EVALUATION OF THE MUTAGENICITY OF AMINOGLYCOSIDE ANTIBIOTICS IN *SALMONELLA TYPHIMURIUM* AND *SACCHAROMYCES CEREVISIAE*

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The mutagenicity of aminoglycoside antibiotics (KM, AKM, DKB, RSM, AMK, GM, TOB) has been studied in cells of the bacteria *Salmonella typhimurium* and in the yeast *Saccharomyces cerevisiae*. The bacterial strains (AMES') monitor reverse mutation (point mutation) and the yeast strain D5 monitors mitotic crossing-over, mitotic gene conversion and point mutation. None of these antibiotics demonstrated any mutagenic activities in either the bacteria or the yeast.

Aminoglycoside antibiotics, kanamycin (KM), aminodeoxykanamycin (AKM), dibekacin (DKB), ribostamycin (RSM), amikacin (AMK), gentamicin (GM) and tobramycin (TOB) are widely used antibiotics, which are known to effect protein synthesis in bacterial cells. In this study we examined the mutagenicity of these drugs by using sensitive *in vitro* microbial tests with *Salmonella typhimurium* and *Saccharomyces cerevisiae*.

Salmonella testers are well-known AMES' strains monitoring reverse mutations ($his^- \rightarrow his^+$). *S. cerevisiae* strain D5, isolated by F. K. ZIMMERMANN assesses mitotic crossing-over.

Materials and Methods

Salmonella typhimurium test system

The *Salmonella* mutagenicity test has been reviewed. *S. typhimurium* test strains TA1535–1538, TA100 and TA98 were obtained from Dr. T. KADA, National Institute of Genetics, Mishima. TA1535 detects base pair mutagens, and TA1536–1538 detect frameshift mutagens. TA100 and TA98 were developed by adding the R-factor plasmid pKM101 into TA1535 and TA1538.[2] All six strains lack excision repair and contain the *gal* and *rfa* mutations (Fig. 1). The bacteria were stored and grown as outlined by AMES *et al.*[1]

Each aminoglycoside antibiotic was dissolved in distilled water (8 mg (base)/ml) and tested directly for mutagenic activity in the qualitative assay.

Saccharomyces cerevisiae test system

Diploid strain D5 obtained from Dr. S. NAKAI, National Institute of Radiological Sciences, Anagawa, contains two different alleles of the gene locus *ade* 2 and can be used for the visual screening of mitotic crossing-over. These alleles differ in their extent of colony pigmentation engendered on low adenine media, and they complement each other to the effect that the diploid is white (Fig. 2). Procedures we used in this study were that described by ZIMMERMANN.[3] The incubation time in liquid was six hours.

Final concentration of each aminoglycoside antibiotic in incubation media was 2 mg(base)/ml.

Positive controls for induced mutations with either test system employed 4-nitro quinolin-1-oxide (4NQO), N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and mitomycin C (MT-C).

Aminoglycoside antibiotics

The following aminoglycoside antibiotics were investigated: kanamycin (KM), aminodeoxy-

Fig. 1. Characteristics of tester strains of *Salmonella typhimurium* (AMES).

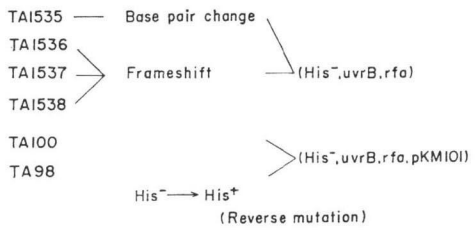
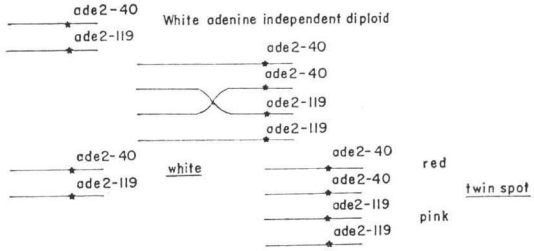


Fig. 2. Mitotic crossing-over in strain *Saccharomyces cerevisiae* D5 (ZIMMERMANN).



kanamycin (AKM), dibekacin (DKB), ribostamycin (RSM), amikacin (AMK), gentamicin (GM), tobramycin (TOB).

Results

All of the aminoglycoside antibiotics were negative in the qualitative *Salmonella* test. The

Table 1. Results of AMES' test in *S. typhimurium*.

	TA1535	TA1536	TA1537	TA1538	TA100	TA 98
KM	−*	−	−	−	−	−
AKM	−	−	−	−	−	−
DKB	−	−	−	−	−	−
RSM	−	−	−	−	−	−
AMK	−	−	−	−	−	−
GM	−	−	−	−	−	−
TOB	−	−	−	−	−	−
4NQO	−	−	−	−	+++	++
MNNG	+++	−	−	−	+++	+
MT-C	−	−	−	−	−	−

* −: <20 colonies. ++: >100 colonies. +++: >500 colonies.

Table 2. Morphology of colonies arising from cells of *S. cerevisiae* D5.

	Total colonies counted	White	Red	Pink	W/R	W/P	R/P	W/R/P	Total aberrants
KM	12,890	12,837	17	5	29	2	0	0	53 (0.411)
AKM	13,063	13,059	0	2	2	0	0	0	4 (0.031)
DKB	8,953	8,945	1	2	3	2	0	0	8 (0.089)
RSM	12,833	12,825	0	4	3	1	0	0	8 (0.062)
AMK	12,894	12,882	3	5	4	0	0	0	12 (0.093)
GM	14,238	14,228	1	1	2	6	0	0	10 (0.070)
TOB	13,852	13,838	1	4	3	6	0	0	14 (0.101)
4NQO	12,412	11,894	94	148	164	109	3	0	518 (4.173)
MNNG	12,106	11,877	55	85	53	35	1	0	229 (1.892)
MT-C	11,506	11,359	36	40	44	20	7	0	147 (1.278)

* 4NQO 0.1 mcg/ml; MNNG 10 mcg/ml; MT-C 20 mcg/ml; Aminoglycosides 2 mg/ml.
** Incubation time: 6 hours (YEPglucose, pH 7.0, 30°C).
*** Spontaneous frequency of aberrants in our laboratory is under 0.8%.

summary of the results is shown in Table 1. Revertants appearing as a ring of colonies around each disc were counted. 4NQO was mutagenic in TA100 and TA98, and MNNG was mutagenic in TA 1535, TA100 and TA98. In this study MT-C was false negative in the six *Salmonella* strains.

A summary of data obtained after screening the aminoglycoside antibiotics in the presence of *S. cerevisiae* D5 for potential genetic damage is presented in Table 2. The results were negative indicating that these antibiotics were not capable of causing mitotic crossing-over and mutations. The three mutagens, 4NQO, MNNG and MT-C were apparently capable of causing mitotic recombination in the D5 strain of *S. cerevisiae*.

Discussion

None of the aminoglycoside antibiotics tested were mutagenic in the systems employing *S. typhimurium* strains TA1535–1538, TA100 or TA98 or *S. cerevisiae* D5. The two carcinogenic agents, 4NQO and MNNG are clearly capable of inducing mutations in *S. typhimurium* and *S. cerevisiae*, and the carcinogenic antibiotic mitomycin C was clearly mutagenic only in yeast. McCann *et al.* indicated that mitomycin C needs excision repair to show its mutagenic activity in *S. typhimurium* and is weakly mutagenic in *S. typhimurium* strain *his* G46/pKM101 with excision repair.[2]

The D5 colonies of red and pink sectors arise from mitotic recombination.[4] The aminoglycoside antibiotics did not induce recombination. However, mutagenic treatment of *S. cerevisiae* D5 causes not only the formation of colonies with red and pink sectors but also colonies with white and pink, white and red double sectors or other colonies that are entirely pink or entirely red. These aberrant colonies are caused by mitotic gene conversion, point mutations, chromosomal deletions and aneuploidy. Each total frequency of aberrant colonies in experiments with the three carcinogens is apparently high as compared with the aminoglycoside antibiotics.

As the reverse mutation detects particular types of genetic alterations and the test organisms differ in their sensitivity towards the induction of mutations by different mutagens,[5] the mutation screening in both *S. typhimurium* and *S. cerevisiae* are suitable for genetic safety evaluations.

From the above results we can conclude that the seven antibiotics (KM, AKM, DKB, RSM, AMK, GM, TOB) do not have mutagenic activity and these antibiotics, which are not almost metabolized in mammals, appear to be genetically safe drugs.

References

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