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# Effects of Environmental Factors on Growth and Morphology of *Mycoplasma pneumoniae*

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# *Mycoplasma pneumoniae*의 成長과 形態에 미치는 環境要素的 影響

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## ABSTRACT

*Mycoplasma pneumoniae* was examined for growth characteristics and morphology when cultivated in several media supplemented with a variety of sera and under different atmospheric conditions. Different formula of the medium as well as different sources of lot numbers of the serum in the same medium exhibited varying effects on growth rate and adherence. When the organisms were cultivated in SSR-2 medium in a normal atmospheric environment or under a facultative anaerobic condition provided with carbon dioxide, they developed filamentous cells with heavy growth, whereas mainly round-shaped cells were produced under strict anaerobic conditions of hydrogen and carbon dioxide. Both morphologies of the organism were transformed by switching the incubation environments. An inverted phasecontrast microscopy using modified petri dishes was excellent to observe single cells and useful to follow the development of the cells. Growth, turbidity, and pH curves were examined under the best conditions obtained Typical *M. pneumoniae* colonies developed on a solid medium and produced clear plaques when overlaid with sheep blood agar.

## INTRODUCTION

Mycoplasmas are the smallest free-living organisms known to date and their fundamental biology is not yet completely understood. The small size of the organism makes them difficult to study by a regular light microscopy. Many improved light microscopic techniques(Bredt, 1968; Bredt and Bierther, 1974; Hubbard and Kite, 1971) have been used to examine the live organisms and electron microscopy(Boatman, 1973; Freundt, 1969; Kim *et al.*, 1977; Muse *et al.*, 1976) has proved most valuable for observation of these small cells. However, the former does not have satisfactory resolving power for examination of the microbes and the latter requires that the specimens should be desiccated and dead. Furthermore, mycoplasmas have no cell walls and are

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so plastic that staining them for better visibility under the light microscope has been thought to distort or disrupt their morphology (Freundt, 1969). Another drawback in the study of mycoplasmas is the complexity of their growth media.

*Mycoplasma pneumoniae*, the only mycoplasma species known to be pathogenic for humans, has not been conclusively studied in growth, morphologic, and replication parameters, because of the reasons described previously. Since the first cultivation of the organism on a cell-free medium by Chanock *et al.* (1962), different formula of the medium have been in troduced and revised(Difco Lab. 1972; Hayflick 1969; Somerson *et al.* 1973). The serum has been demonstrated to be the most essential requirement for the medium. The main components of the serum are fatty acids and cholesterol(Maniloff and Morowitz 1972; Razin 1978), but their functions are not totally understood. The atmospheric environment for cultivation of the organism has been partially studied. Although Kraybill and Crawford(1965) found that the methylene blue additive coupled with anaerobic conditions had no adverse effect on the growth of *M. pneumoniae*, Eaton and Low(1967) reported that the organism was inhibited by strict anaerobiosis with nitrogen. *M. pneumoniae* is now known to grow well aerobically or in an anaerobic atmosphere of carbon dioxide and nitrogen(Maniloff and Morowitz 1972).

To obtain better cultivation conditions of *M. pneumoniae*, present work was carried out to investigate the effects of several commercial sera in different media on the growth characteristics of the organism. The effects of different atmospheric conditions on the growth and morphology of the organism were also examined with an inverted phase-contrast using modified petri dishes.

## MATERIALS AND METHODS

### Organism

*Mycoplasma pneumoniae* strain CL-8 isolated at Children's Hospital, Columbus, Ohio was used in this study. After storage at −70°C, the stock culture was subjected to two passages for full recovery of the metabolic activities in SSR-2 broth medium(Somerson *et al* 1973) in prescription bottles. In early stationary phase, culture fluids were drained and the organisms adhered to glass surface were harvested and concentrated by scraping into fresh broth medium at one-tenth their original volume. This culture was used as an inoculum.

### Culture media

Four different formula of broth medium, Difco PPLO broth medium(Difco Lab. 1972), Hayflick mycoplasma broth medium(Hayflick 1969), Standard PPLO broth medium(Chanock *et al.* 1962), and SSR-2 mycoplasma broth medium(Someson *et al.* 1973), were examined by supplementing them with 8 different commercial sera (Table 1). SSR-2 mycoplasma agar medium(Somerson *et al.*, 1973) was used for counting colony forming units(CFU) of the cultures. In some instances, horse serum(KC Biol. Inc., lot #27060) was substituted for the bovine serum fraction of the SSR-2 medium(Kim *et al.* 1977).

### Growth experiments

Thirty milliliters of different broth