

MICROBIAL APPROACH OF RAT COLONIC MICROFLORA - A DANGEROUS STRINGENT ANAEROBE FOR TARGETING TO COLON

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ABSTRACT

The present study was aimed to isolate and characterize the colonic content. So made an attempt to isolate the ceecal content from the healthy rat and grown in the culture medium. The microbial approach and characterization of the colonic microflora present in the healthy rat ceacum was identified for its features. We reported here the gram type of bacteria, presence of flagella, catalase test, thermal death time, bacterial growth curve, colony forming unit and bio chemical reactions such as methyl red test, citrate utilization test, voges proskauer test, also we reported that the ceecal matter should contain a dangerous strict anaerobe as *bacteroids* in abundant. The characteristic information which is disclosed in this research would be more help full for targeting the drugs to colon.

KEYWORDS: inflammatory bowel disease, *bacteroids*, Ceecal microflora, Chron's disease, microbial characterization, *bafidobacterium*.

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INTRODUCTION

The modified novel drug delivery system has given a method of targeting the drug to colon directly bypassing the GIT track. The colon drug delivery has a number of important implications in the field of pharmacotherapy. Various diseases including inflammatory bowel disease (IBD) can be effectively treated by the local delivery of drug to the large intestine. The treatment of IBD with anti-inflammatory drugs is particularly improved by their local delivery to the bowel, by this technique absorption of the drugs from the stomach and small intestine can be minimized until the drug reaches the large intestine. The treatment of large intestine disorders, such as Chron's disease, irritable bowel syndrome, colitis, colon cancer and local infectious diseases.² this is mainly used to treat ulcerative colitis, colorectal cancer and amoebiasis.

The gastrointestinal bacteria provide a further means of effecting drug release in the colon. These bacteria predominantly colonise the distal regions of the gastrointestinal tract where the bacterial count in the colon is 10^{11} per gram, as compared with 10^4 per gram in the upper small intestine. Moreover, 400 different species are present. Colonic bacteria are predominantly anaerobic in nature and produce enzymes that are capable of metabolizing endogenous and exogenous substrates, such as carbohydrates and proteins that escape digestion in the upper gastrointestinal tract. Therefore, materials that are recalcitrant to the conditions of the stomach and small intestine, yet susceptible to degradation by bacterial enzymes within the colon, can be utilized as carriers for drug delivery to the colon.¹

Microbial degradable polymers especially a 30 cross linked polymers have been investigated for use in targeting drug to colon¹. The name of the natural polysaccharides and degrading bacteria were listed in the (Table 1).

MATERIALS AND METHODS

Materials

General Glass ware required for microbial work, Mechanical shaker –C. M. Equipments & Instruments , pvt. Ltd, Banglore. Colorimeter- Elico pvt. Ltd, Hyderabad. Laminar airflow-Clean air systems, Madras Incubator –Tempo instruments & equipments pvt.Ltd, Bombay
Hot air oven – York scientific industries, Delhi. Autoclave – Guna enterprises, Madras. Colony Counter- C. M. Equipments & Instruments pvt. Ltd, Banglore. Zone reader- C. M. Equipments & Instruments pvt. Ltd, Banglore

Methods

Isolation of ceacum from healthy rat

We selected healthy rat and general anesthetized. Intestinal region was separated from rest of organs and kept in freshly prepared ringer solution. Finally ceacum was isolated from intestine.

Maintenance of pure culture

The isolated ceacum was cut opened and the ceacal matter was inoculated into sterilized nutrient broth medium in a conical flask. Incubated under anaerobic for 24-48 hrs, maintained by periodic sub culturing.

Grams staining

The grams staining procedure was followed from the standard reference books and the reports were given in the results and discussion section¹.

Motility determination by hanging drop technique

The determination of the motility was done using hanging drop method by and the report was given in the results and discussion section².

Bacterial growth curve

Taken two conical flasks with 100 ml of sterilized Nutrient Broth in each flask. One ml of ceacum culture was inoculated in to one conical flask and other kept as control. Placed both the conical flask under mechanical shaker. Withdrawn 2 ml of ceacum culture from the flask at every 4 hrs interval till 48 hrs and measured the absorbance at 610 nm by using colorimeter. Plotted graph between absorbance Vs time in hrs and bacterial growth curve was determined⁵.

Catalase test

Prepared Nutrient agar slant. Inoculated the ceacum culture over the slant. Incubated for 24-48 hrs under anaerobic condition. After 24 hrs, added hydrogen peroxide solution to the ceacum culture and noted down the result⁴.

Thermal death time

Prepared sterilized Nutrient Broth and poured into 11 tubes. Inoculated each tube with ceacum culture and incubated for 24-48 hrs under anaerobic condition.

After 24 hrs, taken two petriplates and poured 20 ml of sterilized Nutrient agar medium in each plate. Left the plates for solidification. Divided each Petriplate into 6 segments. Eleven test tubes containing 24 hrs ceacum culture kept in a water bath (60°C). After an exposure of respective time interval, the tube was taken out and inoculated on the respective segment of plate. Incubated the plates for 24-48 hrs in an incubator under aerobic condition and the observations were tabulated³.

Starch hydrolysis test

Prepared starch agar plates. Inoculated on the plate by streak plate method.

Incubated the plates at 37°C for 24 hrs for sufficient growth. After incubation, flooded the plate with iodine solution. Hydrolysis indicated by clear zones around the growth and unchanged starch gives blue colour. The medium should preferably contain no glucose, as this may diminish starch hydrolysis. The iodine normally used for Gram's stain is suitable⁴.

Methyl red (MR) test

Taken MRVP broth in two test tubes. Inoculated one tube with ceacum culture and another act as control. Incubated the tubes at 37° C for 96 hrs under anaerobic condition. Added 5 drops of methyl red indicator. Observed the colour change⁴

Citrate utilization test

Prepared few slants of simmon citrate agar. Inoculated the slant with ceacum culture. Incubated at 37°C for 48 hrs under anaerobic condition. Examined the tube for result⁴.

Voges proskauer(VP) test

Taken VP broth in two test tubes. Inoculated one tube with ceacum culture and kept another as control. Incubated at 37°C for 48 hrs. Added 10 drops of VP I reagent & drops of VP II reagent to the test tubes. Gently shaken the tube, waited for 15-30 min to complete the reaction. Observed the colour change⁴.

RESULTS AND DISCUSSION

Characterization of colonic micro flora

Grams staining: The presence of pink colored, rod shaped organisms indicates that the isolated organism was found to be Gram negative. The organism was found to possess no flagella, i.e., the given microorganism

was found to be stationary when observed under microscope. Thus it was confirmed that the organism was non-motile. Bacterial growth curve: The graph was plotted between absorbance and time in hrs, which illustrates the different phases of a colonic bacterium. **(Figure 1)**

Catalase test was found to be negative for the ceacum culture. Upon addition of hydrogen peroxide, gas bubbles were not evolved which indicates the absence of Catalase enzyme. Thermal death time: It was found to show susceptibility to heat till 10 minutes, but 12 minute segment does not show any presence of growth, which indicates the growth, was terminated from 12 minute onwards. Starch hydrolysis test: The production capacity of ceacum culture to produce the enzyme to hydrolysis starch was found to be negative. Upon addition of iodine solution produces blue color. Methyl red test: The micro flora present in ceacum culture does not have capacity to produce the acids. Absence of red colour produces yellow color. Citrate utilization test: shows negative, did not utilize glucose. Voges proskauer test shows negative, did not produce pink to rose color.

Upon characterization it was identified as strict facultative anaerobe had more virulence at the initial stage of inoculation finally its virulence was reduced a while which made us to handle easily at the end of the e research work .The isolation and characterization of colonic microflora was extensively studied with microbiological aspects. During the work, it was found to produce some harmful effects to our project volunteers as it is considered as potent strict anaerobe will degrade many of the natural polysaccharides reported by Krishnaih *et al.*, for colon targeted drug delivery system. The isolation and characterization of colonic microflora is the base for CTDDS (colon targeted drug delivery system), would be more helpful for the future works in CTTDS.

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Table 1 - Name of the Polysaccharide and degrading bacteria in the colon

| S.no | Name of the Polysaccharide | Name of the bacterial species that degrade the polysaccharide |
|------|----------------------------|---|
| 1 | Amylose | <i>Bacteriods, Bifidobacterium</i> |
| 2 | Arabinogalactan | <i>Bifidobacterium</i> |
| 3 | Chitosan | <i>Bacteriods</i> |
| 4 | Chondroitin sulphate | <i>Bacteriods</i> |
| 5 | Cyclodextrins | <i>Bacteriods</i> |
| 6 | Dextran | <i>Bacteriods</i> |
| 7 | Pectin | <i>Bacteriods, Bifidobacterium, eubacterium</i> |
| 8 | Xylan | <i>Bacteriods, Bifidobacterium</i> |
| 9 | Guargum | <i>Ruminococcus</i> |

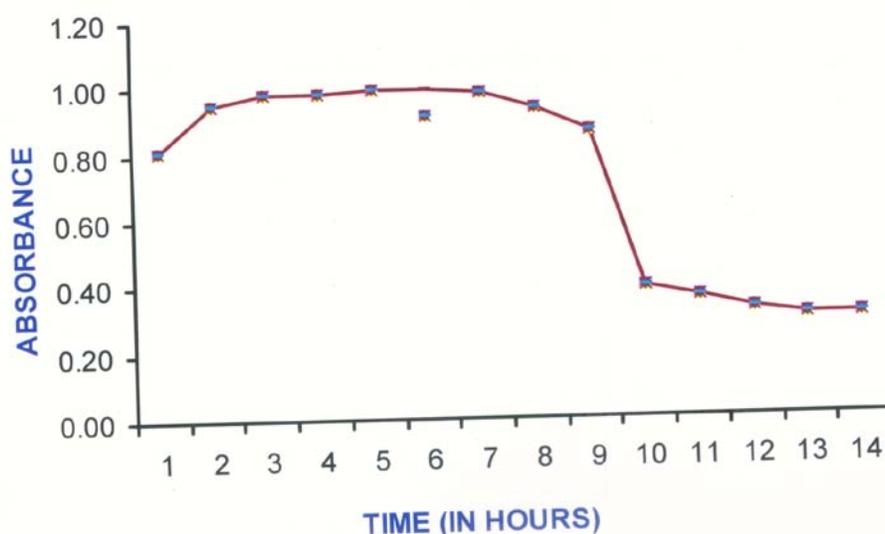


Figure 1 – different phases of growth curve of ceacum culture

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