Thermal Injury and Recovery of Selected Microorganisms

LLOYD D. WITTER
Department of Food Science
University of Illinois
Urbana 61801

In 1943 a past president of our society, Gene Nelson, observed that bacteria exposed to sublethal heating require more restrictive conditions for growth than unheated bacteria (32). During the next two decades a number of such observations were made. Some of these studies suggested the possibility of recovery from injury (2, 10, 22, 27), but a clearcut concept of recoverable injury had not been formulated. The state of the knowledge in 1963 was recorded in an excellent symposium on survival of bacteria printed in the December issue of J. Appl. Bacteriol. This same year Michael Stiles won the American Dairy Science Association student paper competition by reporting the heat injury and recovery of Staphylococcus aureus. He suggested a new approach to the problem of cell injury and recovery by monitoring recovery in the presence of inhibitors to determine the nature of the injury lesion (42). Using this approach, a fellow student, John Iandolo, made the significant discovery that recovery was inhibited by the RNA synthesis inhibitor, Actinomycin D (26). This important discovery initiated a fascinating series of papers on possible mechanisms of injury and recovery.

Iandolo, now at Kansas State University, and his students continued work on S. aureus (25, 36, 37), while the mechanism of Salmonella typhimurium injury and recovery was pursued at the University of Illinois under the leadership of Z. John Ordal (11, 12, 33, 38, 44). By the early 1970’s the general mechanisms were described fairly well. Heat injury of either organism was accompanied by complete degradation of the 30S ribosomal particle and a slight alteration in the 50S particle. These two particles make up the ribosome that is responsible for protein synthesis in the cell. In turn these particles are composed of RNA and proteins; for our purposes, the RNA having a sedimentation coefficient of 23S is associated with the 50S ribosomal particle and the RNA having a sedimentation coefficient of 16S is associated with the 30S ribosomal particle. During heating, the 23S RNA remained intact, but the 16S was degraded completely.

Repair of either S. aureus or S. typhimurium required eventual synthesis of 16S RNA and reassembly of the 30S ribosomal particle. In both organisms the 30S particle was reassembled with protein from the heat degraded ribosomes; the proteins needed for reassembly did not arise from synthesis during recovery. However, S. typhimurium required protein synthesis during recovery but not for ribosome assembly. During recovery the cells synthesized 17S RNA, and protein synthesis was required for 17S RNA to mature into 16S RNA.

Only two facts of general significance have emerged from the collective literature. First, bacteria subjected to sublethal treatment contain repairable lesions. Second, bacteria subjected to sublethal stress become hypersensitive to secondary stress (47). In addition, two lesions have been observed consistently. The first is the leakage of cytoplasmic constituents, indicating damage to the cytoplasmic membrane. The second universally observed lesion is degradation of ribosomes and rRNA.

Other lesions have been observed in injured microorganisms but much less consistently. The correlation of DNA breaks and their repair to cellular injury and recovery is convincing but has been observed limitedly (18). Certainly, inactivation of selective enzymes may play a role in injury (6, 43). Inactivation of catalase may even vie for the rank of a third universally observed lesion (29).

Since recognition of the injured state, there has been a continuing concern with environmental conditions necessary for selective detection and counting of injured organisms. Most of the early papers were content to define and redefine the problems; more recently, interest has turned to doing something about

Received December 7, 1979.
them. Stressed cells may be hypersensitive to normally acceptable diluents (19, 20) or to the warm agar used in plating (28, 35). Therefore, care in selecting diluent and surface plating instead of pour plating is recommended for stressed cells. Preincubation, of course, allows for recovery of injured cells. Since preincubation in liquid media requires choosing the exact time between when recovery is complete and growth commences, it is suggested that solid media be used (35). One versatile method of preincubation is to collect the organisms on a filter pad, incubate the pad on a nonselective medium for preincubation, then transfer the pad to an appropriate selective medium (17). This method has been used successfully with pasteurized milk (13). Several methods have used preincubation on the surface of a nonselective medium and then overlay with the selective medium for colony development (23, 39). If an enzyme can be found which is unique to the bacterium being counted, a nonselective medium can be used in the most-probable-number technique with the presence of the enzyme determining the positive tubes. This procedure was used to count Escherichia coli with glutamate decarboxylase as the indicator enzyme (30).

Perhaps the most successful medium in detecting stressed organisms has been the Baird-Parker medium for detecting S. aureus (4). Not only is it selective and differential, but it also appears not to present a secondary stress to injured S. aureus. Contributing to the success of this medium is pyruvate to cope with hydrogen peroxide. Hence, another approach to counting stressed organisms is the appropriate supplementation of the media. One such supplement is the addition of lysozyme to media to be used in detection of injured sporeformers (1). Another supplement effective in counting either injured sporeformers (21) or nonsporeformers (7, 16, 29) is catalase. Catalase recently has been employed as a key ingredient in a successful alternative medium to the Baird-Parker medium; the Baird-Parker medium is expensive and is perhaps not the medium for dairy products (3, 41).

An injured cell provides a unique opportunity to study the assembly of ribosomes since during recovery essentially the entire cell population is engaged in this activity. Ribosome assembly in S. aureus (25, 37) and in S. typhimurium (45) has been investigated with injured cells. More recently, development of a gentle lysis procedure for S. aureus (15) and painstaking establishment of the most suitable conditions for separation of ribosomal subunits or of RNA species has permitted careful examination of the assembly of ribosomes during recovery (14). Pulse-chase labeling showed two RNA species of large molecular weight which decreased during recovery with simultaneous appearance of precursor 23S and precursor 16S RNA which eventually matured to 23S and 16S RNA. Three ribonucleoprotein particles (49S, 36S, and 30S) were isolated from recovering cells. During recovery the 36S particle decreased with the simultaneous increase in the 49S and the 30S particles. It further was shown that some protein synthesis was needed for maturation of 49S particle to 50S. Uninjured cells growing normally assembled ribosomes by the same process in recovering cells but in roughly 20 min rather than 180 min.

Anyone with further interest in this exciting subject can consult any of a number of fine reviews written since 1975 (8, 24, 31, 46, 47). A particularly good review series is in Advances in Applied Microbiology for 1978 (1, 5, 9, 34, 40).

REFERENCES
8 Busta, F. F. 1976. Practical implications of injured
microorganisms in food. J. Milk Food Technol. 39:138.
