, using chromatography on DEAE-cellulose (Grigorov *et al*., 1983). Fadel and El-Batal (2000) studied the effect of gamma rays on *A. niger* wild type strain they obtained active enhanced isolates producing high level of amylolytic enzymes.

Haq (2004) investigated that citric acid production by some selected mutant strains of *A. niger* from cane molasses in 250 ml Erlenmeyer flasks. A conidial suspension of *A. niger* GCB-75, which produced 31.1 g/l citric acid from 15% (w/v) molasses sugar, was subjected to UV-induced mutagenesis. Among the 3 variants, GCM-45 was found to be a better producer of citric acid (50.0 ± 2a) and it was further improved by chemical mutagenesis using *N*-methyl, *N*-nitro-*N*-nitrosoguanidine (MNNG). Out of 3,2-deoxy-d-glucose resistant variants, GCMC-7 was selected as the best mutant.

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