



Comparative analysis of various media and food source on culturing of the eukaryotic microbe *Blepharisma*

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ABSTRACT

Blepharisma is a heterotrich ciliate used as a model system in cytological, biochemical and physiological investigations. The species of this genus exhibit variability with regard to pigment colour and size. The *Blepharisma* sp. was initially isolated from river Yamuna at Okhla Bird Sanctuary, Delhi and maintained in laboratory conditions. Present study was carried out to ascertain the best suitable medium and food source in order to maintain a high yield of cells under laboratory conditions. Six different media (Pringshiem's Medium, Hay Medium, Four different potable waters from water purifiers) and four different food sources (Wheat, Rice, Cabbage and Hay) were used for culturing and maintenance of *Blepharisma* cells. Cell number, size, behaviour and extent of pigmentation were taken into consideration for the comparative analysis. Results showed that the best culturing medium was from one of the purifiers and the best food source was autoclaved wheat grain. The cell size was optimum and growth and pigmentation of the cells was maximum in this combination of medium and food source. If a large quantity of cells is required, wheat as the food source was best whereas rice was for high amount of pigment.

Keywords: *Blepharisma*, Ciliate culturing, Hay medium, Pigment

INTRODUCTION

Blepharisma is a distinctive rose-colored ciliate which is easily identifiable under the light microscope due to the presence of the subpellicular pigment blepharimin. It has a long macronucleus and several micronuclei.

Species of *Blepharisma* show extensive variation because they are greatly affected by nutrition (size, shape, ciliary rows etc) causing taxonomic confusions in describing the species. The subgeneric groups are based chiefly on the macronuclear configuration. More than 30 species of the genus *Blepharisma* have been reported from fresh, brackish and sea water and from the soil of many parts of the world (1), (5), (6), (10)-(12). The cells are widely used in curriculum as model organisms for

cytological or physiological investigations apart from its taxonomic, morphological, morphogenetic, biochemical and molecular investigations as a heterotrich ciliate (2), (10), (15).

Cells of *Blepharisma* are spindle shaped, flattened laterally when not stuffed with food, very flexible but non-contractile. The size varies from 150x27 to 350x55 μm , in-vivo, length/width ratio is about 6/1. One single contractile vacuole is located in posterior body without collecting canals, about 25 μm in diameter, near which, food vacuoles emit digested food or debris through pore like anus in body posterior (14). The physiology of CV functioning can easily be observed under a light microscope. The cultures are supplied even commercially for the purpose of usage in undergraduate classrooms (9), (17).

Blepharisma cells are usually grown in Hay medium or lettuce extract with added bacteria as food. The cells may turn cannibalistic or undergo encystment under unfavourable conditions. The present study was undertaken to find the most suitable medium and food source which should be easily available, economical and provide the best support for the growth of these cells.

METHODOLOGY

In-vitro culture: Water sample was collected from the river Yamuna (28.5700°N, 77.3023°E) at Okhla Bird Sanctuary, Delhi in the month of July, 2014. Identification of the freshwater ciliate isolated from the sample was done in-vivo under stereoscopic microscope. Clonal culture of *Blepharisma* species was maintained in the laboratory at 22-23°C in Pringsheim's medium (3). Boiled cabbage was added to the medium to promote the growth of bacteria which served as the primary food source for the ciliates.

Experimental Media: All the selected media were boiled for 30 min (could also be autoclaved) and were used for culturing experiments after cooling to room temperature.

- 1) Pringshiem's medium (PM): 5ml of Stock solution in 1L of distilled water [Stock solution concentration- $\text{Ca}(\text{NO}_3)_2$ - 4gm/100mL, KCl-520mg/100mL, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -400mg/100mL, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ - 40mg/100mL].
- 2) Hay Medium: Small amount of dried hay was pre-washed with tap water and boiled in 700 ml of tap water for about 15-20 minutes, cooled to room temperature and filtered through whatman filter paper.
- 3) Medium A: Pure-it (Purifier connected to water supply at Dwarka)
- 4) Medium B: Kent (R.O. purifier connected to water supply at Dwarka)
- 5) Medium C: Direct supply water from Noida.
- 6) Medium D: Aquafresh (R.O. purifier Connected to Ground Water from Hamdard Nagar).

Food Source: Four different food sources were used for culturing of *Blepharisma* cells.

- 1) Autoclaved grains of wheat
- 2) Autoclaved grains of rice
- 3) Boiled cabbage leaves
- 4) Hay extract (boiled in tap water)

Experimental set-up: 100 microlitre of well grown *Blepharisma* culture was added to 3 ml of each medium in glass petridishes. The initial number of cells taken was 40 in each petridish (cells were counted under the microscope in per drop of the culture). The 24 petridishes (six media and four different food sources) were maintained at 22-23°C and the experiment was performed in duplicate. Cultures were maintained and observations were recorded everyday for a week.

Cell Counting: Cells were counted in per millilitre of culture taken in a cavity block separately from each experimental petridish. Cell counting was done (beginning after 24 hrs as day 1) in triplicate and the average was taken (if there was any variation in the number) to calculate the total number of cells in total medium of each petridish.

Cell size: Measurement of cell size was done with the help of Ocular Micrometre. The movement of the live cells on a slide was controlled with the help of vaseline petroleum jelly applied on the corners of the coverslip taking care of avoiding any cell distortion.

RESULTS

Blepharisma sp. (Figure-I) grown in six different media (Pringshiem's Medium, Hay Medium, Med. A, Med. B, Med. C, Med. D) provided with four different food sources (wheat, rice, cabbage, hay) for a week exhibited variation in cell number, size and pigmentation with change of medium as well as the food source (Table I-VI, Figure-II).



Figure-I: Photomicrograph of a live cell of *Blepharisma* sp. Scale bar represents 20 μm .

Cell Density/Number

Initially at the time of inoculation there were 40 cells in 3 ml of all media in each petridish. After 24 hours, the cell count changed gradually and varied with the food

source. In Pringshiem's Medium with wheat the cell count increased by 62%, with rice by 25%, with cabbage by 37% and with hay by 50%. In Hay medium with wheat the cell count increased by 62%, with rice and cabbage by 50% and with hay by 37%. In medium A with wheat and rice the cell count increased by 25%, with cabbage by 12% and with hay by 25%. In medium B with wheat and hay the cell count increased by 50% whereas with rice by 25% and with cabbage by 70%. In medium C with wheat and cabbage the count was increased by 50%, with rice by 37% and with hay by 25%. In medium D with wheat the count was increased by 100%, with rice by 75%, with cabbage by 25% whereas with hay by 50%.

On Day 3 and Day 6, in all media with all food sources the cell count was raised to many folds with maximum increase in medium D and wheat (Table I-VI, Figure-II).

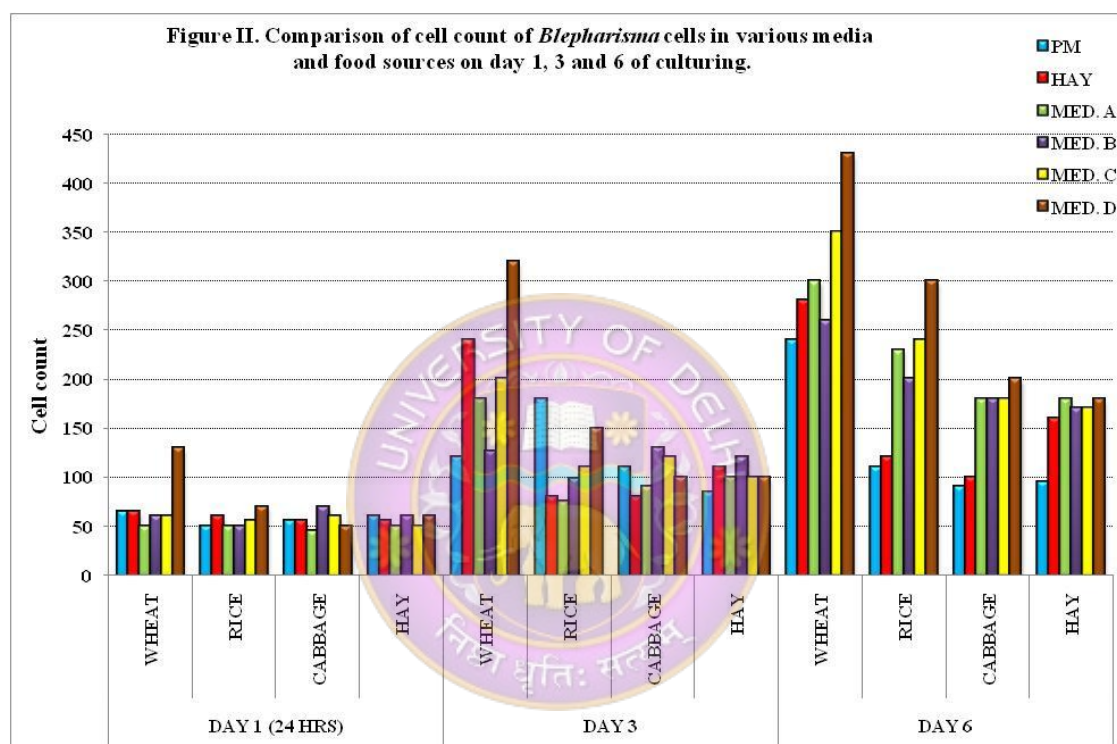


Figure-II Comparison of cell count of *Blepharisma* cells in various media and food sources on day 1,3 and 6 of culturing

Cell Size

Initially the size of the *Blepharisma* cells was same in all the media. After maintaining the cultures for a week the effect on cell size was found to be as follows:

Size of *Blepharisma* cells with respect to food- Wheat > Rice > Cabbage > Hay

Size of *Blepharisma* cells with respect to media- D > B > A > C > PM > Hay

Cell pigmentation

Initially the colour of the *Blepharisma* cells was light pink in all the media with different food sources, but after a week the intensity of pigmentation was affected as follows:

Pigmentation in *Blepharisma* cells with respect to food- Rice > Wheat > Cabbage > Hay

Pigmentation in *Blepharisma* cells with respect to media- D > PM > A > C > B > Hay

Behaviour

There was no significant difference in the behaviour of the *Blepharisma* cells in different media with different food sources. Initially they were scattered throughout the media but later on, most cells started gathering around respective food sources irrespective of the medium used.

Table -I: Effects of various food sources on *Blepharisma* cultured in Pringsheim's Medium.

		Food Source			
		Wheat	Rice	Cabbage	Hay
Day 1 (after 24 hrs)	Number	65	50	55	60
	Size	180 X 27 μ m	180 X 27 μ m	180 X 27 μ m	180 X 27 μ m
	Colour	Light Pink	Light Pink	Light Pink	Light Pink
	Behaviour	Free Swimming	Free Swimming	Free Swimming, More Around Cabbage Leaf	Free Swimming, Evenly Distributed
Day 3	Number	>120	>180	110	85
	Colour	Pink	Pink	Pink	Light Pink
Day 6	Number	>240	110	90	95
	Size	198X 36 μ m	180 X 36 μ m	180 X 27 μ m	126X 32 μ m
	Colour	Dark Pink.	Dak Pink.	Light Pink.	Light Pink.
	Behaviour	Sluggish, gathered around food.	Sluggish, more around food.	Gathered on Cabbage leaf.	Free swimming.

Table-II: Effects of various food sources on *Blepharisma* cultured in Hay Medium.

		Food Source			
		Wheat	Rice	Cabbage	Hay
Day 1 (after 24 hrs)	Number	65	60	60	55
	Size	180 X 27 μ m	180 X 27 μ m	180 X 27 μ m	180 X 27 μ m
	Colour	Light Pink	Light Pink	Light Pink	Light Pink
	Behaviour	Free Swimming	Free Swimming	Free Swimming, More Around Cabbage Leaf	Free Swimming, Evenly Distributed
Day 3	Number	240	80	>80	110
	Colour	Pink	Pink	Pink	Light Pink
Day 6	Number	280	120	100	>160
	Size	180X 45 μ m	180 X 36 μ m	180 X 27 μ m	126X 29 μ m
	Colour	Dark Pink.	Pink.	Light Pink.	Light Pink.
	Behaviour	Sluggish, gathered around food.	Sluggish, gathered around food.	Free Swimming, more on	Free Swimming, evenly

				Cabbage leaf.	distributed.
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Table-III: Effect of various food sources on *Blepharisma* cultured in Medium A

		Food Source			
		Wheat	Rice	Cabbage	Hay
Day 1 (after 24 hrs)	Number	50	50	45	50
	Size	180 X 27µm	180 X 27µm	180 X 27µm	180 X 27µm
	Color	Light Pink	Light Pink	Light Pink	Light Pink
	Behaviour	Free Swimming	Free Swimming	Free Swimming, More Around Cabbage Leaf	Free Swimming, Evenly Distributed
Day 3	Number	180	75	90	100
	Color	Pink	Pink	Pink	Light Pink
Day 6	Number	>300	230	>180	>180
	Size	216 X36µm	180 X 27µm	180X 27µm	180 X 32µm
	Color	Dark Pink.	Pink.	Pink.	Light Pink.
	Behaviour	Sluggish, gathered around food.	Sluggish, gathered around food.	Free Swimming, More on Cabbage Leaf	Free Swimming

Table-IV: Effect of various food sources on *Blepharisma* cultured in Medium B

		Food Source			
		Wheat	Rice	Cabbage	Hay
Day 1 (after 24 hrs)	Number	60	50	70	60
	Size	180 X 27µm	180 X 27µm	180 X 27µm	180 X 27µm
	Color	Light Pink	Light Pink	Light Pink	Light Pink
	Behaviour	Free Swimming	Free Swimming	Free Swimming, More Around Cabbage Leaf	Free Swimming, Evenly Distributed
Day 3	Number	126	98	130	120
	Color	Pink	Pink	Pink	Light Pink
Day 6	Number	260	200	180	170
	Size	196 X 45µm	216 X 54µm	200 X 27µm	180 X 28µm
	Color	Dark Pink.	Pink.	Light Pink.	Light Pink.
	Behaviour	Sluggish, gathered around food.	Sluggish, gathered around food.	Free Swimming, More on Cabbage Leaf	Free Swimming

Table-V: Effects of various food sources on *Blepharisma* cultured in Medium C.

		Food Source			
		Wheat	Rice	Cabbage	Hay
Day 1 (after 24 hrs)	Number	60	55	60	50
	Size	180 X 27µm	180 X 27µm	180 X 27µm	180 X 27µm
	Color	Light Pink	Light Pink	Light Pink	Light Pink
	Behaviour	Free Swimming	Free Swimming	Free Swimming, More Around Cabbage Leaf	Free Swimming, Evenly Distributed
Day 3	Number	200	110	120	100
	Color	Pink	Pink	Pink	Light Pink
Day 6	Number	>350	>240	>180	>170
	Size	198 X 54µm	180 X 36µm	180 X 27µm	185X 29µm
	Color	Dark Pink.	Dark Pink.	Light Pink.	Light Pink.
	Behaviour	Sluggish, gathered around food.	Sluggish, more around rice grain.	Free Swimming, More on Cabbage Leaf	Free Swimming, evenly distributed.

Table-VI: Effects of various food sources on *Blepharisma* cultured in Medium D

		Food Source			
		Wheat	Rice	Cabbage	Hay
Day 1 (after 24 hrs)	Number	80	70	50	60
	Size	180 X 27µm	180 X 27µm	180 X 27µm	180 X 27µm
	Color	Light Pink	Light Pink	Light Pink	Light Pink
	Behaviour	Free Swimming	Free Swimming	Free Swimming, More Around Cabbage Leaf	Free Swimming, Evenly Distributed
Day 3	Number	320	150	100	100
	Color	Pink	Pink	Pink	Light Pink
Day 6	Number	>430	>300	>200	>180
	Size	216 X 45µm	180 X 36µm	180 X 27µm	185X 32µm
	Color	Dark Pink.	Dark Pink.	Light Pink.	Light Pink.
	Behaviour	Sluggish, gathered around food.	Sluggish, more around food.	Free Swimming.	Free Swimming, evenly distributed.

DISCUSSION

Cell count

Initially all the media with different food source were provided with equal number of cells. After 24 hours the cell count increased in all the media. At the end of 6th day, number increased significantly with maximum number of cells in Medium D with wheat as food source.

Cell size

Initially the size of the *Blepharisma* cell was about 180×27µm in the inoculums. But when the cells were observed on day 6, the cells in Medium D with wheat as food source were largest and cells in Hay Medium were shortest. Therefore, for maintaining a culture with good growth and for cytological studies where large number of cells are required, Medium D with wheat as food source can be used for better results.

Pigmentation

The highly pigmented cells were observed with Rice as food source. Light sensitive property of *Blepharisma* cells also depends on the extent of pigmentation and there are many reports related to the photoreceptor mechanism in various species of *Blepharisma* and the pigment extraction and characterization from various species of *Blepharisma* (4), (7), (8), (16), (18). For extraction of pigment and photoreceptor mechanisms, Medium D with Rice as food source can be used to get better results.

Behaviour

In the beginning of the experiment, cells in all the media with different food sources were free swimming, but the behaviour of cells changed gradually gathering more around the food source may be because the cell number increased significantly and so the bacteria available in the free medium must have reduced forcing the cells to gather around or on the food source.

CONCLUSION

From the experiments performed, it is concluded that maximum growth of *Blepharisma* cells was in Medium D and the maximum density of cells with Wheat as the food source.

For the pigment analysis and photoreceptor property, Rice as the food source is favorable.

For cytological studies where a large quantity of cells is required, Wheat as the food source could be used along with Medium D.

The chemical analysis of the water used from various water purifiers needs to be done further in order to evaluate the differences in various media used.

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