Role of Glucose in Enhancing the Temperature-Dependent Growth Inhibition of *Escherichia coli* O157:H7 ATCC 43895 by a *Pseudomonas* sp.

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*Escherichia coli* O157:H7 has become a concern to health authorities, industry, regulators, and consumers in recent years (19). The pathogen has been involved in an increasing number of food-borne disease outbreaks linked to the consumption of various foods, including undercooked ground beef (2), unpasteurized milk (12), fresh produce (1), and acidic foods (5). Its survival in acidic foods may be due to acid adaptation (14) or to the natural selection of *Escherichia coli* O157:H7 in foods (19). Competition by the natural flora (11, 24) or by protective cultures (10) may assist in controlling the pathogen during the processing and storage of some foods. *Pseudomonas* spp. are important potential competitors (9) due to their predominant growth in the storage temperature range (i.e., 0 to 15°C) of most perishable food products, including fresh meat (22), milk (23), fish (22) and produce (16). Some *Pseudomonas* species may further exert a siderophore-mediated inhibition against food-borne pathogens (6). Saprophytic *Pseudomonas* spp. are also prevalent in water, soil, and bovine-associated (e.g., cattle feedlot, manure, and hide) environments, which are the primary reservoirs of *E. coli* O157:H7 (19). Recently, Janisiewicz et al. (11) showed that an antagonistic *Pseudomonas syringae* inoculated into wounds of apples prevented the growth of *E. coli* O157:H7. Similarly a high level of background flora inhibited the growth of *E. coli* O157:H7 in ground beef by a factor of 1 to 3 logs at 10 to 12°C; however, this inhibition was maximized anaerobically rather than aerobically due to the natural selection of *Lactobacillus* instead of *Pseudomonas* under limited-oxygen conditions (17, 24). Additional coinoculation studies with *Pseudomonas* strains, though, have shown only weak or no inhibitory effects of these bacteria on *E. coli* O157:H7 in skim milk (20) and ground beef (21), especially at abusive storage temperatures. We have only scanty information on the growth kinetics of *E. coli* O157:H7 in mixed cultures with *Pseudomonas* spp. in broth (9) or model (e.g., liquid) foods (20, 25) under conditions controlled to allow better elucidation of their interactions. *Pseudomonas fragi* did lengthen the lag phase of *E. coli* O157:H7 by ca. 3 h in brain heart infusion broth of neutral pH incubated at 15°C, but it had no effect on either the growth rate or the maximum population density (MPD), or even the lag phase at 37°C (9). Thus, competitive effects by *Pseudomonas* spp. should be considered at temperatures at or below 15°C. Also, the potential role of glucose in altering such low-temperature-dependent competitive interactions has yet to be addressed, given that conversion of glucose to gluconate by *E. coli* O157:H7 in foods may enhance the acid tolerance of the pathogen due to adaptation (4). Therefore, this study was undertaken to evaluate the growth in broth of *E. coli* O157:H7 in coculture with a *Pseudomonas* strain of meat origin at four different temperatures (e.g., 5, 10, 15, and 25°C) and to compare it with the corresponding responses of the pathogen grown in pure culture under the same conditions. The goal was to determine whether supplementation of the culture broth with glucose could alter the timing and magnitude of competitive effects of *Pseudomonas* on *E. coli* O157:H7 over a wide range of incubation temperatures. **Bacterial strains.** The strains used in this study included a rifampin (RIF) (100 μg/ml)-resistant derivative of *E. coli* O157:H7 ATCC 43895 (an acid-resistant, raw-meat isolate originally implicated in a hemorrhagic colitis outbreak caused by hamburgers) (2) and a *Pseudomonas* sp. isolate of meat origin. The latter strain was isolated as a predominant natural.
colonies grew as a pure culture on TSAYE plates. Whenever
grew
glucosylated citrate, and produced acid from glucose and arabinose
which formed creamy-white to pale-yellowish, circular, convex
in our laboratory. The isolate was characterized as a gram-
contaminant from cured pork bologna during previous studies
in our laboratory (VOL. 68, 2002 GLUCOSE ANTAGONISM OF
E. coli O157:H7 and Pseudomonas putida) by the API 20E (Biomerieux, Marcy-l’Etoile, France) code identification system. In accordance with this identification,
colonies of the strain were surrounded by an orange pigment appearing on TSAYE plates after 24 h of incubation at
30°C. Before use in the experiments, both the Pseudomonas
and E. coli O157:H7 strains were activated by the transfer of
0.05 ml of frozen (−70°C) stock cultures (suspended in 20%
glycerol) to 10 ml of tryptic soy broth (BBL) with 0.6% yeast extract (TSBYE) and overnight incubation at 30°C, followed by a second subculturing in TSBYE at 30°C for 24 h. An incubation temperature of 30°C instead of 37°C was selected for E. coli O157:H7 in order to obtain inocula of both bacterial competitors prepared under the same culturing conditions.

**Culture preparation and growth conditions.** The growth of E. coli O157:H7 in pure culture, in both the absence and the presence of glucose, was monitored following inoculation of the pathogen (10⁶ CFU/ml) into individual screw-cap bottles (Nalgene; Nalge Co., Rochester, N.Y.) containing 50 ml of 
glucose-free tryptic soy broth (BBL) with 0.6% yeast extract (TSBYE) and then plated in duplicate on TSAYE or on
G and TSBYE/H11002 before being autoclaved. To monitor the growth of E. coli O157:H7 in coculture with Pseudomonas sp., a second series of 50-ml TSBYE/G flasks were inoculated with the pathogen, as described above, and then 10⁵ CFU of the Pseudomonas strain was added to each of the cultures. Four series of pure and mixed cultures were prepared and then distributed into corresponding incubators set at 5, 10, 15, and 25°C. Incubation was done for up to 14 days without agitation.

**Microbiological and pH analyses.** The levels of microbial growth and pHs of the cultures were determined at 0, 2, 4, 7, and 14 days after inoculation. Samples (1 ml) from each culture were serially diluted with 9 ml of 0.1% buffered peptone water (Difco) and then plated in duplicate on TSAYE or on
TSAYE supplemented with 100 mg of RIF (Sigma) per liter (TSAYE+RIF) to determine the total number of colonies of both competing bacteria and of E. coli O157:H7, respectively. Colonies on agar plates were enumerated after incubation at 30°C for 48 h. No selective medium for the Pseudomonas strain was used because its colonies were clearly distinguishable from those of E. coli O157:H7 on the basis of their appearance (more convex and less than half the size of the characteristically large colonies of the pathogen) and pigment formation. Furthermore, in most treatments, the Pseudomonas sp. out-grew E. coli O157:H7 by such high levels that pseudomonad colonies grew as a pure culture on TSAYE plates. Whenever needed, colonies of E. coli O157:H7 on TSAYE were selectively enumerated and their numbers were subtracted from the total numbers of colonies to obtain separate counts for both competitors; this result was validated by flooding colonies on TSAYE plates with API 20E oxidase reagent to confirm a negative reaction of those counted as E. coli O157:H7. The pHs of the cultures were measured with a digital pH meter (Accumet 50; Fisher Scientific, Houston, Tex.) equipped with a glass electrode (Hanna Instruments, Ann Arbor, Mich.). Experiments were performed in triplicate, microbiological counts were expressed as log CFU per milliliter, and the mean and standard deviation values were calculated.

**Growth of E. coli O157:H7 in pure culture.** When in pure culture, E. coli O157:H7 ATCC 43895RIF+ counts on TSAYE and TSBYE+RIF plates were similar at all incubation temperatures, indicating that RIF had minimal, if any, inhibitory effect on pathogen growth. Therefore, only data from plates with TSBYE+RIF are presented (Fig. 1 and 2) for E. coli O157:H7 to evaluate its growth in pure versus mixed cultures. In pure culture, E. coli O157:H7 grew abundantly, irrespective of glucose supplementation, and finally exceeded 8 log CFU/ml at 10°C (Fig. 1), 15°C (Fig. 2), and 25°C (data not shown) but not at 5°C, where no growth was observed (data not shown). As expected, increasing the incubation temperature enhanced the growth of the pathogen. At 5°C, the E. coli O157:H7 populations declined from days 7 to 14, with average population declines being 0.6 log CFU/ml greater in the presence of glucose (TSBYE+G) than in its absence (TSBYE–G) (data not shown). This finding confirmed the considerable inactivation effect of growth-arresting, cold temperatures on E. coli O157:H7 observed when the pathogen was incubated at 4°C in normal TSBYE (which contains 0.25% glucose) (7), while it further indicated that this effect may be enhanced at increased glucose concentrations. At higher incubation temperatures permitting growth, the effects of glucose appeared to change in response to the growth rate of the pathogen, while at 10°C, the slowly growing populations of E. coli O157:H7 were larger in pure cultures with glucose from days 2 to 14 (Fig. 1). This growth pattern tended to be reversed after 4 days at 15°C (Fig. 2), while it was reversed throughout incubation at 25°C (data not shown).

**Effect of glucose on growth of E. coli O157:H7 in mixed cultures.** Overall, the growth of E. coli O157:H7 was retarded and its MPD was suppressed in coculture with the Pseudomonas strain incubated at 10°C (Fig. 1), 15°C (Fig. 2), and 25°C (data not shown) compared to the growth and MPDs of corresponding pure cultures. Once again, the pathogen did not grow at 5°C. The competitive ability of the Pseudomonas strain was shown to be highly dependent on the presence or absence of glucose, while it was simultaneously greater at lower growth temperatures (Fig. 1 and 2). Indeed, while E. coli O157:H7 populations exceeded 7 log CFU/ml at 7 days in mixed cultures without glucose at 10°C, its growth was strongly inhibited by Pseudomonas sp. in cocultures with glucose (Fig. 1). The inhibitory effects of the pseudomonad strain on E. coli O157:H7 in the presence of glucose at 15°C (Fig. 2) were weak compared to those at 10°C and resulted in the biphasic growth of the pathogen. By analyzing day 1 samples from some of the experiments at various intermediate time intervals (18 to 24 and 36 h of incubation) (data not shown), we found that this
biphasic growth pattern occurred because *E. coli* O157:H7 colonies multiplied to approximately 7 log CFU/ml within 24 to 48 h at 15°C and then the numbers always declined. Thus, there was an early noninteractive growth of the two competitors in the presence of glucose, followed first by an inactivation of *E. coli* O157:H7, which peaked by day 4, and then by a second phase of growth beginning within the next 3 days (Fig. 2). Because abundant growth of *E. coli* O157:H7 without a diauxic pattern occurred in mixed cultures without glucose at 15°C, it was concluded that glucose stimulated the inhibitory activity of the *Pseudomonas* sp. In contrast, at 25°C, the *Pseudomonas* sp. had weak, if any, inhibitory effects on the growth of *E. coli* O157:H7 while glucose did not assist this strain in inhibiting or retarding pathogen growth (data not shown). This result indicated a high competitiveness of *E. coli* O157:H7 at 25°C, irrespective of the presence of glucose.

**Growth of Pseudomonas sp. in mixed cultures.** The population of the *Pseudomonas* sp. exceeded 9 log CFU/ml at all temperatures of incubation. Thus, its CFU, which were at least one to several logs higher than those of the pathogen, could easily be enumerated on TSAYE at 5°C (data not shown), 10°C (Fig. 1), and 15°C (Fig. 2). Enumeration of *Pseudomonas* sp. on TSAYE plates was more tedious at 25°C, especially in cocultures without glucose, which had high numbers of *E. coli* O157:H7 colonies; thus, the simple confirmatory tests described above were used. Notably, in cultures with glucose at 25°C, an early (day 2 to 4) predominance of *Pseudomonas* sp. was succeeded by a late (day 7 to 14) predominance of *E. coli* O157:H7, completed at day 14 (data not shown).

**Effect of microbial growth on culture pH.** Inoculated TSBYE–G cultures had initial pH values of 7.3 to 7.4, which showed restrictive reductions (e.g., 0.0 to 0.4 pH unit) during
growth, irrespective of incubation temperature and of the type (pure or mixed) of culture (data not shown). This was clearly due to the absence of glucose, which resulted in minimal, if any, acid formation by either bacterial competitor. In contrast, pH reductions in TSBYE+G cultures were far more pronounced and were strongly affected by the incubation temperature and the culture type (Fig. 3). Growth of E. coli O157:H7 in pure culture caused great decreases in pH due to the fermentation of glucose into acid, which intensified when the incubation temperature increased from 10 to 15 and 25°C; however, no pH decreases occurred at 5°C due to the absence of pathogen growth (Fig. 3). In contrast, the pH did decrease to 6.0 in mixed cultures at 5°C, indicating the ability of Pseudomonas sp. to produce acid from glucose under refrigeration. Accordingly, the faster decreases of pH in mixed cultures than in pure TSBYE+G cultures from days 0 to 7 at 10°C (Fig. 3) indicated that acidification of the former culture was primarily due to the rapid growth and metabolic activity of Pseudomonas sp. rather than to the slow growth of E. coli O157:H7 at 10°C (Fig. 1). At 15°C, however, the patterns of pH decrease for the pure and mixed cultures were reversed (Fig. 3), indicating the ability of E. coli O157:H7 in pure culture to produce acid (e.g., lactate) and also the still highly competitive effect of Pseudomonas sp. in reducing acidification by its early predominant growth (Fig. 2) and potentially by rapid conversion of available glucose into a different acid, probably gluconate. Conversely, pure and mixed cultures at 25°C showed similarly rapid and extensive pH reductions (Fig. 3), a result consistent with the inability of Pseudomonas sp. to inhibit E. coli O157:H7 at that temperature.

The results of this study indicated that supplementation with 1% glucose enhanced the ability of a Pseudomonas sp. meat isolate to inhibit the growth of E. coli O157:H7 in a mixed culture in broth. Glucose-dependent inhibitory effects appeared to be acid mediated and were strongly affected by temperature, as they were maximal at 10°C, weakened at 15°C, and minimal at 25°C. It is well documented that most Pseudomonas spp. readily oxidize glucose to gluconate and oxygluconate in synthetic media (26) or food extracts, such as meat extracts (8). In fact, this catabolic pathway has been shown to offer a great competitive advantage to Pseudomonas over other spoilage bacteria in fresh meats stored in air or under a high oxygen tension (13, 18, 22). The Pseudomonas strain used in this study did oxidize glucose to gluconate, as shown by the API 20E method. Thus, although the catabolism of glucose was not monitored, published data in correlation with the strain's API profile and pH changes (Fig. 3) suggest that levels of gluconate inhibitory to E. coli O157:H7 were formed in vitro by Pseudomonas sp. in mixed TSBYE+G cultures, mainly at 10 and 15°C. Notably, Massa et al. (15) reported in vitro growth inhibition of enterotoxigenic E. coli and Salmonella enterica serovar Derby by glucose oxidase, which was due to the production of gluconate from glucose in the presence of the enzyme. Thus, the present results compare favorably with the results of that study (15), except that in this study the growth inhibition of E. coli O157:H7 was achieved in vitro through microbial oxidation of glucose to gluconate and through competition of two species rather than enzymatically by the addition of glucose oxidase. Additional studies, including gluconate quantification and evaluation of gluconate-negative pseudomonad strains, are needed as controls to better clarify the glucose effect.

**Applied aspects.** The results of this study suggest that glucose may play an important role in enhancing the inhibition of E. coli O157:H7 by Pseudomonas spp. in foods. It needs to be stressed, however, that a complete growth inhibition of the pathogen at 10°C, and to a lesser extent at 15°C, occurred in mixed broth cultures with 1% glucose. Thus, the concentrations of glucose naturally present in fresh meat (e.g., 0.1 to 0.5%) (18) or as a supplement (e.g., 0.2%) in minced meat in the study of the effects of microbial association on spoilage (13) may be insufficient for Pseudomonas spp. to inactivate or fully suppress the growth of E. coli O157:H7. Indeed, a weak growth inhibition of E. coli O157:H7 by Pseudomonas spp. or
the background flora may be expected in culture broth (9) or foods (17, 20, 21) stored in air without any previous glucose supplementation. Consistent with this expectation, we have recently shown that the inhibitory effects of the *Pseudomonas* strain used in this study against *E. coli* O157:H7 ATCC 43895RF+ are reduced in mixed cultures grown in normal TSBYE with 0.25% glucose at incubation temperatures of 10 to 25°C and are finally decreased to levels (unpublished data) similar to those of the cocultures without glucose (Fig. 1 and 2).

In summary, supplementation of foods with up to 1% glucose may be required to achieve inhibition of *E. coli* O157:H7 by *Pseudomonas*, especially in fresh foods such as raw meat, fish, and produce. Given, however, that numbers of naturally occurring colonies of *E. coli* O157:H7 on meat are much lower (e.g., <10 cells/g) than the 10^5 CFU/ml inoculated in this study, glucose levels lower than 1% (e.g., 0.5%; evidently >0.25%) may also be adequate for inhibition of this pathogen. The exact level is unknown and may strongly depend on the type, pH, and overall quality of meat; thus, this issue requires scientific verification. More studies are therefore needed to evaluate whether the spraying of carcasses or primary cuts of meat with various levels of glucose or the introduction of glucose into minced meat prior to aerobic refrigerated storage can enhance the biocontrol of *E. coli* O157:H7 and other pathogens by naturally occurring or selected *Pseudomonas* strains without adverse effects on product quality. A possible limitation of this approach may be the high numbers of colonies of *Pseudomonas* spp. required for substantial inhibitory effects, given their association with offensive types of meat, milk, and fish spoilage (22, 23). However, increased levels of added glucose, which is preferentially converted to gluconate by selected *Pseudomonas* spp., may reduce the rate and extent of deamination of amino acids and other metabolic activities and thus may delay putrid spoilage (13). On this basis, monitoring in situ glucose metabolism and potentially other inhibitory abilities of pseudomonads at low temperatures (e.g., ≤15°C) may contribute to the control of *E. coli* O157:H7 and may enhance the safety and quality of fresh meat and other foods.

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**REFERENCES**


