Abstract

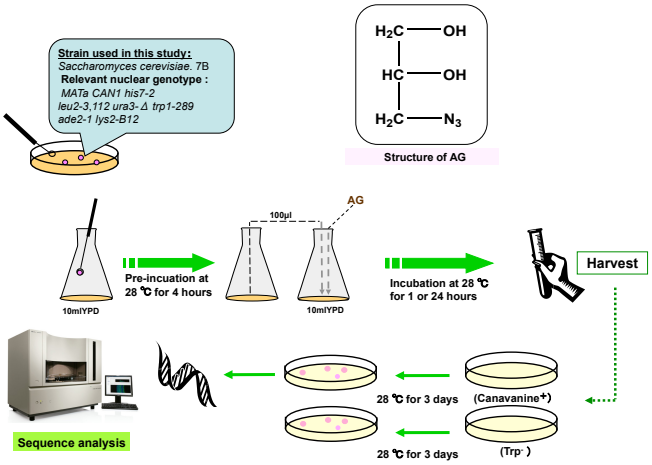
Sodium azide, a strong mutagen in barley and pea, has been successfully employed in mutation breeding for the induction of specific types of gene mutations in crop plants. Mutagenesis by azides has been studied since a long time ago. Recently, organic aliphatic azido derivatives including azido acids, azido alcohols and azido saccharides have been found to be strongly mutagenic in *Salmonella*, *E. coli* and *Saccharomyces cerevisiae*. However, the precise mechanism of their mutagenic action remains poorly understood.

In this study, the mutagenic activity of one of the organic azides 3-azido-1,2-propanediol (azidoglycerol, AG) has been determined in the 7B haploid strain of *Saccharomyces cerevisiae*.

At 3 mM AG increased the frequency of forward mutations to canavanine resistance 231 times and the frequency of reversions to tryptophan independence 356 times during a 24 h growth period. Under the non-growth conditions, AG treatment for 1 h resulted in dose-dependent increase of forward mutations but reversions were not induced. The forward mutations to canavanine resistance were increased 95 times at 50 mM AG under these conditions. Next we analyzed the mutation spectrum at the *CAN1* locus and found that AG treatment strongly enhanced G:C to A:T base substitutions.

Our results suggest that AG is significantly mutagenic in *Saccharomyces cerevisiae*, and that the type of the induced mutations is exclusively G:C to A:T transitions.

Method



Results

S. cerevisiae 7B after 1 h or 24 h AG treatment under growth conditions

Treatment Time [hour]	Concentration of AG [mM]	Living cells (×10 ^{6}/ ml) after 24 h treatment	Growth (%)	Mutant frequencies [per 10 $^{-7}$ living cells]	
				Canavanine resistant	Tryptophan prototrophs
24	0	110±6.8	100	6.74 ±2.98 (6)	0.38 ±0.34 (6)
	0.2	107±22.0	97	170.06 ±14.80 (6)	18.66 ±15.08 (6)
	0.5	105±10.0	97	266.69 ±29.96 (6)	25.00 ±4.90 (6)
	1	86±7.4	78	536.02 ±97.95 (6)	82.43 ±17.77 (6)
	3	59±3.0	53	1556.53 ±179.72 (6)	135.23 ±11.71 (6)
1	0	94±2.8	100	6.91 ±2.26 (2)	1.33 ±1.13 (2)
	0.5	106±22.2	113	40.00 ±18.53 (3)	1.88 ±1.03 (3)
	5	76±11.2	80	138.41 ±24.55 (2)	7.95 ±7.39 (2)
	50	85±23.4	90	659.44 ±79.94 (2)	2.80 ±2.23 (2)

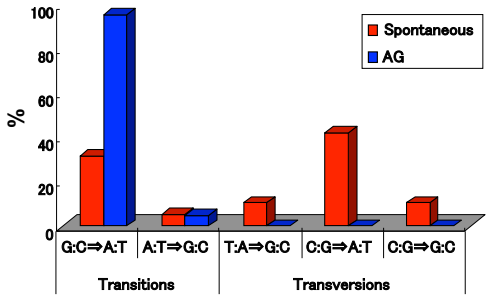
Results represent data pooled from experiments with 9-11 plates per dose. Numbers in parentheses indicate the number of independent experiments. mean±SD.

Spectra of sequenced mutations in the CAN*1 gene*

Base substitutions induced by AG in *CAN1* mutants

Nucleotide position	Nucleotide substiution	DNA sequence at the site of mutation	Nucleotide position	Nucleotide substiution	DNA sequence at the site of mutation
296	G → A	CTT GGT GGT	268	C → T	AAG CAA AGA
296	G → A	CTT GGT GGT	268	C → T	AAG CAA AGA
296	G → A	CTT GGT GGT	268	C → T	AAG CAA AGA
314	G → A	TCA GGT CTT	1166	C → T	TCT GCC GCA
353	G → A	GCC GGT CCA	1426	C → T	ATG CAA GCT
509	G → A	AAT GGT TAC	1633	C → T	TTT CAA TGC
585	G → A	TTT GGT CGT	1600	A → G	TCT ATT TTC
671	G → A	TAC GGT GAA			
671	G → A	TAC GGT GAA			
671	G → A	TAC GGT GAA			
671	G → A	TAC GGT GAA			
896	G → A	GAA GGT ACT			
896	G → A	GAA GGT ACT			
1208	G → A	TTT TGG CTT			

Comparison of mutation spectrum in *CAN1*



Discussion

Mechanism of azide mutagenesis