CATALASE ACTIVITY IN *LEPTOSPIRA*

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ABSTRACT

RAO, P. J. (University of Illinois, Urbana), A. D. LARSON, AND C. D. COX. Catalase activity in *Leptospira*. J. Bacteriol. 88:1045-1048. 1964.—A number of serotypes of *Leptospira* were found to possess catalase activity, although considerable variation in activity existed among various serotypes. Catalase activity of *L. pomona* was reduced by inhibitors commonly employed for arresting catalase activity in other biological systems. Catalase activity was increased three to five times by growing cultures under conditions of oxygen availability; however, aeration had no beneficial effect on total viable cell crop. The relationship of oxygen to metabolism and future studies on virulence of the leptospirae is discussed.

Leptospirae are classified as aerobic spirochaetes (Breed, Murray, and Smith, 1957). However, there have been published reports based upon empirical observations that leptospirae were microaerophilic. Such observations were those of Dinger (1932) and Czekalowski, McLeod, and Rodican (1953), who observed bands of leptospiral growth below the surface of semisolid medium. Czekalowski et al. (1953) were unable to demonstrate catalase activity by the addition of hydrogen peroxide to leptospiral cultures. Subsequently, Fulton and Spooner (1956) described the presence of cytochrome components in *L. icterohaemorrhagiae*, which indicated that catalase should be present. However, Armstrong and Goldberg (1960) flooded agar plates containing leptospiral colonies with dilute hydrogen peroxide and failed to see evidence of catalase activity. In the same year, Faine (1960) clearly demonstrated catalase activity in pathogenic leptospirae. This report serves to confirm findings of Faine (1960) and Fulton and Spooner (1956), and also records some additional observations on the properties of leptospiral catalase.

MATERIALS AND METHODS

Leptospiral serotypes were obtained from departmental stock cultures. Colonies of leptospirae were produced on the medium of Cox and Larson (1957) with no added red-cell lysate, and leptospirae were subcultured and grown in the medium of Cox (1955). Total viable counts were determined by colony counts (Larson et al., 1959). Cells were grown under high oxygen tension in 300-ml volumes of medium in 3-liter Fernbach flasks. Cells were grown under reduced oxygen tension in 100-ml volumes of medium in 100-ml screw-capped milk dilution bottles. Oxygen content of culture medium under both conditions of oxygen availability was measured with an oxygen meter (model 51; Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). Determinations were made during the late logarithmic and early stationary phases of growth. Media used for growing cells were inoculated with 1% volume of a mature culture and were incubated at 30 C until maximal visible growth was achieved. Cells were harvested by centrifugation at 10,000 × *g* and were washed once with distilled water. The resulting pellet of cells was suspended in distilled water, and 1.0 ml of the suspension was used to prepare dilutions for assaying the catalase activity of whole cells. Another 1.0-ml sample of the suspension was dried on a tared watchglass at 110 C for 3 hr to determine dry weight. Cellular extracts were prepared from cell suspensions by treatment in a 10-kc Raytheon sonic oscillator for 10 min, followed by centrifugation at 10,000 × *g*. Dry weights of the cellular extracts were determined in the same manner as that employed for whole cells. Catalase activity is expressed as microliters of O₂ per minute per milligram, and was assayed at 30 C by the manometric method of Lawrence and Halvorson (1954) with the use of 158 μmoles of H₂O₂ per ml and phosphate buffer

at pH 7.2. Cells and cellular extracts were diluted sufficiently to release approximately 30 μ liters of O_2 every 5 min.

RESULTS AND DISCUSSION

Preliminary studies on the distribution of catalase in leptospirae were accomplished by flooding petri plates containing leptospiral colonies with 3% hydrogen peroxide. All serotypes studied were capable of destroying hydrogen peroxide as determined by formation of gas bubbles, although some serotypes possessed more catalase activity than did others (Table 1). Colonies of aerobic bacteria such as *Pseudomonas fluorescens* destroy hydrogen peroxide much more

rapidly than do colonies of leptospirae. This is probably dependent in part on the relative amounts of cellular protoplasm present in the colonies, since leptospiral colonies apparently contain as many cells as do colonies of other bacteria (Cox and Larson, 1957).

Substrate concentration of 158 μ moles of hydrogen peroxide per ml, employed by Lawrence and Halvorson (1954), evidently saturates the catalase of *L. pomona* cells without inhibition or destruction of catalase which occurs at higher concentrations (Table 2). Maximal catalase activity in whole cells of *L. pomona* occurred over a broad pH range of 6.0 to 8.0. The absence of a sharp peak for optimal activity could be the result of using whole cells rather than a cellular extract, although Faine (1960) reported a sharp pH optimum near 7.0.

The oxygen content of culture medium incubated under conditions of high oxygen availability was close to saturation. Uninoculated medium contained 6.5 ppm of dissolved oxygen, and the inoculated medium during the late logarithmic and early stationary phases of growth was always near 5.4 ppm. The uninoculated medium incubated under conditions of low oxygen availability contained 6.4 ppm of oxygen, but the inoculated medium during the late logarithmic and early stationary phases of growth did not contain sufficient oxygen for accurate measurement (less than 0.2 ppm). The figures for dissolved oxygen were not corrected for the salt effect on the solubility of oxygen, and the figure of 6.5 ppm probably represents oxygen saturation for the medium at 30 C in an atmosphere of air.

Cells from six different serotypes grown under conditions of high oxygen tension possessed three to five times the catalase activity of cells grown under reduced oxygen tension (Table 3). Similar results were obtained with sonically prepared cellular extracts from cells grown under the two different conditions of oxygen availability. Thus, catalase activity in leptospirae is dependent on the availability of oxygen during growth. Enzymes responsible for the transfer of electrons to oxygen were shown to be adaptive in microorganisms (Ephrussi and Slonimski, 1950; Grunberg-Manago and Gunsalus, 1953; Englesberg, Levy, and Gibor, 1954). The fact that catalase activity is adaptive in leptospirae is indirect evidence that such may be the case with the entire cytochrome system.

The above findings indicate that studies con-

TABLE 1. Catalase activity of different cultures of *Leptospira*

Rapid gas evolution*	Slow gas evolution
<i>L. icterohaemorrhagiae</i> (B) and (K)	<i>L. biflexa</i>
<i>L. canicola</i>	<i>L. icterohaemorrhagiae</i> (C)
<i>L. canicola</i> (R) and (Hond Utrecht)	<i>L. pomona</i> (Wickard)
<i>L. pomona</i> (pomona)	<i>L. mitis-johnson</i>
<i>L. pomona</i> (C-164) (S-91) and (C-B)	
<i>L. bataviae</i>	
<i>L. grippityphosa</i> (21)	
<i>L. autumnalis</i>	
<i>L. hebdomadis</i>	
<i>L. pyrogenes</i>	
<i>L. hos</i>	

* Rapid and slow gas evolution observed visually after flooding leptospiral colonies with 3% hydrogen peroxide.

TABLE 2. Effect of hydrogen peroxide concentration on catalase activity of whole cells of *Leptospira pomona*

H_2O_2 μ moles	Oxygen evolved*
15	2.5
30	5.2
60	13.8
90	14.0
120	13.6
150	13.8
200	11.6
250	9.7

* Expressed as microliters of oxygen evolved per minute per milligram.

cerned with the catalase and cytochrome components of leptospirae should take into account oxygen availability during growth. Difficulty in demonstrating oxygen uptake by leptospirae with suspected substrates may be due to the manner in which the organisms were cultivated (Helprin and Hiatt, 1957); more significant results by respirometer studies might be obtained if the cells were grown under conditions of good oxygen availability to fully develop terminal respiratory mechanisms. Catalase activity has been correlated with the virulence of microorganisms (Merz, 1938; Huddleson and Stahl, 1943; Jawetz and Meyer, 1944; Ghatak, Saxena, and Agarwala, 1958; Faine, 1960), and such studies with the leptospirae should involve care to control oxygen tension during growth. However, good control of oxygen tension in such studies may require perfection of media devoid of serum components, since in our experience aeration by rotary shaking or bubbling devices precipitates considerable quantities of serum proteins and interferes with the harvest of clear cell preparations.

The effect of various inhibitors and treatments on the catalase of *L. pomona* extract is recorded in Table 4. These results are similar to those published by Faine (1960) for leptospiral catalase and also catalases from other sources. Complete inactivation by heating indicates that all catalase activities reported here were due to enzymatic activity rather than to the presence of inorganic materials in the cellular extracts. Antiserum from the rabbit did not inhibit catalase activity, which may indicate that none of the antibodies formed by immunization with whole leptospiral cells was directed against the catalase; or, if catalase

TABLE 3. *Effect of oxygen during growth on the catalase activity of whole cells of leptospirae*

Culture	Growth under reduced oxygen tension	Growth under high oxygen tension
<i>Leptospira biflexa</i>	8*	43
<i>L. canicola</i> (Hond-Utrecht).....	156	447
<i>L. icterohaemorrhagiae</i> ...	90	330
<i>L. grippityphosa</i> (21)....	935	4,000
<i>L. grippityphosa</i> (36K)...	605	2,094
<i>L. pomona</i> (pomona).....	144	403

* Results expressed as microliters of oxygen evolved per minute per milligram.

TABLE 4. *Inhibition of catalase activity in a cellular extract of Leptospira pomona*

Inhibitor	Per cent inhibition
Sodium azide (0.01 M).....	100
Sodium cyanide (0.01 M).....	100
Ethylenediaminetetraacetate (0.2 M)...	40
Hydroxylamine (0.15 M).....	100
Hydroxylamine (0.015 M).....	100
Potassium fluoride (0.01 M).....	0
Heating (2 min at 100 C).....	100
Homologous <i>L. pomona</i> antiserum (rabbit, 0.2 ml).....	0

TABLE 5. *Effect of aeration on total viable cell count of Leptospira pomona*

Conditions of growth	Initial viable count/ml after inoculation ($\times 10^2$)	Viable count/ml after 240 hr of incubation* ($\times 10^6$)
Cultures grown in 100-ml volumes in 250-ml Erlenmeyer flasks on rotary shaker (25 C).....	1.30	7
Same as above but not shaken.....	1.80	7.4
Cultures grown in 100-ml volumes in 100-ml milk dilution bottles; not shaken (25 C).....	1.60	5.7

* Plate counts were performed every 24 hr and reached a maximum in 240 hr.

antibodies were present, the catalase-antibody complex was still enzymatically active.

Although increase in oxygen availability stimulated catalase activity, aeration did not increase the total viable cell crop (Table 5). Small starting inocula were used in order that any beneficial effect of aeration would be apparent. These results indicate that the limiting factor for growth may be some nutritional element rather than an energy-yielding reaction in which the cytochrome system is involved. With similar findings, Fulton and Spooner (1956) used a serum-containing medium and could not demonstrate a beneficial effect of oxygen on the growth of *L. icterohaemorrhagiae*.

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