Effect of Metallic Cations on the Viability of Phenol-treated *Escherichia coli*

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Five metallic cations (Fe³⁺, Cr³⁺, Ca²⁺, Mg²⁺, Mn²⁺; concentration range, 1.85 × 10⁻⁴ to 37 × 10⁻⁴ M) were incorporated individually as chlorides into nutrient broth and agar media used for the recovery of phenol-treated *Escherichia coli*. The effects observed varied with the concentration and the ionic species. In nutrient agar, Fe³⁺ and Cr³⁺ were generally beneficial but were toxic at 37 × 10⁻⁴ M. Of the divalent ions tested, Ca²⁺ and Mg²⁺ usually gave higher counts in nutrient broth, except at a concentration of 9.25 × 10⁻⁴ M, whereas the effect of Mn²⁺ was rather variable. Two possible explanations are suggested to explain these effects. Toxic materials may be removed from the media by the precipitates formed on the addition of Fe³⁺ or Cr³⁺, or, in the case of the divalent ions, the integrity of the bacterial cell membranes may be maintained.

Flett et al. (2) and Jacobs and Harris (4) reported that inclusion of ferric chloride in the recovery medium is beneficial to the revival and recovery of bacteria after treatment with bactericides. In addition, Jacobs and Harris (5, 6) showed that Fe³⁺, Cr³⁺, Al³⁺, Mg²⁺, and Ca²⁺ are beneficial to bacteria and aid the revival of both phenol- and o-cresol-treated *Escherichia coli* and *Staphylococcus aureus*.

Many different species of bacteria exhibit sensitivity toward their environment after exposure to bactericides, and the survival and subsequent multiplication of such damaged cells may depend upon several related or isolated factors. The purpose of this investigation was to obtain further information on the effect of cations included in the nutrient media on the recovery of phenol-treated *E. coli*.

**Materials and Methods**

Organism and its treatment with phenol. The organism used in this investigation was a laboratory strain of *E. coli* type 1, isolated from water.

The growth from a 24-hr nutrient agar culture was washed, suspended in water, and exposed to 1% (w/v) phenol at 20 C for 35 to 90 sec to give a mortality greater than 90%. Survivors were counted after diluting 10⁶ times in distilled water.

Addition of cations to the recovery media. Solutions of the chlorides of Fe³⁺, Cr³⁺, Ca²⁺, Mg²⁺, and Mn²⁺ were prepared, and these solutions were added either to nutrient agar or to nutrient broth prior to sterilization (at 115 C for 30 min) to yield concentrations of 37, 18.5, 9.25, 3.7, and 1.85 × 10⁻⁴ M. These concentrations were based on the concentrations used originally by Flett et al. (2), namely, 0.03% FeCl₃, 18.5 × 10⁻⁴ M.

Counting techniques. The nutrient broth contained 16 g (per liter) of Lab-Lemco granules no. CM15 (Oxoid) and was solidified with 15 g (per liter) of New Zealand agar when required. Surface viable plates were prepared by use of 10 replicate 0.017-ml drops, and these plates were counted after 24 hr at 37 C. When viable counts were performed in the absence of agar, drops were distributed on 8-cm membrane filters (Oxoid) supported for incubation on a no. 17 Whatman filter paper pad containing 5 ml of nutrient broth.

**Results**

Effect of cations in solid media. The results are given in Table 1. When untreated *E. coli* was used, the count was not affected significantly, with the exception of the markedly depressed counts with Fe³⁺ at 37 × 10⁻⁴ M.

When phenol-treated *E. coli* was used, the response depended upon the concentration and species of ion. Both Fe³⁺ and Cr³⁺ had the greatest effects, and, in concentrations of 18.5 × 10⁻⁴ M and below, the counts were much higher than the counts on the corresponding medium without added cations. At a concentration of 37 × 10⁻⁴ M, both Fe³⁺ and Cr³⁺ were toxic. Of the divalent ions, only Mg²⁺ and Ca²⁺ were beneficial; the optimal concentrations of these substances are 9.25 × 10⁻⁴ M and 37 × 10⁻⁴ M, respectively. Mn²⁺ was markedly toxic to the treated cells, and this effect increased throughout...
the concentration range although it did not affect untreated cells.

Effects of cations in fluid media. The experiments described above were repeated, in the absence of agar, using membrane filters to deduce the effects of the agar. In untreated organisms (Table 2), the mean count was significantly different from the control in only two cases (Mg²⁺, 9.25 × 10⁻⁴; Ca³⁺, 3.7 × 10⁻⁴ M).

As with the nutrient agar, the results obtained with phenol-treated suspensions depended on the ion concentration. Both Fe³⁺ and Cr³⁺ demonstrated the most dramatic effects, particularly at 18.5 × 10⁻⁴ M, but owing to variation between replicates the differences were not statistically significant. It is believed that a failure to demonstrate significant differences resulted from an inability to control the experimental conditions (e.g., the extent of treatment and the incubation temperature) with sufficient precision to produce consistent data from very sensitive systems. However, in all trials of a series, the responses were generally consistently favorable or unfavorable and it is reasonable to assume that the direction, if not the magnitudes, of the mean responses reasonably reflected the situation.

With the exception of Mn²⁺, which gave rather variable responses and was toxic at 37 × 10⁻⁴ M and 9.25 × 10⁻⁴ M, and Mg²⁺ and Ca²⁺ which produced no effect at 9.25 × 10⁻⁴ M, all other ions were beneficial at all concentrations. The responses at the lowest concentrations (3.7 × 10⁻⁴ and 1.85 × 10⁻⁴ M) were similar.

Harris and Richards (3) reported that the count of phenol-treated E. coli was always lower on membrane filters than on nutrient agar. In the present experiments, the addition of Fe³⁺ to the broth increased the membrane filter count, and we wanted to determine whether this improvement nullified the normal poor response on the filter and was similar for the broth and agar media. Accordingly, phenol-treated E. coli was cultivated on four different media, nutrient agar and nutrient broth with and without Fe³⁺ (18.5 × 10⁻⁴ M), the broth being used with the membrane filters as before. The counts were similar to those obtained in previous experiments; i.e., Fe³⁺ potentiated the counts on both the nutrient agar and the nutrient broth. Analysis of variance showed that the effect of Fe³⁺ was significant and both media showed a similar responsiveness.

**Discussion**

It has been known for a considerable time that many metallic cations affect bacteria and other microorganisms, the type of effect depending upon concentration. Low concentrations of ions are stimulatory whereas high concentrations are inhibitory (7). An analogous situation occurred in phenol-treated cells exposed to media containing trivalent cations, although the mechanisms involved were undoubtedly different. It is noteworthy that the effects of Fe³⁺ and Cr³⁺...
were different in the two media; when untreated organisms were used, these ions, at a concentration of $37 \times 10^{-4}$ M, had no effect in the nutrient broth, whereas in the agar medium this concentration of Fe$^{3+}$ was appreciably toxic and this concentration of Cr$^{3+}$ was slightly so. This order of activity (Fe$^{3+} >$ Cr$^{3+}$) was similar with phenol-treated suspensions, for which the ions were definitely toxic in the agar at $37 \times 10^{-4}$ M but were probably beneficial in the nutrient broth.

When autoclaved in media, both ferric and chromic chlorides produced a flocculent precipitate which may function as an adsorbent for toxic materials or may remove such materials by coprecipitation.

However, the divalent cations were not precipitated, so they must influence the revival of phenol-treated E. coli by other mechanisms; e.g., they may prevent the uptake of cationic material by the cell by direct competition for adsorption sites. Riemersma (8) suggested that binding of metallic cations by yeast could increase the stability of the cell membrane, thus hindering lysis by surfactants. The action of vancomycin on E. coli is adversely affected by Mg$^{2+}$, and Russell and Thomas (9) attributed this to competition between the drug and the cation for the same sites on the bacterial cell. Another possibility is that cations may reduce the permeability of damaged cell membranes, thus limiting leakage of materials vital to the cell for its recovery, or, conversely, preventing the diffusion of toxic substances into the cell. In this connection, divalent cations such as Mg$^{2+}$ are involved in maintaining the integrity of bacterial cell membranes, thus preventing lysis (1). Although the mechanisms may be obscure, it is clear that the addition of cations to media may be advantageous for bacterial revival and growth.

**Literature Cited**


