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Resuscitation Effects of Catalase on Airborne Bacteria

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Catalase incorporation into enumeration media caused a significant increase (>63%) in the colony-forming abilities of airborne bacteria. Incubation for 30 to 60 min of airborne bacteria in collection fluid containing catalase caused a >95% increase in colony-forming ability. However, catalase did not have any effects on enumeration at high relative humidities (80 to 90%).

A change in cellular water content due to desiccation and dehydration represents the most fundamental potential stress to airborne bacteria (5, 6). Dehydration may cause structural damage to bacterial membranes (8). Stressed bacteria may be unable to grow under conditions that are otherwise conducive to growth (3). In order to repair the damage caused by stress and reach a fully viable state, stressed bacteria may have to undergo resuscitation, a process which may require the addition of specific resuscitation agents such as betaine, pyruvate, catalase, and peptone to media (1, 2, 10, 11, 13). Previous studies in our laboratory have shown the positive effects of the osmoprotectant betaine on the colony-forming abilities of airborne bacteria (13). Catalase has been used to increase the enumeration efficiencies of physically and chemically stressed microorganisms (4, 7, 14). Catalase acts by removing or degrading hydrogen peroxide (H_2O_2), which is lethal to cells. Cellular catalase activity is reduced by stress, thus resulting in the accumulation of H_2O_2, and the inability of the cells to destroy this toxic compound may lead to loss of colony-forming ability (9, 14). The present study was initiated to test the effects of catalase on enumeration and resuscitation of air-stressed bacteria.

Experiments were conducted separately at a waste water treatment plant (WWTP) in Albany, Oreg., and in a greenhouse at the Willamette Research Station of the U.S. EPA Environmental Research Laboratory in Corvallis, Oreg., on a representative mixed airborne bacterial population and on aerosolized *Pseudomonas syringae* TLP2, respectively.

Effects of catalase on recovery. Airborne bacteria from the WWTP were collected directly in slit samplers (New Brunswick Scientific, Edison, N.J.) by using Luria-Bertani (LB) agar (Difco Laboratories, Detroit, Mich.) plates with and without 1,000 U of catalase (Sigma Chemical Co., St. Louis, Mo.), filter-sterilized, and surface spread 30 min prior to use. This experiment was repeated three times with eight replicates per experiment for each amendment.

P. syringae TLP2 (obtained from Steve Lindow, University of California, Berkeley, Calif.) was grown in LB broth for 18 h at 30°C on a shaker. Cells were harvested, washed three times with 10 mM phosphate buffer (4.0 g of KH_2PO_4 and 13.6 g of K_2HPO_4 per liter of distilled water; pH 7.2) and resuspended in distilled water to ca. 10^6 CFU/ml. The washed-cell suspension was sprayed using a CO_2-pressurized sprayer (R & D Sprayers, Inc., Opelousas, La.) in the greenhouse and collected in Andersen samplers (Andersen Samplers, Inc., Atlanta, Ga.) on LB agar with and without catalase. This experiment was repeated twice with eight replicates of each amendment. A one-way analysis of variance (SAS) (15), performed on the log-transformed data, indicated a significant increase in the colony-forming abilities of airborne bacteria after amendment with catalase. Results are presented as mean $\log_{10}$ CFU per cubic meter with 95% confidence intervals (Table 1). The average percent increase in the number of CFU per cubic meter was found to be 104% for WWTP bacteria and 63% for *P. syringae* after catalase amendments.

Resuscitation of stressed bacteria by catalase. Airborne bacteria from the WWTP were collected for 20 min in eight all-glass impingers (AGIs; Ace Glass Inc., Vineland, N.J.) containing 20 ml of 10 mM phosphate buffer. After 20 min, four of the eight AGIs received 1,000 U of catalase. At 0, 30, and 60 min after catalase addition, samples were removed from all the AGIs and plated on LB agar. Plates were incubated at 30°C for 48 h. This experiment was repeated four times. Similar experiments were done with *P. syringae*. To determine the extent of catalase-induced resuscitation of airborne bacteria, a repeated-measures analysis of variance was performed on the differences between counts for each of the sampling times. (Results are presented as mean CFU per cubic meter ± 1 standard error and as percent increase in counts over the 0-min counts [Table 2].)

Incubation of airborne bacteria with 1,000 U of catalase for 30 min caused an increase of 98.4% in the colony-forming abilities of bacteria from the WWTP. By 60 min, this increase was 112.9% (Table 2). Similar results were obtained with *P. syringae* (110.9% after 30 min; 112.1% after 60 min).

Effects of high RH on catalase-induced resuscitation. *P. syringae* was aerosolized at 80 to 90% relative humidity (RH)

TABLE 1. Effects of catalase on enumeration of airborne bacteria collected at a WWTP and of *P. syringae* sprayed into a greenhouse

Catalase added	Avg $\log_{10}$ CFU/m^3 (± 95% confidence limits)[a]	
	WWTP bacteria[b]	*P. syringae*[c]
No	2.897 (±0.038)	3.339 (±0.042)
Yes	3.207 (±0.038)	3.552 (±0.042)

[a] Obtained from Fisher's least significant difference test (SAS) (15).
[b] At (average) 17°C and 51% RH.
[c] At (average) 22°C and 49% RH.

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TABLE 2. Catalase-induced resuscitation of airborne bacteria collected at a WWTP and of aerosolized *P. syringae*

Time (min)	WWTP bacteria[a]		*P. syringae*[b]	
	Avg CFU/m^3 (± SE)	% Increase	Avg CFU/m^3 (± SE)	% Increase
0	281.50 (±23.56)		112.68 (±5.34)	
30	558.50 (±73.28)	98.40	237.67 (±14.22)	110.93
60	599.24 (±59.20)	112.87	239.00 (±7.29)	112.11

[a] At (average) 20°C and 48% RH.
[b] At (average) 23°C and 40% RH.

and collected in AGIs as described above. After catalase addition, samples were plated on LB agar. Data analysis was done as described above.

As shown in Table 3, there was a significant reduction in the resuscitation efficiency of *P. syringae* aerosolized at high RHs. After a 30-min incubation with catalase, the colony-forming ability increased by only 25.5%, and after 60 min, this increase was only 5.3%.

Previous studies in our laboratory have shown that aerosolization causes stress in bacteria, as indicated by a reduction in viable counts over time (12). We show here that the addition of 1,000 U of catalase to media causes a significant increase in enumeration of airborne bacteria. In a number of studies, exogenously added catalase has been found to increase colony-forming abilities of stressed bacteria, since cellular catalase activity is reduced by stressful conditions (7, 14). We have shown that incubation of air-stressed bacteria with catalase significantly increases colony-forming ability, indicating that catalase may facilitate repair of some of the damage caused by aerosolization. It should be noted that at least a part of the increase in counts at the WWTP may be due to the increased survival of anaerobic and microaerophilic organisms, since a mixed population was sampled.

TABLE 3. Effect of 80 to 90% RH on catalase-induced resuscitation of aerosolized *P. syringae*

Time (min)	With catalase		Without catalase	
	Avg CFU/m^3 (± SE)	% Increase	Avg CFU/m^3 (± SE)	% Increase
0	62.69 (±6.76)		62.33 (±9.59)	
30	78.69 (±7.79)	25.52	64.00 (±10.52)	2.68
60	66.00 (±5.54)	5.28	61.67 (±11.47)	−1.06

Resuscitation ability was significantly reduced at high humidities (80 to 90%). This may be because the bacterial membrane is minimally damaged at high RH and prevents leakage of intracellular catalase as well as entry of exogenous catalase.

In summary, catalase addition increases the colony-forming abilities of air-stressed bacteria and facilitates resuscitation. However, catalase-induced resuscitation is greatly reduced at high RHs.

REFERENCES

1. **Andrew, M. H. E., and A. D. Russell.** 1984. The revival of injured microbes, p. 1–384. Academic Press, Inc., New York.
2. **Baird-Parker, A., and E. Davenport.** 1965. The effect of recovery medium on the isolation of *Staphylococcus aureus* after heat-treatment and after storage of frozen or dried cells. J. Appl. Bacteriol. **28:**390–396.
3. **Bissonnette, G. K., J. J. Jezeski, G. A. McFeters, and D. G. Stewart.** 1975. Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Microbiol. **29:**186–194.
4. **Brewer, D. G., S. E. Martin, and Z. J. Ordal.** 1977. Beneficial effects of catalase or pyruvate in a most-probable-number technique for the detection of *Staphylococcus aureus*. Appl. Environ. Microbiol. **34:**797–800.
5. **Cox, C. S.** 1987. The aerobiological pathway of microorganisms, p. 172–201. John Wiley and Sons, Inc., New York.
6. **Cox, C. S.** 1989. Airborne bacteria and viruses. Sci. Prog. **73:**469–500.
7. **Flowers, R. S., S. E. Martin, D. G. Brewer, and Z. J. Ordal.** 1977. Catalase and enumeration of stressed *Staphylococcus aureus* cells. Appl. Environ. Microbiol. **33:**1112–1117.
8. **Hambleton, P.** 1971. Repair of wall damage in Escherichia coli recovered from an aerosol. J. Gen. Microbiol. **69:**81–88.
9. **Kovacs, E., J. Lantos, and H. Mazarean.** 1966. The effects of phage infection on the catalase induction of the *Staphylococcus aureus* culture. Experientia **22:**802–803.
10. **Larsen, P. I., L. K. Sydnes, B. Landfald, and A. R. Strom.** 1987. Osmoregulation in *Escherichia coli* by accumulation of organic osmolytes: betaines, glutamic acid, and trehalose. Arch. Microbiol. **147:**1–7.
11. **Le Rudulier, D., A. R. Strom, A. M. Dandekar, L. T. Smith, and R. C. Valentine.** 1984. Molecular biology of osmoregulation. Science **224:**1064–1068.
12. **Marthi, B., V. P. Fieland, M. Walter, and R. J. Seidler.** 1990. Survival of bacteria during aerosolization. Appl. Environ. Microbiol. **56:**3463–3467.
13. **Marthi, B., and B. Lighthart.** 1990. Effects of betaine on enumeration of airborne bacteria. Appl. Environ. Microbiol. **56:**1286–1289.
14. **Martin, S. E., R. S. Flowers, and Z. J. Ordal.** 1976. Catalase: its effect on microbial enumeration. Appl. Environ. Microbiol. **32:**731–734.
15. **SAS Institute, Inc.** 1990. SAS/STAT users' guide, vol. 1, p. 209–245. SAS Institute, Inc., Cary, N.C.