Thermocins of *Bacillus stearothermophilus*

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Several strains of *Bacillus stearothermophilus* produced bacteriocin-like substances or thermocins which were lethal for thermophilic bacilli. Thermocins were detected in shake cultures of these microorganisms grown at 58 C in nutrient broth (Difco) supplemented with 0.3% yeast extract and 0.1% dextrose. Culture filtrates were assayed for antibacterial activity by the method of G. Ivánovics and L. Alföldi (J. Gen. Microbiol. 16:522, 1957).

Of the 22 strains of *B. stearothermophilus* screened for activity, 12 produced substances which were growth inhibitory to one or more strains. In contrast to the thermocins of NU-1 and NU-2, which showed the widest range of activity, agents produced by NU-4, NU-37, and NCA-1492 were highly specific, each being lethal for only one strain of those examined. The inhibitory effect of thermocins appeared to be limited to only a few strains of the thermophilic species studied. Several cultures of mesophilic bacilli and cocci screened for sensitivity were not affected by these agents. Unlike *B. megaterium* strains, which are sensitive to their own megacins (G. Ivánovics, Bacteriol. Rev. 26:108, 1962), thermophilic strains were resistant to their re-

FIG. 1. Time course of thermocin production by *Bacillus stearothermophilus* NU-10.

TABLE 1. Thermocins of *Bacillus stearothermophilus*

Thermocinogenic strains	Sensitive strains	Culture condition ^b	Thermocin concn units/ml ^c
NU-1	NU-10, -18, -24, -37, -44, -47, NCA-1493, -2184	NL	200
NU-2	NU-1, -7, -10, -18, -24, -34, -35, -37, -39, -44, -47, NCA-1493, -2184	NL	20
NU-4	NCA-2184	L	400
NU-7	NU-41, -44	NL	10
NU-10	NU-18, NCA-2184	NL	1,400
NU-23W	NU-1, -4, -18, -41, -44	NL	10
NU-34	NU-1, -2, -35, -37, -47, NCA-2184	NL	20
NU-37	NU-1	NL	20
NU-41	NU-10, -12, -18, NCA-1493, -2184	L	100
NU-44	NU-12, -18, -35, NCA-1493, -2184	L	10
NCA-1373	NU-1, -2	L	10
NCA-1492	NU-39	PL	10

^a NU (University of Nebraska) and NCA (National Canners Association) strains.

^b Autolytic activity of thermocinogenic strains after 18 hr of incubation at 58 C. L = lysis; PL = partial lysis; NL = no lysis.

^c The unit of thermocin refers to the reciprocal of the last dilution which partially inhibits growth of the indicator lawn.

spective thermocins, and in certain instances they were resistant to substances produced by all other cultures (Table 1). Iso-antagonistic effect was observed in 4 of the 22 strains tested. In cultures of NU-10, the rate of thermocin release was low during the logarithmic phase of growth and reached a maximal level in the early stationary period (Fig. 1). Cessation of metabolic activities of the cell by depletion of nutrients or by exposure to lethal doses of ultraviolet light terminated the release of thermocins. Mass lysis of certain thermocinogenic cultures either spontaneously (Table 1) or after ultraviolet irradiation did not enhance thermocin titer.

The growth-inhibitory activities of all the thermocins studied were not affected by ficin, pepsin, trypsin, ribonuclease, deoxyribonuclease,

chloroform, ether, heating for 5 min at 70 C, or acid treatment to pH 2. These agents were non-dialyzable and did not sediment on centrifugation at $100,000 \times g$ for 2 hr. Thermocins were readily concentrated by 0.6 ammonium sulfate saturation in the cold.

The possibility that the observed antibacterial activity of these organisms is due to their temperate phage is rather unlikely, mainly because: (i) the culture filtrates of thermocinogenic strains, assayed by the conventional methods of phage assay, did not produce plaques on their respective thermocin indicators; (ii) strain NU-4, which produces a thermocin against NCA-2184, releases a lysogenic phage which does not infect the thermocin indicator (F. Shafia and T. L. Thompson, *J. Bacteriol.* **87**:999, 1964).