

A New Laboratory Cultivation of *Paramecium bursaria* Using Non-Pathogenic Bacteria Strains

Tomasz Bator

Department of Microbiology, Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Kraków, Poland. E-mail: tbator@ap.krakow.pl

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In most studies dealing with the laboratory cultivation of paramecia (*Paramecium bursaria*), *Klebsiella pneumoniae* bacteria are used to inoculate the medium. However, *Klebsiella pneumoniae* is a typical pathogen, and its use is always associated with a risk of infection. The aim of the present research was to examine non-pathogenic bacteria strains as components of the medium for *Paramecium bursaria*. The paramecia were incubated on lettuce infusions bacterized with different bacteria strains: *Bacillus subtilis* DSM 10, *Bacillus megaterium* DSM 32, *Escherichia coli* DSM 498, *Micrococcus luteus* DSM 348. A strain derived from the natural habitat of *Paramecium bursaria* was used as the control one. Experiments were conducted under constant light and in the dark. Paramecia cells were counted under a stereomicroscope on consecutive days of incubation. The obtained results show that the most intensive growth of *Paramecium bursaria* occurs in the presence of *Escherichia coli* DSM 498. The use of this strain as a component of the medium allows one to obtain a high number of ciliates regardless of the light conditions. It can be concluded that the *Paramecium bursaria* cultivation procedure can be modified by using the non-pathogenic bacteria strain *Escherichia coli* DSM 498 instead of *Klebsiella pneumoniae*.

Key words: *Paramecium bursaria*, Cultivation, Bacteria

Introduction

The ciliate *Paramecium bursaria* (paramecia) with numerous algae (*Chlorella* sp.) occurring in its cytoplasm represents a symbiotic association which is a model example of obligatory endosymbiosis. This complex is used very often as material in various research. The *Paramecium bursaria* cell may obtain nutrients from endosymbiotic algae but heterotrophic feeding plays a very important role in its metabolism (Bator, 2005; Pado and Bator, 2001). For this reason the infusion of lettuce (*Lactuca sativa*), which is used as a typical medium for *Paramecium bursaria*, is additionally inoculated with bacteria which become food for paramecia cells. In most cases, in research connected with cultivation of *Paramecium bursaria*, *Klebsiella pneumoniae* bacteria are used to inoculate the lettuce infusion (Hosoya *et al.*, 1995; Kadono *et al.*, 2006; Tanaka *et al.*, 2005). However, *Klebsiella pneumoniae* is a typical pathogenic bacterium, which is a common cause of hospital infections (Hansen *et al.*, 1998). It may cause pneumonia, infections of the digestive and urinary system, and infections of bones and joints. Moreover, *Klebsiella pneumoniae* is one of the etiologic factors for meningitis (Harvey *et al.*, 1999). It follows that

using these bacteria as a component of the medium for *Paramecium bursaria* cultivation is always associated with a risk that results from the pathogenic nature of *Klebsiella pneumoniae*.

In our laboratory we have used a wild bacteria strain derived from the natural habitat of *Paramecium bursaria* to inoculate the lettuce infusion. Unfortunately, we still have not found out exactly which bacterial species occur in this material. Although the aforementioned bacterial strain gives satisfactory results in laboratory cultivation of *Paramecium bursaria* (Bator and Pado, 2009), it does not guarantee full control of conditions of conducted experiments. Therefore, I decided to examine the usefulness of selected non-pathogenic bacteria strains in laboratory cultivation of *Paramecium bursaria*. The aim of this research was to check the possibility of using unequivocally identified and also safe microbiological material as a component of the medium for *Paramecium bursaria*.

Material and Methods

The ciliate *Paramecium bursaria* from a laboratory culture kept for many years in the Department of Microbiology at the Pedagogical

University of Cracow, Poland (Pado and Bator, 2001) was used as the research material. The non-pathogenic bacteria strains *Bacillus megaterium* DSM 32, *Bacillus subtilis* DSM 10, *Escherichia coli* DSM 498, and *Micrococcus luteus* DSM 348 were received from the German culture collection (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig). Additionally, a bacteria strain derived from the natural habitat of *Paramecium bursaria*, isolated from a sample of water collected from a natural pond in Marcówka (Malopolska province, Poland) was used as the control strain (Pado and Bator, 2001).

In the initial phase of the experiments all bacteria strains were rejuvenated by transfer into Petri dishes containing sterile enrichment agar medium. The dishes with bacteria cultures were incubated at 37 °C for 24 h. Then, each bacteria strain was transferred with an inoculating loop into a flask containing 50 ml of a newly prepared sterile lettuce infusion (Pado and Bator, 2001). Flasks with bacteria strains were incubated at 37 °C for 72 h. In order to determine the intensity of the bacteria growth, measurements of optical density in each culture were made after 72 h using a SPEKOL 11 spectrophotometer (Carl Zeiss) equipped with a nephelometric attachment. The sterile lettuce infusion was used as a blank sample. Measurements for each bacteria strain were conducted at the appropriate wavelengths which were established on the basis of data from the literature: *Bacillus megaterium* – 578 nm (Wang *et al.*, 2005), *Bacillus subtilis* – 600 nm (Warner and Lolkema, 2002), *Escherichia coli* – 550 nm (Pedro *et al.*, 2003), *Micrococcus luteus* – 600 nm (Mukamolova *et al.*, 2002), the control strain – 580 nm (Bator, 2005). Next, the precisely calculated volume of the bacteria cell suspension was taken from the liquid cultures and transferred into 50 ml of the newly prepared sterile lettuce infusion. The volume of the cell suspension did not exceed 5 ml and was calculated on the basis of the optical density values in order to obtain a similar cell density for each bacteria strain. The bacterized lettuce infusions were incubated at 37 °C for 24 h and then were used as the media for cultivation of the paramecia.

In order to determine the possibility of the ciliates' growth in the presence of the examined bacteria strains, three cells of *Paramecium bursaria* were taken from the stock culture and transferred with a glass micropipette into a watch glass

containing 4 ml of the lettuce infusion inoculated with the selected bacteria strain. Six cultures for each bacteria strain were made. Three of them were incubated under constant white fluorescent light (1.02 W/m²) and three were incubated in the dark. The watch glasses were placed in Petri dishes, lined with wet filter papers in order to minimize the evaporation of water. The experimental cultures prepared in this way were incubated for 21 d. Cultures containing bacteria strains isolated from the natural habitat of *Paramecium bursaria* (Pado and Bator, 2001) were treated as control samples. In all cultures paramecia cells were counted under a stereomicroscope on consecutive days of incubation. Next, the cell division coefficient for each day of incubation was calculated by dividing the number of paramecia cells noted on a given day by 3 (the initial number of paramecia cells). After completion of incubation the mean value of cell division coefficient was calculated on the basis of the values collected during the incubation period. In this way, the average cell division coefficient was obtained. This is the parameter used to describe the intensity of the paramecia culture growth during 21 d of incubation (Bator and Pado, 2009). The values of the average cell division coefficient obtained from the experimental cultures were compared with the results from the control cultures (*t* test) and expressed as the percentage of the control sample. The described procedure was repeated 5 times for each bacteria strain.

Results and Discussion

Conducted experiments showed distinct differences in the growth of paramecia cells, depending on the bacteria strain present in the medium.

The growth of *Paramecium bursaria* in the presence of *Bacillus subtilis* DSM 10 (Fig. 1A) and *Micrococcus luteus* DSM 348 (Fig. 1B) proceeded similarly as in the presence of the control strain, independently of the light conditions. The value of the average cell division coefficient, expressed as the percentage of the control sample, in the presence of *Bacillus subtilis* DSM 10 was 97.26% under constant light and 104.19% in the dark. In the case of *Micrococcus luteus* DSM 348 it was 96.78% and 102.24%, respectively. No statistically significant differences between these values and the data obtained in control cultures were noted (Fig. 2).

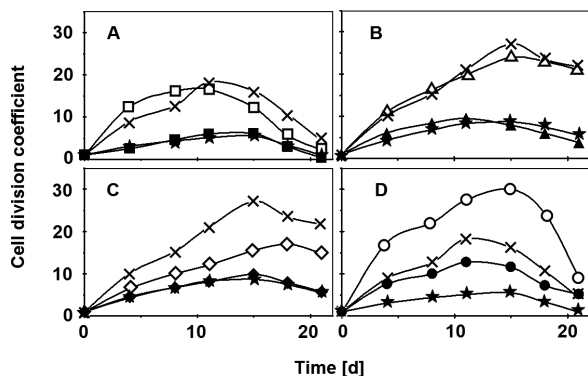


Fig. 1. *Paramecium bursaria* growth in the presence of *Bacillus subtilis* DSM 10 (A; squares), *Micrococcus luteus* DSM 348 (B; triangles), *Bacillus megaterium* DSM 32 (C; diamonds), *Escherichia coli* DSM 498 (D; circles) and a control strain (crosses and asterisks); white marks and crosses, incubation under constant white light; black marks and asterisks, incubation in the dark; mean values.

The influence of the presence of *Bacillus megaterium* DSM 32 on *Paramecium bursaria* growth was dependent on the light conditions (Fig. 1C). In the dark the number of paramecia cells during incubation was comparable with that of the control cultures, and the average cell division coefficient was 105.58%. But under constant light a statistically significant decrease in *Paramecium bursaria* growth intensity was observed, and the average cell division coefficient was merely 64.38% (Fig. 2).

Very interesting results were obtained in cultures incubated on the medium containing *Escherichia coli* DSM 498. The growth of *Paramecium bursaria* in the presence of this bacteria strain proceeded definitely more intensively than in the control cultures. The number of paramecia cells exceeded the results obtained in the presence of the control strain almost twofold during the whole period of incubation under constant light as well as in the dark (Fig. 1D). The average cell division coefficient for *Paramecium bursaria* incubated in the presence of *Escherichia coli* DSM 498 was 176.49% under constant light and 239.88% in the dark (Fig. 2).

The obtained results show that *Paramecium bursaria* can feed on a diverse range of bacterial food. This is a further confirmation of the highly developed adaptive abilities of this organism, which were reported earlier (Bator, 2005). But

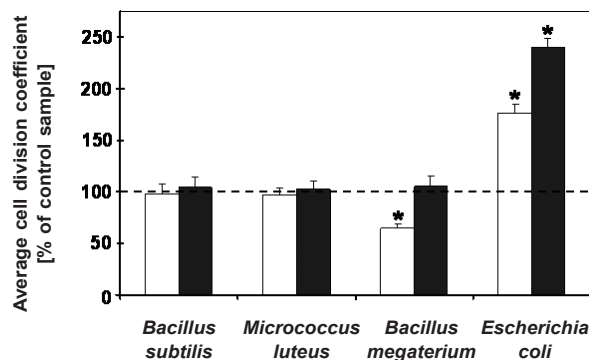


Fig. 2. Intensity of *Paramecium bursaria* growth during a 21-day incubation in the presence of examined bacteria strains; white columns, incubation under constant light; black columns, incubation in the dark; mean values with standard deviation; asterisks indicate a significant difference between experimental and control sample ($P < 0.05$; t test).

not each of the applied bacteria strains is used by *Paramecium bursaria* equally.

Apparently *Bacillus megaterium* DSM 32 is an insufficient food for the examined paramecia. This is attested by a distinct reduction of *Paramecium bursaria* growth intensity during incubation under constant light in the presence of this bacteria strain. The capability of growth of *Paramecium bursaria* in the presence of *Bacillus subtilis* DSM 10 and *Micrococcus luteus* DSM 348 at a level comparable to that of control cultures shows that the aforementioned bacteria strains can be used in laboratory cultivation of *Paramecium bursaria*. But the excellent results obtained in the presence of *Escherichia coli* DSM 498 show unequivocally that this bacteria strain is the best quality food for the examined paramecia.

On the basis of the obtained results, it can be concluded that *Escherichia coli* DSM 498 may be successfully applied in the laboratory cultivation of *Paramecium bursaria*. The use of this strain as a component of the medium allows one to obtain excellent results, regardless of the light conditions. The species *Escherichia coli* and *Klebsiella pneumoniae* are closely related and they have a lot of common features. In consequence they are both appropriate sources of nutrients for *Paramecium bursaria*. However, *Escherichia coli* DSM 498, which is a non-pathogenic strain, is significantly safer than the commonly applied bacteria

Klebsiella pneumoniae, and therefore its use reduces the risk of infection.

The high reproducibility of data obtained in consecutive repetitions of the experiments probably results from exact checking of the number of bacteria during the lettuce infusion inoculation in

each series. Perhaps such a procedure should be applied to bacterize the medium for *Paramecium bursaria*. This subject requires further research, focused on the influence of the number of bacteria present in the medium on *Paramecium bursaria* growth.

- Bator T. (2005), Adaptative Abilities of Symbionts on the Example of the Ciliate *Paramecium bursaria*. Scientific Press of Pedagogical University of Cracow, Cracow (in Polish).
- Bator T. and Pado R. (2009), The influence of hypergravity on the *Paramecium bursaria-Chlorella* sp. symbiotic association. *Z. Naturforsch.* **64c**, 743–746.
- Hansen D. S., Gottschau A., and Kolmos H. J. (1998), Epidemiology of *Klebsiella* bacteraemia: a case control study using *Escherichia coli* bacteraemia as control. *J. Hosp. Infect.* **38**, 119–132.
- Harvey D., Holt D. E., and Bedford H. (1999), Bacterial meningitis in the newborn: a prospective study of mortality and morbidity. *Semin. Perinatol.* **23**, 218–225.
- Hosoya H., Kimura K., Matsuda S., Kitaura M., Takahashi T., and Kosaka T. (1995), Symbiotic algae-free strains of green paramecium *Paramecium bursaria* produced by herbicide paraquat. *Zool. Sci.* **12**, 807–810.
- Kadono T., Uezu K., Kosaka T., and Kawano T. (2006), Altered toxicities of fatty acid salts in green paramecia cultured in different waters. *Z. Naturforsch.* **61c**, 541–547.
- Mukamolova G. V., Turapov O. A., Kazarian K., Telkov M., Kaprelyants A. S., Kell D. B., and Young M. (2002), The *rpf* gene of *Micrococcus luteus* encodes an essential secreted growth factor. *Mol. Microbiol.* **46**, 611–621.
- Pado R. and Bator T. (2001), *Paramecium bursaria* as a typical example of vegetal-animal symbiosis. Adaptation of wild cultures to laboratory conditions. *Pol. J. Environ. Stud.* **10** (Suppl. I), 32–37.
- Pedro M. A., Young K. D., Hölftje J. V., and Schwarz H. (2003), Branching of *Escherichia coli* cells arises from multiple sites of inert peptidoglycan. *J. Bacteriol.* **185**, 1147–1152.
- Tanaka M., Ishizaka Y., Tosuji H., Kunimoto M., Hosoya N., Nishihara N., Kadono T., Kawano T., Kosaka T., and Hosoya H. (2005), A new bioassay for toxic chemicals using green paramecia, *Paramecium bursaria*. In: *Environmental Chemistry: Green Chemistry and Pollutants in Ecosystems* (Lichtfouse E., Schartzbauer J., and Robert D., ed.). Springer Verlag, Berlin, pp. 673–680.
- Wang W., Hollmann R., Fürch T., Nimtz M., Malten M., Jahn D., and Deckwer W. D. (2005), Proteome analysis of a recombinant *Bacillus megaterium* strain during heterologous production of a glucosyltransferase. *Proteome Sci.* **3**, 4.
- Warner J. B. and Lolkema J. S. (2002), Growth of *Bacillus subtilis* on citrate and isocitrate is supported by the Mg²⁺-citrate transporter CitM. *Microbiology* **148**, 3405–3412.