Viability of Airborne *Salmonella newbrunswick* Under Various Conditions

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Abstract

Death rates of *Salmonella newbrunswick* aerosolized from distilled water or skimmilk into a closed chamber were determined at 10 and 21°C and 30, 50, 70, 90% relative humidity (RH). Initial death rates (natural logarithms of slopes of survivor curves without negative sign) for the first 20 min at 10°C ranged from 0.0112 to 0.0346 at 90 and 30% RH, and at 21°C from 0.0141 to 0.056. The D values corresponding to these death rates, ranged from 41 rain at 21°C and 30% RH to 206 rain at 10°C and 90% RH. The secondary death rates for the 20 to 90 rain aerosol age ranged from 0.0057 to 0.0099 at 10°C and 90 or 30% RH, and from 0.0141 to 0.0182 at 21°C and the same relative humidities.

The protection of skimmilk solids was shown by decreases in death rates. The corresponding D values for *S. newbrunswick* aerosolized in skimmilk at 10°C ranged from 245 rain to 404 rain at 90 and 30% RH and at 21°C from 164 to 470 min.

Activation energies for the initial period of death ranged from 3,520 to 7,357 cal per mole at 90 and 30% RH. These values increased to 13,360 and 8,649 cal per mole for the secondary period of death. The negative entropies for the airborne death of *S. newbrunswick* may indicate concentration of solutes in the bacteria. Concentration of solutes or aggregation of macromolecules may result in physiochemical changes which ultimately cause death.

Introduction

The prevention of microbial airborne contamination in food processing areas may be highly important. The time is increasing between packaging and consumption with the present system of mass production and distribution of foods. The possibility for multiplication of organisms from a small contamination is becoming greater to the extent that outbreaks of disease or serious spoilage may occur.

The exclusion of pathogenic organisms such as salmonellae from air of food processing plants is highly desirable. Salmonellosis has received much attention recently because of its public health significance. Yet, little is known about the viability of salmonellae in the airborne state.

This investigation was undertaken to determine the effect of some common factors on the survival of airborne salmonellae.

Information in the literature on sources of food contamination by airborne salmonellae is limited. Although two recent reviews (3, 8) refer to the possibility of contamination of foods from airborne salmonellae, they do not provide specific data. Specific evidence has been shown for airborne contamination by salmonellae in turkey processing plants by Zottola et al. (11). However, less is known about the survival of airborne salmonellae.

Investigators (5) reported that an increase in temperature from 28 to 37°C and in relative humidity (RH) from 15 to 80% increased death rates of *Salmonella pullorum*. *Escherichia coli*, another enteric organism, has been reported by Hayakawa and Poon (7) to have a high death rate in the first second of the airborne state. Williamson and Gotans (10) concluded from their experiments that a change of temperature from 24 to 30°C did not affect greatly the disappearance of *Serratia marcescens*, *Escherichia coli*, *Staphylococcus* and *Streptococcus* species. Ehrlich et al. (6) reported a progressive increase in death rate for airborne *S. marcescens* and *E. coli* between 15 to 40°C, and a significant reduction in death rate of these organisms as a result of temperature increase from 24 to 49°C. *Bacillus subtilis* spores survived well under these conditions. However, at -40°C the three species had fewer survivors than between -18 and 24°C.

Webb (9) claimed that polyhydroxy alcohols, and other organic compounds such as amino
acids, (with hydroxyl groups), amino, and possibly sulfhydryl groups have a protective effect on airborne *E. coli* and *S. marcescens* by replacing bound water to maintain biological integrity. He also found that partial hydrolysates of casein and hemoglobin decreased death rates of these organisms during 0- to 5-hr aerosol ages.

Cox (4) stated that protective agents may form a viscous layer around the cell wall thereby protecting airborne bacteria against the detrimental effects of oxygen. Oxygen (1, 2) has been reported to injure flavin-linked enzymes as the result of free radical activity in bacteria.

**Experimental Procedures**

Figure 1 shows the equipment used for studying the survival of airborne *Salmonella newbrunswick*. This organism originally isolated from nonfat dry milk was grown with $^{32}$P and viability destroyed by heating to 80°C and holding for 15 min. A mixture of the $^{32}$P labeled bacteria and viable cells was suspended in distilled water or skim milk (approximately $10^8$ per ml), aerosolized into a prechamber b) in Figure 1, and passed into a 1.92 x 1.31 x 1.63-m chamber c). The DeVilbiss 40 a) atomizer was operated at 1.45 kg/cm$^2$. A fan d) with the capacity of 22.1 m$^3$ per minute circulated air in the chamber during aerosolization of the organisms into the chamber for 5 min. The chamber was maintained at 10 or 21°C and the relative humidity at 30, 50, 70 or 90% by mist spray and exposure of water or silica gel.

At the end of the 5-min aerosolization and at 10-min intervals during 90 min, 28,316 cm$^3$ of air were taken by the Casella sampler (Fig. 1e). The organisms of each air sampling were deposited on a plate of standard agar to determine the total viable count. Simultaneously from the same location in the chamber, 14,158 cm$^3$ of air were drawn through a membrane filter (Fig. 1f) to determine the total count by radioactivity. A Geiger Muller counter (Fig. 1g) determined radioactivity of samples.

**Results and Discussion**

The survivor curve (% S) for airborne *S. newbrunswick* (Fig. 2) was plotted on the percentage of viable$^1$ count (% T) and total population (% P) by the formula % S = % T/$P \times 100$. The natural logarithms ($\log_e$) of the slopes of the best fit survivor curves were taken to obtain death rates (K values) omitting the negative sign. These K values were also converted into decimal reduction times (D values) so that they might

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$^1$Viable count of airborne bacteria was the colony count on the agar plates. Decrease in this count was attributed to two factors: a) biological, i.e. dying of bacteria in the air, and b) physical removal of bacteria from air due to settling, attraction to the walls, etc.

**Figure 1.** Equipment for studying the survival and sampling of airborne *Salmonella newbrunswick*.

**Figure 2.** Survivors (%S), total population (%P), and viable population (%T) curves of airborne *Salmonella newbrunswick* aerosolized from skim milk at 10°C and 90% relative humidity.
Fig. 3. Survivor curves of airborne _Salmonella newbrunswick_ aerosolized from skimmilk or distilled water at 10 C and 30% relative humidity. (K₁) initial death rate, (K₁₁) secondary death rate, (K₅) single death rate.

be related to thermal resistance data in the literature.

Formula for the relationship between K and D values was:

\[ KD = \log e \frac{N_o}{N_1} \]

where \( N_o \) = percentage of organisms at zero time.
\( N_1 \) = percentage of organisms reduced by 90%.
K = natural logarithm of the slope of the survival curve.
D = time in minutes necessary to reduce the population by 90%.

Straight or broken survivor curves are shown in Figure 3, for the experimental conditions. Accordingly, single (K₅), initial (K₁) for the first 20 min, and secondary (K₁₁), during 20 to 90 min, death rates were calculated.

Effect of relative humidity, temperature and suspending medium. Table 1 (five or six trials for every condition) shows the viability of _S. newbrunswick_ at 10 and 21 C and 50, 70 or 90% RH expressed both as K and D values. The highest death rates for the initial period of the distilled water aerosolized _S. newbrunswick_ were at 21 C and 50 or 30% RH (0.059 and 0.056). The corresponding D values were 39 and 41 min. As the relative humidity was increased to 70 and then to 90% the initial death rates decreased to 0.0288 and 0.0141. The D values were 80 and 163 min.

At 10 C a similar trend (Table 1) was observed with relative humidity but the death rates were fewer, indicating more survivors of airborne _S. newbrunswick_ at 10 than at 21 C. Death rates of the organism decreased substantially during the following 20 to 90 min as shown by the K₁₁ values in Table 1. However, at 21 C and 90% RH there was no change in death rate during 0 to 90 min.

Further increase in D values occurred at both temperatures and the four relative humidities when the organism was aerosolized from skimmilk. However, the trend of death rates was just the opposite compared to the organisms aerosolized in distilled water. Death rates decreased with decreasing relative humidity when the organism was aerosolized from skimmilk.

Of interest is that lower death rates correspond to higher D values indicating up to several hours of survival for _S. newbrunswick_. This is especially applicable to the secondary death rates of cells aerosolized in distilled water or to the single death rates of skimmilk aerosolized cells.

These results in general are similar to those by Webb (9) for airborne _E. coli_ and _S. marcescens_. He recognized initial higher death rates for both organisms during the 0- to 1-hr aerosol age, and lower death rates for the next 1- to 5-hr aerosol age.

The protection of skimmilk in aerosolized _S. newbrunswick_ may be interpreted several ways: a) milk solids may form a protective viscous layer around the organism, retarding evaporation of water from within and the access of oxygen to the internal milieu of the bacteria; b) certain organic compounds in skimmilk such as lactose, peptides, and amino acids may replace bound water necessary to maintain functional structures of various macromolecules as suggested by Webb (9).

The opposite influence of relative humidity on death rates of skimmilk aerosolized _S. newbrunswick_ may be the result of more rapid evaporation of water at the lower relative humidity. The more rapid evaporation of water in turn, would lead to formation of a protective viscous layer more rapidly than at a higher relative humidity. Consequently, faster protection would result in lower death rates at lower relative humidities.

Determination of activation energies and entropies. With D values at 10 and 21 C and
TABLE 1. Death rates (K) and decimal reduction times (D) of *Salmonella newbrunswick* at 10 and 21 C and at four relative humidities.

<table>
<thead>
<tr>
<th>Relative humidity (%)</th>
<th>Distilled water</th>
<th>Skimmilk</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>K&lt;sub&gt;I&lt;/sub&gt;</td>
<td>K&lt;sub&gt;II&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>10 C</td>
<td>21 C</td>
</tr>
<tr>
<td>30</td>
<td>0.0346</td>
<td>0.056</td>
</tr>
<tr>
<td>50</td>
<td>0.0330</td>
<td>0.059</td>
</tr>
<tr>
<td>70</td>
<td>0.0191</td>
<td>0.0288</td>
</tr>
<tr>
<td>90</td>
<td>0.0112</td>
<td>0.0141</td>
</tr>
</tbody>
</table>

At the various humidities, activation energies, \( \Delta E \), were calculated by the relationships:

\[
\frac{\log D_2 - \log D_1}{T_1 - T_2} = \frac{1}{Z} \tag{1}
\]

\[
\Delta E = \frac{2.303 RT_1 T_2}{Z} \tag{2}
\]

where \( D_1 \) and \( D_2 \) are decimal reduction times at the corresponding absolute temperatures \( (T_1 \) and \( T_2 \)); \( R \) gas constant cal/mole/K; \( Z \) = increase in temperature necessary to reduce \( D \) by one log cycle.

Entropy changes associated with bacterial death were calculated by Webb's (9) equation:

\[
K = \frac{K_b}{h} e^{\left(\frac{\Delta S}{R}\right)} e^{\left(-\frac{\Delta H}{R \cdot T}\right)} \tag{3}
\]

where

- \( K \) = death rate
- \( K_b \) = Boltzmann's constant
- \( h \) = Planck's constant
- \( T \) = absolute temperature
- \( \Delta S \) = activation entropy
- \( \Delta H \) = heat of activation assumed equal to \( \Delta E \) (9)
- \( R \) = gas constant in cal/mole

Activation energies and entropies associated with death of airborne *Salmonella newbrunswick* are in Table 2. The activation energies for *S. newbrunswick* resemble those for *S. marcescens* and *E. coli* by Webb (9) both in magnitude and in that they increase from initial to secondary period of death at the respective relative humidities.

The activation energy indicates the relative resistance of the organism at various relative humidities and two temperatures. These activation energies are associated with the chemical or physicochemical processes or both which result in bacterial death in air. The increase of activation energy from the initial period of death to the secondary period may be indicative of the bacterial population having a natural distribution of degree of resistance to airborne conditions. Therefore, those bacteria with less resistance in air will require less energy to cause death. Conversely, the survivors being more resistant will require more energy to cause death.

The similarity in magnitude of activation energies for *S. newbrunswick*, *S. marcescens* and *E. coli* (9) may indicate the resistance of airborne gram negative organisms as a group.

The negative entropies also resemble those for *S. marcescens* and *E. coli* by Webb (9) both in magnitude and in sign although the

<table>
<thead>
<tr>
<th>Relative humidity (%)</th>
<th>Activation energies</th>
<th>ΔS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E&lt;sub&gt;I&lt;/sub&gt;</td>
<td>E&lt;sub&gt;II&lt;/sub&gt;</td>
</tr>
<tr>
<td>30</td>
<td>7,357</td>
<td>8,649</td>
</tr>
<tr>
<td>50</td>
<td>8,760</td>
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<td>70</td>
<td>6,230</td>
<td>10,775</td>
</tr>
<tr>
<td>90</td>
<td>3,520</td>
<td>13,360</td>
</tr>
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</table>

\( E_I \) are activation energies during initial and \( E_{II} \), secondary periods of death; \( S_I \) and \( S_{II} \) are entropies for same periods.

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secondary death rates were associated with increased negative entropies. The negative entropies may indicate concentration and crystallization of internal solutes or tightening of molecules as suggested by Webb (9). Therefore, decrease in entropy during the airborne state resulting from either concentration of internal solutes or tightening of macromolecules or both may indicate considerable alteration in the physical arrangement of intracellular entities. Alteration of tertiary structures of enzymes, ribosomes, genetic material (deoxyribonucleic acid) as well as the cytoplasmic membrane during the airborne state and subsequent rehydration may profoundly affect the normal functions leading to loss of viability of bacteria.

Conclusions
Death rates of airborne S. newbrunswick are influenced by suspending media used for aerosolization, relative humidity, and temperature of air.

The broken survivor curves of distilled water aerosolized S. newbrunswick may indicate less and more resistant portions in the populations of these bacteria in the airborne state.

D values of several hours are associated with airborne S. newbrunswick at higher relative humidities when the organism is aerosolized from distilled water. Survivors increase when the organism is sprayed from skim milk, especially at low relative humidities. Long survival of airborne S. newbrunswick may be a potential public health hazard in food packaging areas.

Activation energies associated with the death of distilled water aerosolized S. newbrunswick indicate moderate resistance of the organism. The negative activation entropies may indicate concentration of solutes which can result in physicochemical changes leading to bacterial death in air.

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References