

Activation of Nisin Production by UV-Irradiation in a Nisin-Producing Strain of *Streptococcus lactis*<sup>1</sup>

Nisin is an antibacterial substance, produced by certain strains of the common dairy organism, *Streptococcus lactis* (MATTICK and HIRSCH<sup>2</sup>). It is extensively used for the preservation of processed cheese by preventing growth of clostridia. Nisin is also known to lower the heat treatment of various other canned dairy products (FOWLER and McCANN<sup>3</sup>).

Although some attempts have been made in recent years to produce mutants of lactis acid bacteria for increased acid and flavour production (BANNIKOVA and PYATNITSYNA<sup>4</sup>, GRINEVICH<sup>5</sup>, KUULA et al.<sup>6</sup>), there is paucity of information in regard to increase in nisin production by mutation techniques in lactic streptococci, except for one report (CSISZAR and PULAY<sup>7</sup>). In the present study, an attempt has been made to isolate some high nisin-producing mutants in a strain of *S. lactis* by irradiation.

**Experimental procedures.** *Streptococcus lactis*-6, a nisin-producing strain isolated locally was used. Cells harvested from a 6-h-old broth culture were washed and suspended in phosphate buffer (pH 7.0, 0.02*M*) and adjusted to an optical density between 0.55 to 0.66, before using for irradiation.

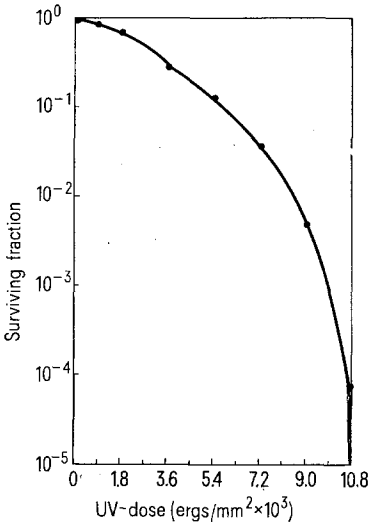
A Mineralight<sup>8</sup> Lamp with twin 4-watt germicidal tubes and built-in filter to give 95% of the output at 2537 Å

was used as the radiation source. 20 ml cell suspension was used and kept stirred with a magnetic stirrer during irradiation. Dose rate was measured with a UV-dose-rate meter (M/s Ultraviolet Products Inc., California, USA).

After plating control and experimental samples on yeast dextrose agar, a survival curve was prepared by plotting the log number of survivors against UV-dose ranging from 0–10,800 ergs/mm<sup>2</sup>. Survivors isolated at random from petri plates showing 1.0–0.1% survival, were compared with the non-irradiated control for variations in nisin and acid production.

For nisin production, isolates were grown in yeast dextrose broth for 16 h at 30°C and nisin content was determined according to the procedure of TRAMER and FOWLER<sup>9</sup>. Titratable acidity was estimated in 8 h old cultures by titrating a known aliquot of the sample against 0.1*N* NaOH.

**Results and discussion.** The ultraviolet survival curve of *Streptococcus lactis*-6 is illustrated in the Figure. An UV-dose of 9,000 ergs/mm<sup>2</sup> gave less than 1.0% survival and mutants were isolated from such petriplates. Definite increase in nisin production to the extent of 50 and 100% was evident after first and second treatment with UV-irradiation. After third treatment, a downward trend in nisin production was noticed, although the range in nisin production was much higher compared with the non-irradiated parent culture (Table). CSISZAR and PULAY<sup>7</sup> reported 10 to 15 times increase in nisin production by UV-irradiation in a nisin-producing strain of *Streptococcus lactis*. The differences in degree of increase in nisin production in the 2 studies may perhaps be due to inherent differences in the nisin-producing ability of the 2 cultures. It is of interest to note that UV-irradiation had no effect on acid production by the culture after first and second exposures to UV-irradiation, although it slowed down



Survival of *Streptococcus lactis*-6 with UV-irradiation.

<sup>1</sup> NDRI Publication  
<sup>2</sup> A. T. R. MATTICK and A. HIRSCH, *Nature*, Lond. **154**, 551 (1944).  
<sup>3</sup> G. G. FOWLER and B. McCANN, *Aust. J. Dairy Technol.* **26**, 44 (1971).  
<sup>4</sup> L. N. BANNIKOVA and I. N. PYATNITSYNA, 18th Int. Dairy Congress, IE, 123 (1970).  
<sup>5</sup> A. G. GRINEVICH, *Microbiology* **35**, 831 (1966).  
<sup>6</sup> RAYANA, *J. Dairy Sci.* **54**, 331 (1971).  
<sup>7</sup> R. K. KUULA, B. RANGANATHAN, S. M. DUTTA and H. LAXIMANA.  
<sup>8</sup> J. CSISZAR and G. PULAY, 14th Int. Dairy Congress **2**, 423 (1956).  
<sup>9</sup> M/s Ultraviolet Products, California, USA.  
<sup>9</sup> J. TRAMER and G. G. FOWLER, *J. Sci. Fd. Agric.* **15**, 522 (1964).

Increase in nisin production in *S. lactis*-6 after exposure to UV-irradiation

No. of exposure	Survival (%)	No. of survivors examined	Nisin production* (Ru/ml)	Titrateable acidity (%)
Untreated		Parent	Avg. 500	0.49
1st	0.17	18	Avg. 750	0.48
			Range 500–1000	0.48–0.49
2nd	0.26	22	Avg. 1028	0.48
			Range 500–1500	0.45–0.50
3rd	5.2	17	Avg. 639	0.42
			Range 375–1000	0.40–0.51

\* Reading unit as defined by Mattick and Hirsch<sup>2</sup>.

after third exposure (Table). BANNIKOVA and PYATNITSYNA<sup>4</sup> reported an increase in acid production by UV-irradiation in *S. lactis*, while KUULA et al.<sup>6</sup> observed decrease in acid-producing ability in a strain of *S. diacetilactis* by the same method of treatment. The nisin-producing character thus appears to be independent of acid-producing ability of the culture.

*Streptococcus lactis*-6 exhibited a higher percentage of survival namely, 5.2% after third exposure as against 0.17 and 0.26% after first and second exposures respectively, thereby indicating development of higher percentage of resistant population in the culture although the dosage was kept constant.

**Zusammenfassung.** Isolierung von Mutanten mit erhöhter Nisin-Produktion aus einem weiteren *Streptococcus lactis*-6 Stamm durch UV-Bestrahlung (9000 erg/mm<sup>2</sup>). Die Produktion des Antibiotikums nahm nach der ersten Bestrahlung um 50%, nach der zweiten Bestrahlung um 100% zu, während nach der dritten eine Abnahme beobachtet wurde.

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29 August 1972.

## Effects of d-Amphetamine upon Open Field Behaviour in Two inbred Strains of Mice

BALB/cJ mice are regarded as more emotional than C57BL/10J mice because they defecate more and ambulate less in the open field<sup>1,2</sup>. They also have a higher concentration of serotonin and norepinephrine in brain stem and lower concentration of norepinephrine in the hippocampus and the pyriform cortex as compared with C57BL/10J<sup>3-5</sup>.

In order to elucidate some of the relationships between these differences in brain chemistry and the behavioral differences of these two strains of mice it would be of value to test the effects upon behavior of a drug such as amphetamine that acts upon release and uptake of brain monoamines (c.f. COSTA and GARATTINI<sup>6</sup>).

This paper reports differences between BALB/cJ and C57BL/10J mice in their response to d-amphetamine as measured by the open field test.

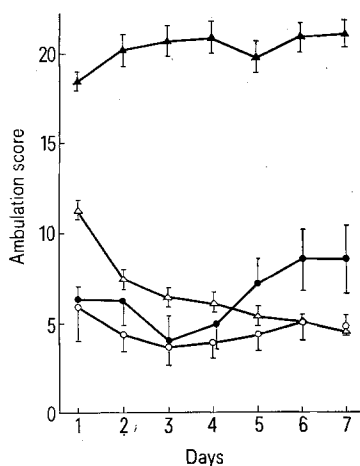
**Methods.** Male mice of the strains BALB/cJ and C57BL/10J were received from Jackson Laboratory at weaning age and housed 5 to a cage for 3 to 4 weeks to acclimate. Treatment was started at the age of 40 to 50 days. The open field used was a square surface 24 × 24 inch surrounded by walls 8 inch high; the walls and floors are white, and the floor is divided into 36 squares by thin lines. 12 BALB/cJ and 14 C57BL/10J mice were given an i.p. injection of 5 mg/kg of d-amphetamine

sulfate (in 0.1 ml 0.9% NaCl). The controls (15 BALB/cJ and 13 C57BL/10J) received an injection of the same volume of saline solution. Each animal was tested individually 15 min after injection; mice were placed into one corner of the open field and allowed to explore it for 3 min, and the number of lines crossed during that time was recorded as the ambulation score. The open field was wiped clean with a wet sponge and paper towel after testing each mouse. All tests were done between 09.30 h and 12.30 h. This whole procedure was repeated daily for each mouse on each of 7 consecutive days. Ambulatory activity was expressed as the square root of the number of lines crossed during a 3 min period.

**Results.** C57BL/10J mice ambulated more than BALB/cJ mice on the first 4 days (Figure), in agreement with results of other authors<sup>1,3</sup>. The difference was largest on day 1 and became gradually smaller until there was no significant difference in ambulation between the controls of the 2 strains on days 5 to 7. This was due to the fact that C57BL/10J mice became habituated to the open field and explored less after the first day while BALB/cJ did not.

Amphetamine caused a highly significant increase (between 300 and 1000%) of ambulatory activity of C57BL/10J mice with respect to C57BL/10J controls on all 7 days ( $p < 0.001$ ); the rate of locomotion remained constant throughout the 7 days of treatment, and there was no sign of habituation to the novel environment. Amphetamine treated BALB/cJ mice did not show greater ambulatory activity during days 1 to 4 than their controls, but there was a moderate increase in their ambulation (between 200 and 300%,  $p < 0.05$ ) during days 5, 6, and 7.

**Discussion.** The increase in ambulation induced by d-amphetamine in C57BL/10J mice seems to reflect locomotor hyperactivity rather than an increase in exploration, because the animals were about as hyperactive in their home cages as in the open field and because there was no drop in activity, no habituation to the open field, throughout the seven days of testing.



Ambulation in the open field of male BALB/cJ and C57BL/10J mice after an injection of d-amphetamine (5 mg/kg). Data are square roots of the numbers of squares crossed during 3 min. Vertical bars represent standard errors of the mean. BALB/cJ: amphetamine ●; saline ○. C57BL/10J: amphetamine ▲; saline △.

<sup>1</sup> N. D. HENDERSON, *Anim. Behav.* 15, 364 (1967).

<sup>2</sup> J. C. DEFRIES and J. P. HEGMANN, in *Contributions to Behavior-Genetic Analysis-The Mouse as a Prototype* (Eds. G. LINDZEY and D. D. THIESSEN; Appleton-Century-Crofts, New York 1970), p. 23.

<sup>3</sup> J. W. MAAS, *Science* 137, 621 (1962).

<sup>4</sup> J. W. MAAS, *Nature*, Lond. 197, 255 (1963).

<sup>5</sup> H. S. SUDAK and J. W. MAAS, *Nature*, Lond. 203, 1254 (1964).

<sup>6</sup> E. COSTA and S. GARATTINI, *International Symposium on Amphetamines and Related Compounds* (Raven Press, New York 1970).