Recycling of Culture Media by Re-melt & Re-plating Method

Shaikh Zubair Ahmed, Mohseen Khan

Abstract— Culture media is widely used in cultivation and identification of microorganisms from sample food, water, soil, pharmaceutical product etc .and discarded after the use. In our present paper we proposed the technique to reutilize the media. As per our objective average weight of *E.coli* is 2 Pico gram and average plate of culture media contains approximately 1 gram of nutrition. All nutrition is not utilized by the bacteria within period of incubation of 24hrs to 48 hrs used in the pathogens except *Mycobacterium tuberculosis* which require 2 days to grow. As Global market of culture media is 16.85 billion dollar. And as Bactria does not utilize all the nutrition so it can be recycled by simple remelt and re-autoclaving method. Growing on same media after complete incubation of first culture. It maybe economically feasible and eco-friendly as less of it will be discharged and most of it will be recycled and used in the lab.

Index Terms— Agar-agar, culture media, recycling, re-melting, re-plating, re-utilize

1 INTRODUCTION

In 1860, Pasteur was the first to use a culture medium for growing bacteria in the laboratory. This medium consisted of yeast ash, sugar and ammonium salts. In 1881, W. Hesse used his wife's agar (considered an exotic food) as a solidifying agent for bacterial growth. During the period 1857-1878, both Louis Pasteur and Joseph Lister published significant papers on their extensive studies on fermentation. By 1887, a simple device called the Petri dish revolutionized microbiology.[1-2-3] with the invention of the Petri dish, the focus turned to culture media formulations. With all the research being performed, scientists began to replace gelatin with agar because it was resistant to microbial digestion and liquefaction. Agar was discovered in 1658 by Minora Tarazaemon in Japan. In 1882[4], Koch was the first to use agar in microbiology. Agar is a phycocolloid, water-soluble polysaccharide, extracted from a group of red-purple marine algae (Class Rhodophyceae) including Gelidium and Gracilaria.[4] Production of agar in the United States was started just before the beginning of World War II as a strategic material.[4-5]In the 1940s, bacteriological-grade agar manufactured by the American Agar Company of San Diego, California, served as reference agar for the evaluation of the characteristics of other culture media components, such as peptones. After this major breakthrough industry of culture media got a stand up and microbiology become best emerging field of biology.[6] Cultivation of bacteria becomes easy in laboratory. Pioneers of culture media in the market are BD's microbiology, Difco laboratories, Baltimore's Hynson Wescott and Dunning (HW&D) and Johnston Laboratories, Inc., in 1952, the formulation of the U.S. version of Lowenstein-Jensen Medium was introduced, launching the prepared tubes.[7] In 1960, the line of prepared culture media was completed by introducing commercially-prepared plated media. And readymade ingredients Trypticase™ Peptone, a pancreatic digest of casein, and Phytone™ Peptone, a papaic digests of soybean meal, ingredients which are employed in Trypticase Soy Agar, Trypticase Soy Broth and many other media.[8] Commercial market of culture media is about 16.85 billion US dollar.[9]

Material and Method

Culture media
Nutrient agar form Himedia™ Company Mumbai

Method

Day 1

100 ML of Nutrient agar prepared in flask Autoclaved and plated on sterile Petri plate.
After solidification fresh culture of four different bacteria (E.coli, B.subtilis .B.megateriam) is streaked on the plate using Nicrom wire loop and kept in BOD incubator four 24hrs incubation.

Day 2

After incubation of plates colony characters has been noted and colonies removed from the plate by using glass slide. Solid agar cut into pieces by cutter and jelly of agar is redropped in conical flask.
Flask is replaced in autoclave and autoclaved on 121^{0}C for 30mins. After autoclave replate on new Petri plates. After solidification fresh culture same previous bacteria streaked on the plate and kept in the incubator
Same procedure was followed till 5days.

Shaikh Zubair Ahmed M.sc microbiology Maulana Azad Post-Graduate & Research Cetre Dr.RafiqZakariaCampusRauzaBaghAuranbad.Maharashtra, India Email:shaikhzubairahmed@outlook.com
Mohseen khan M.Sc microbiology Dr. Rafiq Zakaria centre for higher learning and research Dr.RafiqZakariaCampusRauzaBaghAuranbad.Maharashtra

Technique

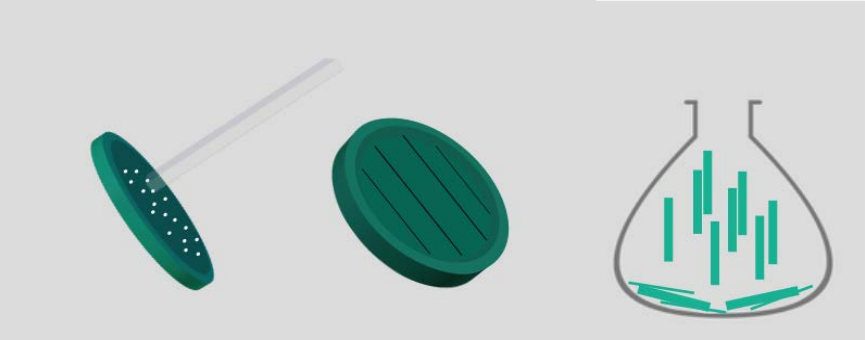

Fig: colonies removed By glass slide Agar cut into pieces pieces droped into flask

Result

Plates of second day showing good growth as compared to first day. Approach towards the recycling of culture media is giving good result. It shows that media can be recycled and agar is the main component which is helping in solidification of media. Agar was easily recycled in our experiment and no changes was observed till second day but after three days colour of media was getting darker because of continuous heat treatment to media due to charring of media components and also interaction of media components. Chemical changes are less now direct inhibition of growth. As per our objective all nutrition of media is not utilized by the bacteria is first day. Nutrition is present in the plates till day five but it got decreased after recycling.

Discussion

Without addition of any nutrition plates of second and third day showing good growth. Some inhibitory metabolic product need to purify to grow bacteria in natural condition. Size of colonies is different in the all recycled plates due to inhibitory or essential trace element which is consumed in the first growth. Analysis of purity is major in way to recycle the media as fresh. Question arising about the purity or porosity of agar which gets decreased after the remelting.

Figure 2: Fresh Media plate S.aureus

Figure 3: Fresh Media Plate E.coli

Figure-4: Second day media Plate E.coli

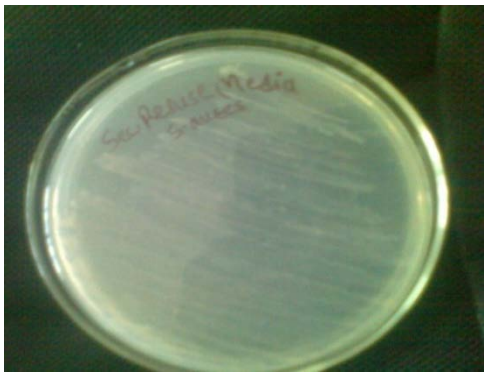

Figure-5:SeconddaymediaPlateS.aureus

1. Conclusion

There is significant result of recycling of media, metabolic products of microorganism are not hindering the growth cycle. Result of day2 shows that Nutrition of culture media is not completely utilized in first cycle. It remains present after 24 hours of incubation. Essential trace elements need to be analyzed , trace elements has major role in the growth of microorganism & agar which is major and costly component of culture media is easily recyclable, plate of second day shows no changes in the color and all properties of agar is intact as fresh till day three. Chemical analysis of Reused media the topic of further research has great potential. If media is recycled it can save money, labour, time and environmental hazard which is arising from genetic changes in the microbes. Control of super pathogen bugs, hospital infections etc.

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