THE INFLUENCE OF CULTIVATION CONDITIONS ON THE GROWTH OF *MYCOPLASMA HOMINIS*

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Abstract

This study is dedicated to the comparative analysis of the efficiency of cultivating Mycoplasma hominis using surface and submerged methods and assessing the impact of growth characteristics of horse serum on bacterial biomass production during surface cultivation.

Keywords: *Mycoplasma hominis, colony, solid media, broth cultivation.*

Introductions. *Mycoplasma hominis* – is a species of pathogenic bacteria that can colonize the human genital tracts and be associated with inflammatory processes, pregnancy complications, neonatal infections, and infertility. Mycoplasmas are the smallest self-replicating organisms that lack a cell wall and their small genome sizes limit their biosynthetic capabilities, making mycoplasmas quite demanding in terms of in vitro cultivation. The classical diagnostic approach involves isolating *M.hominis* in selective media, and isolated immunogenic proteins are components of test kit for specific antibody determination [1, 2].

The study aimed to investigate the cultivation conditions of *M.hominis* and analyze the influence of different nutrient medium components on cell growth.

Materials and methods. The culture of *M. hominis* was isolated from a positive clinical sample confirmed in a culture test. After inoculating the samples onto a selective diagnostic agar medium, a series of passages were conducted to obtain a pure culture. The isolated culture was identified using two different PCR test kits.

In this study, cultures were grown using the submerged method and agarized nutrient media (surface cultivation). Surface cultivation was conducted on standard PPLO medium (Oxoid, cat. CM0403B) with 1% agar, containing arginine, yeast extract, prepared according to the protocol [3] and different batches of horse serum (Biowest lot S00P1, Biowest lot S00PM, Sigma lot 19D017) at 37°C for 2-3 days. A cell suspension from the agar surface was after obtained in 1 ml of liquid medium.

For submerged cultivation, the suspension from the agarized medium was inoculated into a liquid PPLO medium with the same supplements as the agarized medium. Cultivation was carried out at 100 rpm and 37°C for 5 days, and the marker of mycoplasma presence in the culture fluid was a color change of the phenol red.

The evaluation was performed by inoculating a sample of culture fluid or agar cell suspension onto Petri dishes, Colonies were counted in 5-15 microscope fields of view, and the data was converted to the cell per 1 ml of nutrient medium. For statistical analysis, the Mann-Whitney U-test and ANOVA test were performed.

Results and discussion. In the initial stage, the efficiency of cultivating *M.hominis* by surface and submerged methods was compared. Colonies with characteristic morphology (Fig.1) appeared on the 2nd day of cultivation on an agarized medium. A cell suspension was needed for subculturing due to colony fragility.

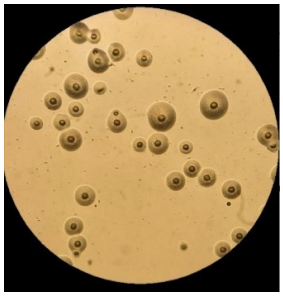

Fig. 1. Appearance of *M. hominis* colonies, magnification 100X.

During submerged cultivation, a change in the indicator color was observed on the 5th day, indicating the accumulation of mycoplasma cells in the culture fluid.

To compare the efficiency of the cultivation methods, cell suspension from the agarized medium and culture fluid were inoculated onto Petri dishes. Calculations showed that during surface cultivation, $6.42 \cdot 10^5$ cells/ml were obtained, while during submerged cultivation, the cell count was $4.02 \cdot 10^5$ cells/ml. The data from both groups were analyzed using the Mann-Whitney U-test ($U_{emp.} = 5$, $U_{0.01} = 7.0$, $U_{0.05} = 13.0$), and the results indicated statistically significant differences between the two groups, with surface cultivation showing higher efficiency.

Additionally, the influence of different batches of horse serum on culture growth was investigated. Some concerns regarding the issue were previously expressed regarding *M. pneumoniae* [4]. When grown on medium with Biowest serum (lot S00PM) the cell concentration was $6.42 \cdot 10^5$ cells/ml , and with Biowest (lot S00P1) it was $5.94 \cdot 10^5$ cells/ml, and with Sigma serum (lot 19D017) $6.56 \cdot 10^5$ cells/ml were counted. An analysis of the data performed using ANOVA ($f_{emp.} = 1.2585$, $p = 0.312$) showed no statistically significant difference between groups, indicating that the investigated sera did not affect culture growth.

Conclusions. A pure culture of *M. hominis* was isolated, and the possibility of its cultivation in liquid and agarized PPLO media was established. It was determined that submerged cultivation is less efficient than surface cultivation and different batches of horse serum in the agarized medium show no effect on the culture growth. It was shown that further research on alternative nutrient components and conditions influencing the growth of *M. hominis* in submerged culture is needed to increase the biomass yield of the culture for the isolation of immunoreactive components.

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