Neuraminidase Activity in *Mycoplasma gallisepticum*

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The whole viable *Mycoplasma gallisepticum* (strain TT) organisms were found to possess neuraminidase activity with a pH optimum of 5.8 on substrates such as human transferrin, human α1-glycoprotein, and rabbit serum. The enzyme operated optimally at pH 4.5 when N-acetylneuraminyl-lactose was used as the test substrate.

*Mycoplasma gallisepticum*, a respiratory tract pathogen of birds, is known to cause hemagglutination of mammalian and avian erythrocytes (1, 5). Sialic acid receptors on the surface of erythrocytes have been reported to be involved in the hemagglutination by *M. gallisepticum* as well as in the hemadsorption reaction with colonies grown on agar of this Mycoplasma species. Nonspecific inhibition of Mycoplasma hemagglutination by chicken serum which is known to possess inhibitors of influenza virus hemagglutination led Roberts (7) to suggest the presence of a "neuraminidase-like enzyme" in a strain of *M. gallisepticum*. He, however, failed to remove the receptors on chicken erythrocytes for this strain of *M. gallisepticum* by neuraminidase treatment. Neuraminidase activity can be precisely measured by assaying its ability to liberate free sialic acid from a substrate containing bound sialic acid. By using this procedure, we were successful in demonstrating the presence of neuraminidase activity in a *M. gallisepticum* strain. The results obtained are reported in this note.

Mycoplasmas (strain TT of *M. gallisepticum* obtained from A. Ruys of Amsterdam, Netherlands) were grown for 2 days at 37°C in 1 liter of Difco PPLO broth medium prepared by the method of Hayflick (2). The organisms were harvested by centrifugation (10,000 × g) for 1 hr, and the packed organisms were suspended in 4 ml of 0.1 M phosphate-buffered saline (containing no Mg-Ca salts; pH 7.4) and shaken to form a homogeneous suspension which was used for assaying the enzyme activity. This preparation had a protein content of 1 mg/ml, as determined by biuret method.

For assay of neuraminidase activity, 0.15-ml portions of Britton-Robinson buffer at different pH values ranging from 3.0 to 10.0 (8), 0.10 ml of rabbit serum substrate, 0.15 ml of test mycoplasma preparation, and 0.05 ml of sterile distilled water or 0.01 M CaCl₂ solution were incubated at 37°C for 4 hr. The reaction was stopped by the addition of 0.1 ml of periodate reagent (0.2 M sodium-meta periodate and 9 M orthophosphoric acid), and the amount of free sialic acid released in 0.2 ml of the reaction mixture was quantitated by Warren's thiobarbituric acid method (10).

![Graph](image1.png)  
**Fig. 1.** Effect of pH on neuraminidase activity of *Mycoplasma* preparation (final pH values of the reaction mixture are given).

![Graph](image2.png)  
**Fig. 2.** Effect of pH on enzymatic activity.
The uninoculated broth medium used for growing the mycoplasmas had no enzyme activity. The data represented in Fig. 1 show the presence of measurable neuraminidase activity in the mycoplasma preparation. The maximum enzyme activity was detected at pH 5.8, there being a progressive decrease in the activity at pH values below and above this level. Presence of Ca²⁺ ions in the reaction mixture significantly stimulated the neuraminidase activity. Mycoplasma preparations heated at 70 C for 50 min showed complete loss of enzyme activity. The pH optimum of the mycoplasma enzyme on other protein substrates, i.e., human transferrin and human α₁-glycoprotein, was found also to be 5.8. The enzyme, however, operated optimally at pH 4.5 when N-acetylmuraminyl-lactose was used as the test substrate (Fig. 2). The Km values, as determined by the Lineweaver-Burk method (4), for the substrates N-acetylmuraminyl-lactose, human transferrin, and human acid α₁-glycoprotein were found to be 3.0 x 10⁻³ M, pH 4.5 (Fig. 3); 3.5 x 10⁻¹ M, pH 5.8 (Fig. 4); and 5.0 x 10⁻⁴ M, pH 5.8 (Fig. 5), respectively. Thus, with the present assay procedure, we have demonstrated the heretofore suspected presence of neuraminidase activity in at least one of the M. gallisepticum strains. The screening of other strains of M. gallisepticum as well as other Mycoplasma species for possible presence of the neuraminidase activity is now underway.

Myxovirus and some bacterial neuraminidases have been reported to be involved in disease processes (3, 6). Perhaps the neuraminidase activity associated with M. gallisepticum may be of significance in the pathogenesis of the infections caused by this organism. Washed suspensions of whole viable organisms of some M. gallisepticum strains are neurotoxic for turkey poult. The possibility that neuraminidase may be the toxic material was conceived by L. Thomas (9), who reported that the toxicity can be neutralized by the specific antibody to organisms only when administered a few minutes after the infection, the suggestion being that irreversible binding between toxin and brain receptors, presumably sialic acid, occurs very early.

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