International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 10; October 2017; Page No. 6817-6819
DOI: http://dx.doi.org/10.24327/ijcar.2017.6819.1022

Research Article

IN-VITRO STUDY ON GROWTH KINETICS OF *HAEMOPHILUS INFLUENZAE*

Navinraj Theneri Irudhayaraj* and Ananda Arone Premkumar

Pasteur Institute of India, Coonoor, Tamil Nadu

A R T I C L E I N F O

Article History:

Received 15th July, 2017
Received in revised form 19th
August, 2017 Accepted 25th September, 2017
Published online 28th October, 2017

Key words:

pH, rpm, OD, *Haemophilus influenzae*

A B S T R A C T

The study aims at optimization of the ideal conditions of pH and rpm with different time intervals for better growth of Haemophilus influenzae. Broth cultures were grown for a period of 24 hours with different pH and rpm. The OD was recorded every six hours to study the growth pattern and growth conditions.The peak OD was recorded with culture at pH of 7.2 and rpm of 200. There have been various reports of invasive infections caused by non type b serotypes and the advent of non-typeable strains and failure of hib vaccinations in such conditions. In this context culturing and rapid diagnosis of strains become vital. Though the nutritional requirements of Haemophilus species are similar there has been no data on the growth patterns. The maximum OD recorded was 0.78 at 24 hours after inoculation.

INTRODUCTION

Haemophilus influenzae is a Gram-negative, coccobacilli. The bacterium belongs to the Pasteurellaceae family and exists in two forms, capsular and non-capsular. Capsular (typeable) forms have a polysaccharide covering and are responsible for the virulence, infection and stimulation of immunity. Six distinct capsular serotypes have been described; they are designated types 'a' through 'f'. Out of the six serotypes the type b accounts for the maximum infections in children which can be prevented by use of vaccine (WHO, 2006), (ACIP 2009) (James P Watt *et al* , 2009). It is transmitted through the respiratory tract from infected to susceptible individuals (Ramachandran *et al*, 2013). Hib also causes potentially severe inflammatory infections of the blood, epiglottis, joints, heart, bones, peritoneum, and trachea. Although this problem occurs worldwide the burden of Hib disease was considerably higher in resource-poor countries (Heikki Peltola, 2000).

Haemophilus influenzae requires strict culturing conditions for its growth. In the under developed countries failure to grow *Haemophilus influenzae* has resulted in poor reporting of disease burden and vaccine coverage (Heikki Peltola, 2000). To manufacture vaccines, it is necessary to produce large quantities of bacteria in large volumes of culture medium from which PRP is extracted and purified (Ghislaine, 2008). Since PRP is a growth related product, low culture density limited the PRP concentration (Hamidi *et al*, 2009).

Corresponding author:* **Navinraj Theneri Irudhayaraj
Pasteur Institute of India, Coonoor, Tamil Nadu

The current study is aimed at analyzing the optimal culturing conditions of pH and rpm by recording the optical density at different time intervals.

MATERIALS AND METHODS

Strain: The bacterial strain used in the study was procured from MTCC-Chandigarh with the designation of MTCC-3826 *Haemophilus influenzae* strain.

Culturing conditions: Culturing was done with Brain Heart Infusion Agar and Broth-Himedia Laboratories supplemented with Hemin and NAD (Himedia Laboratories). 250ml Erlenmeyer flasks were used with Cole-Palmer Shaker **(Fig.1)** kept in a Thermo Fisher CO_2 Incubator. Chocolate Agar plates were prepared with BHI Agar and fresh sterile 5% defibrinated sheep blood. The plates were streaked by scraping cells from master seed lot vial and grown overnight at 37^0C with 5% CO_2.

Strain characterization: Microbiological characterization was done by Grams staining Biochemical characterization by Catalse and oxidase were done with the freshly grown culture.

Broth culture optimization: From the overnight colonies a single colony was taken and inoculated into two 50ml BHI broth supplemented with Hemin and NAD. This was again grown overnight OD recorded and was used as the inoculum for seeding the flasks during the study.

pH: Each set of cultures comprised of four flasks containing 100ml of BHI broth supplemented with Hemin and NAD at various pH 7.0, 7.2, 7.4 and 7.6. The media were autoclaved after pH was set. The pH meter was calibrated before each measurement .The inoculum concentration was kept standard

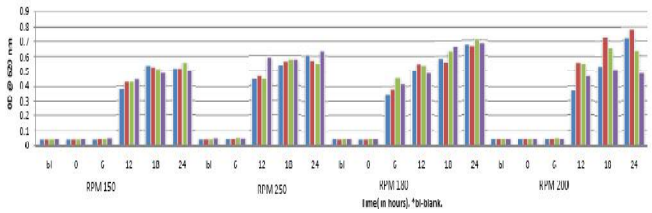
to 100µl. The flasks were set on the shaker and the particular rpm was set.

RPM: A total of four rpm's such as 150, 180, 200 and 250 were studied.

Time intervals: The OD readings were recorded with blank, 6, 12, ,18 and 24 at 620nm with the help of a 96 well plate in a Thermo-scan Ex ELISA reader, the volume being 300µl per well in two wells per sample per reading. The readings were tabulated and growth patterns at altered conditions were studied.

RESULTS

The results obtained gave a detailed report on various parameters. The highest OD recorded was found to be with RPM 200 at pH 7.2 after 24 hours whereas the lowest OD was with RPM 150 at pH 7.4 after 24 hours. The study also throws light on the initiation log phase of cells under these varied culturing parameters. In this context the earliest log phase achieved among the various groups was at 6 hours, RPM 180 and pH 7.4. The various OD recorded at different time intervals, at different pH and varied RPM's are below.



DISCUSSION

There are many investigations made by several research groups regarding the growth of *Haemophilus* species. Though it may vary from serotype a to the recent non-typeable species the nutritional requirements have always been similar. The fact about the supplements required for the growth of these organisms has been well studied, analyzed and established. Since the type b is significant among the prevalent serotypes for causing infections, a lot of work has been done regarding the growth and the consecutive yield of polysaccharide which is the vaccine candidate. In all of these studies the ratio of growth and the yield of the polysaccharide was of primary importance. Wheras the current study claims to analyze the growth and the various factors associated with it such as pH, rpm in respect to various time intervals. These results give us detailed knowledge about how varied parameters can effectively alter growth, initiation of log phase, pH for rapid growth and optimal rpm. The results obtained can be used for other serotypes or non-typeable strains for growth in vaccine production which may in future have outbreaks. Moreover incorporating these methods, problems like low growth and delayed log phase can be prevented. It would be extremely useful when standardizing culture media and deciding upon growth factors and additional supplements for *Haemophilus influenzae* growth. There are many claims of encountering failure in the culturing of *Haemophilus influenzae* which mostly attributes to the quality of media, factors and also failure to adhere to strict culturing parameters.

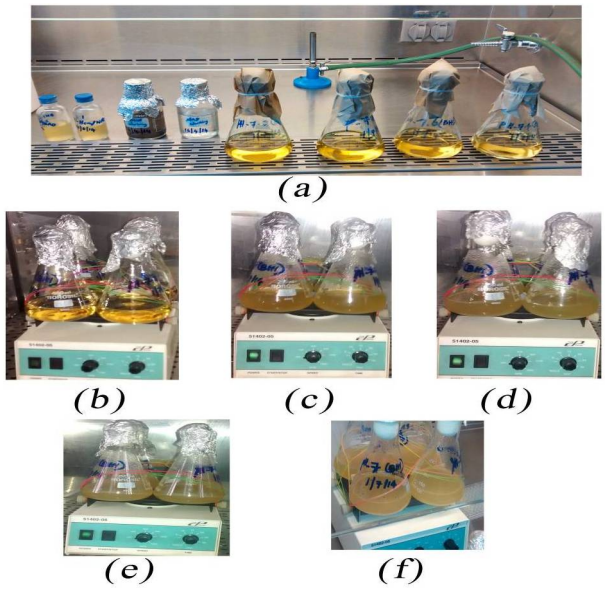


Fig 1 (a)-Sterile broth culture, inoculum and media supplements Hemin and NAD (b) 0 hour culture (c) 6 hour culture (d)12 hour culture (e)18 hour culture (f) 24 hour culture.

REFERENCES

1. WHO position paper on *Haemophilus influenzae* type b conjugate vaccines No. 47, 2006, 81, 445-452.
2. Advisory Committee on Immunization Practices (ACIP), the American Academy Combination Vaccines for Childhood Immunization: Recommendations of the of American Academy Pediatrics (AAP), and the American Academy of Family Physicians (AAFP)-Committee on Infectious Diseases, 1998-1999 Pediatrics 1999;103;1064.
3. Burden of disease caused by Haemophilus influenzae type b in Children younger than 5 years: global estimates. James P Watt, Lara J Wolfson, Katherine O'Brien, Emily Henkle, Maria Deloria-Knoll, Natalie mccall, Ellen Lee, Orin S Levine, Rana Hajjeh,Kim Mulholland, Thomas Cherian, *Lancet* 2009; 374: 903-11.
4. Prospective multi-centre sentinel surveillance for Haemophilusinfluenzae type b & other bacterial meningitis in Indian children- Padmanabhan Ramachandran, Sean Patrick Fitzwater, Satinder Aneja, Valsan Philip Verghese, Vishwajeet Kumar, Krishnamoorthy Nedunchelian, Nitya Wadhwa, Balaji Veeraraghavan, Rashmi Kumar, Mohamed Meeran, Arti Kapil, Sudha Jasmine, Aarti Kumar, Saradha Suresh, Shinjini Bhatnagar, Kurien Thomas, Shally Awasthi, Mathuram Santosham & Aruna Chandran- *Indian J Med Res* 137, April 2013, pp 712-720.
5. Worldwide *Haemophilus influenzae* Type b Disease at the Beginning Of the 21st Century: Global Analysis of the Disease Burden 25 Years after the Use of the Polysaccharide Vaccine and a Decade after the Advent of Conjugates Heikki Peltola Clinical Microbiology Reviews, Apr. 2000, P. 302-317 Vol. 13, No. 2
6. Methods in Molecular Medicine, vol. 71: *Haemophilus influenzae* Protocols Chapter 3 Poje and Redfield
7. Preparation, Characterization, and Immunogenicity of *Haemophilus influenzae* type b polysaccharide protein conjugates- Rachel Schneerson, Osmar Barrera, Ann

Sutton, John B. *Robbins The Journal Of Experimental Medicine* -Vol. 152, 1980- 361.

8. Laboratory Methods for the Diagnosis of Meningitis Caused by Neisseria meningitidis, Streptococcus pneumoniae, And *Haemophilus influenza* Centers for Disease Control And Prevention-August, 1998.
9. Maitre-Wilmotte Ghislaine, Denis Speck, Bachra Rokbi. 2008. Culture medium for haemophilus influenzae type b. US patents Registration No-US20090017074.
10. Chemically Defined Media for Growth of *Haemophilus influenzae* Strains Hannah N. Coleman,Dayle A. Daines, Justin Jarisch, and Arnold L. *Smith Journal Of Clinical Microbiology*, Sept. 2003, p. 4408-4410 Vol. 41, No. 9.
11. Improvement of simple cultivation conditions for polysaccharide synthesis by *Haemophilus influenzae* type b Mickie Takagi1, Teresa Cristina Zangirolami2, Martha Massako Tanizaki1 and Joaquin Communicating Current Research and Educational Topics and Trends in Applied Microbiology, 2007.
12. The growth-factor requirements of *Haemophilus influenzae*.- Holt, L.B. *J. gen. Microbiol.* 27, 317-322 (1961).
13. Klein, R.D. and G.H. Luginbuhl (1979) Simplified media for the growth of *Haemophilus influenzae* from clinical and normal flora sources. *J. Gen.Microbiol.* 113(2), 409-411.
14. Hamidi Ahd, Michel Francois Beurret. 2009. Process for producing a capsular polysaccharide for use in conjugate vaccines. De staat der nederlanden, vert. door de minister v. us patent Registration No-7582459 B2.

How to cite this article:

Navinraj Theneri Irudhayaraj and Ananda Arone Premkumar (2017) 'In-Vitro Study on Growth Kinetics of Haemophilus Influenzae', *International Journal of Current Advanced Research*, 06(10), pp. 6817-6819.
DOI: http://dx.doi.org/10.24327/ijcar.2017.6819.1022

****