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USE OF OXYRASE® ENZYME TO ENHANCE RECOVERY OF *ESCHERICHIA COLI* O157:H7 FROM CULTURE MEDIA AND GROUND BEEF

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Summary

Escherichia coli O157:H7 is a bacterium that has caused great concern in the meat and food industry during the last few years because of several, well-publicized, disease outbreaks, including the incident at the Jack-in-the-Box fast food chain in Seattle, Washington. The organism can cause severe sickness and even death in certain population groups. To better assure meat safety, federal meat inspection is focusing on developing rapid methods to detect this disease agent and others. Oxyrase is a commercially available enzyme that can accelerate the growth of some bacteria. Current techniques for isolation and culturing of *E. coli* O157:H7 from foods require an enrichment period of 18 to 24 hours, thus limiting their usefulness for perishable foods that are marketed quickly. Our investigation found that Oxyrase shortened required enrichment periods in broth culture only. The enzyme was less effective in sterilized ground beef.

(Key Words: *Escherichia coli* O157:H7, Oxyrase, Ground Beef, Rapid Methodology, Meat Safety.)

Introduction

The January 1993 food poisoning outbreak in the northwestern United States resulting from eating undercooked ground beef contaminated with *Escherichia coli* O157:H7 has focused attention on the need for rapid methods for detecting meat-borne human pathogens. The Food Safety and Inspection Service of the Department of Agriculture is examining procedures to rapidly identify fresh meat and meat products harboring pathogenic (disease-causing) microorganisms. A scientifically based inspection system utilizing microbial testing to support current visual inspection is likely to result. Such a system will mandate improving existing microbiological procedures and developing better ones for detection of various food-borne pathogens. These tests need to be rapid, economical, sensitive, accurate, and simple.

Escherichia coli O157:H7 was identified as a human pathogen in 1982. Several food-related illness outbreaks have been attributed to this microorganism over the last decade. Many have been linked to consumption of under-cooked contaminated ground beef. Symptoms of infection include hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome (severe kidney failure); these manifestations often lead to lifelong disability or death. A limited number of surveys have indicated that about 4% of fresh, retail, ground beef is contaminated with *E. coli* O157:H7. However, because the organism is difficult to recover from foods, the incidence may be higher. Several screening methods for the isolation and/or enumeration of *E. coli* O157:H7 have been developed. Most are too complex and time-consuming to be useful in meat inspection. *E. coli* O157:H7 is usually present in foods in low numbers and is accompanied by competitive microbial populations, including other *E. coli* strains requiring differentiation. These factors mandate selective enrichment procedures that allow *E. coli* O157:H7 cells to repair injuries and grow to high enough numbers for detection, normally extending testing times by 18 to 24 hours. Techniques to reduce the time for enrichment, during which

very low numbers of *E. coli* O157:H7 are increased to detectable levels, are needed in regulatory detection of this pathogen.

Oxyrase enzyme derived from ruptured *E. coli* cells speeds up growth of several bacterial groups by removing oxygen from the growth medium. Several studies at Kansas State University have demonstrated that Oxyrase increases growth rates of certain bacteria, especially those found in the digestive tract of animals (i.e., *E. coli*, *Salmonella*, and *Campylobacter*). We undertook this study to evaluate incorporating Oxyrase into an enrichment medium designed to speed up the recovery of low levels of *E. coli* O157:H7 from culture media and sterile ground beef.

Experimental Procedures

We worked with four strains of *E. coli*, both O157 and non-O157 serotypes, isolated from meats or humans. Brain Heart Infusion (BHI) broth was used as an enrichment medium for all experiments. After specified enrichment periods, McConkey Sorbitol Agar (MSA) was used as a selective plating medium to isolate and count *E. coli* strains. In Experiment 1, flasks containing BHI broth with (*oxy* +) or without (*oxy* –) 0.1 unit/ml of Oxyrase were inoculated with approximately 1 *E. coli*/ml of broth. After inoculation, flasks were incubated in a 37°C water bath and sampled hourly from 0 to 10 h and after 12, 14 and 24 h by plating on MSA. Differences in *E. coli* growth rates in *oxy* + and *oxy* – BHI broth were determined. In Experiment 2, 10 sterile ground beef samples were prepared and placed into sterile plastic bags. BHI broth was added, and samples were mixed for 2 min in a lab blender. Oxyrase (0.1 unit/ml) was added to five of the samples (*oxy +)*. One *E. coli* O157:H7 strain (B) and one non-O157 strain (D) were investigated; strain B at initial levels of 1, 10 and 100 cells/g and strain D at 10 cells/g of meat. Samples were incubated in bags at 37°C, and *E. coli* counts performed hourly from 0 to 12 h and at 14, 16, 18, and 24 h.

Results and Discussion

Oxyrase increased the growth rate for all three pathogenic *E. coli* strains tested in BHI broth, when they were initially present at <1 cell/ml. However, we noticed some variation in the way different strains responded to Oxyrase supplementation. Where differences were noted, they resulted from a shortening of the lag phase (initial stage of growth) of the organisms. However, with nonpathogenic strain D, Oxyrase suppressed growth during the initial 4 h (Figure 1). Strain D numbers increased rapidly in *oxy* - broth during this period. This suppressive effect was overcome by the sampling at 5 h. This observation might be important in developing methods to specifically detect pathogenic strains of *E. coli*.

When pathogenic *E. coli* strain B was inoculated into sterilized meat, no enhancement in growth of the organism occurred. The level of Oxyrase may need to be greater than the 0.1 unit/ml we used, because of possible inhibitory effects of meat on the enzyme. Secondly, autoclaving ground beef to achieve sterility may reduce dissolved oxygen in the samples enough to nullify the benefits of Oxyrase. The use of cooked meat medium for cultivating anaerobic bacteria supports this hypothesis.

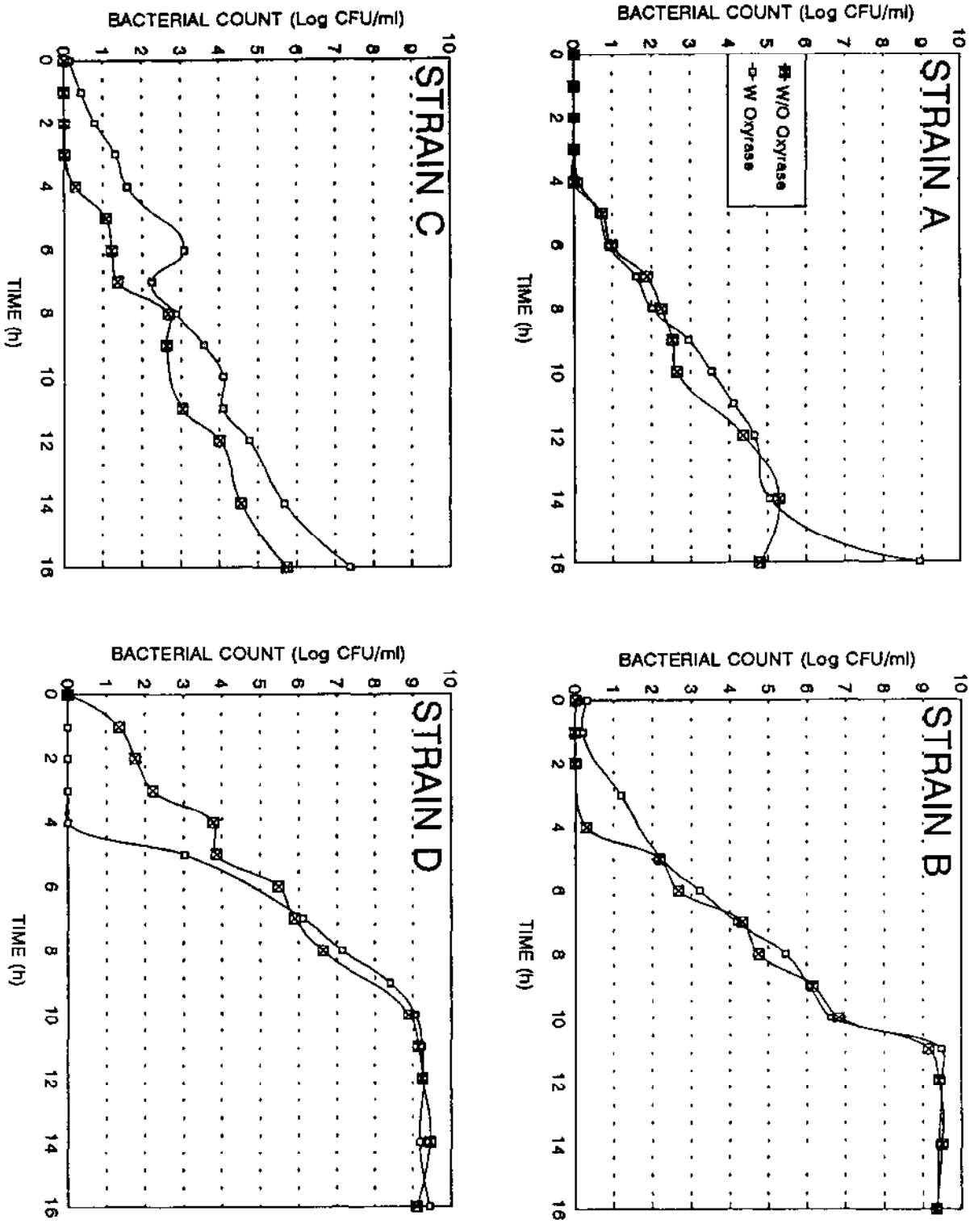

Figure 1. Effect of Oxyrase™ on the Growth of *E. coli* Strains Inoculated at 1 CFU/mL into BHI Broth at 37°C