Bacterial concentration and diversity

Science, Biology

The objective of this particular paper was to study the results that were extracted when bacterial communities were formed. These bacterial communities were formed through the process of the reproducibility of small volume of repeat sampling from replicate bioreactors with stabilized continuous-flow chicken cecal bacterial communities. The results referring to the bacterial concentration and diversity were then analyzed by phenotypic, biochemical and ribotype analysis. To grow bacteria a stableenvironmentis the most essential requirement this stable and a constant environment is known as steady-state conditions.

This allows bacterial cultures to be obtained in a reproducible manner for batch consistency. The cultural efficacy was determined by taking an assumption that the aliquot taken from the cultures were identical and therefore did not overall affect the results to be determined by the particular experiment (bacterialculture). Mixed samples of avian cecal material were used to establish replicate bioreactor cultures. Repetitive samplings of the planktonic components were done to determine if all aliquots had the like bacterial contents within the same bioreactor.

Consistency was seen during this repetitive sampling process but changes were seen in the specific composition of the resulting communities that initiated from one supply of cecal assortment. These are the basis on which the whole experiment and the methodology are based on. METHODOLOGY: • Bioreactor and Sampling Design: The cecal contents were extracted from 150 birds (chicken) and then thoroughly mixed under sterile anaerobic conditions. Three replicate bioreactors (Bioflo® 110 Fermentor/Bioreactor, New Brunswick Scientific Co, Inc.

, Edison, NJ) were used. The steady-state conditions were maintained by keeping the cultures under continuous-flow conditions at a flow rate of 0. 8 ml/min and also flushed with carbon dioxide that was free of any oxygen. For the first 48 hours the pH of the bioreactor was maintained to a stable 6. 2 ± 0. 3. Then for 3 weeks the cultures were allowed to reach equilibrium the planktonic component was sampled 11 times during this 3 weeks period. After this period 1ml aliquots were collected for analysis. i. e. pH measurement , bacterial isolation etc.

• Bacterial Isolation and Preliminary Identification: The material obtained from the bioreactor was sampled and some of the bacterial cultures obtained were quantified by growth of a 10? l aliquot on selective media in triplicate. The triplicate had a 5% sheep blood and was used to determine hemolytic reactions and for the recovery and the enumeration of the aerobic microbial species. The identification and the isolation of the aerobic bacteria was done by streaking the 10 µl aliquots onto TS-blood agar , Brilliant Green Agar, BGA; Becton Dickinson, Sparks, MD), CHROMagar E.

coli and Orientation, MacConkey, mEnterococcus, and Rogosa plates. These plates were then incubated for 24 hours at thirty seven degrees. Likewise anaerobic bacteria were isolated too but the streaking was done onto Brucella-blood agar, Phenylethyl alcohol agar (Becton Dickinson, Sparks, MD), Veillonella, and BBE plates. The plates were then incubated anaerobically for 48-72hours at the same temperature. These bacteria were also tested for aero tolerance. • Enumeration:

The total aerobic and anaerobic population levels were enumerated by serial dilation onto TS-blood agar, MacConkey, mEnterococcus agars or Brucella-

blood agar plates, respectively. • Ribotype Characterization: Isolates from the bacterial lawns were collected and analyzed by using RiboPrinter® Microbial Characterization System following the manufacturer's instruction using lytic enzymes. Endonuclease EcoRI was used to cleave the DNA and gel electrophoresis was used to separate the fragments and analysis was done using a modern hybridization blotting technique.

The DNA hybridized was labeled rRNA operon prob derived from Escherichia coli, and the bands were detected by chemiluminescence. The image formed was captured and transferred to the RMCS database and data were normalized to a standard marker set. The images were compared with the 6448 EcoRI riboprint patterns in the DuPont database and a 900 EcoRI riboprint pattern custom in-house database (USDA, ARS, College Station, TX). • Data Analysis: For each set of combined cecal material the above mentioned 3 replicate bioreactor were established.

These were then analyzed for enumeration and characterization (eleven per bioreactor). The statistics were represented in tabular form. Commercially available software was used to analyze and calculate data. Differences in cfu/ml were compared among the replicate bioreactors. MAIN RESULT: The collecting of bacteria cultures or any other organism is greatly affected by the sample size and the frequency of organisms being sampled in a particular environment. Some of the processes or the methods described above also have limitations due to different constraints that govern their working e.

g. enumeration. Enumeration of bacteria is affected by many factors including individual species growth rates, fitness of each competing species

etc. therefore the bacteria produced or grown in a selective media maybe less productive when exposed to competition from many other species in a non-selective media culture. The probability of collection is greatly affected by the spatial distribution of organisms. As a rule the sampling requirement must increase as the degree of unit aggregation increases.

Enumeration is also affected by aggregation and may account for some of the variation reported in the bacterial quantification. Therefore considerations should be given to the sampling size when using aliquots for inoculation from cultures with known aggregating species. An important thing to mention here that whatever the sampling technique is used there are also certain limitations associated with sampling. It is not only quite difficult to harvest all species comprehensively but our present technological inability also creates a hindrance because we are unable to artificially culture all bacterial species.

It was also seen that the efficiency of detection of pathogenic bacteria is affected by dilution i. e. a lower efficiency of detection was achieved where the prevalence of the target bacteria was diluted. A threshold quantity of specific bacteria maybe required for the proper mix. Adjustments in population density, adhesion and diversity which occur during culturing period greatly affects thisobservation. CONCLUSION: The aim of this study or experiment was to determine reproducibility of small volume repeat sampling with the help of a bioreactor.

Basically bacterial concentration and diversity were the two important factors that were being concentrated these two quantities were analyzed within stabalised continuous-flow chicken cecal bacterial communities

initiated by replicate aliquots taken from thoroughly mixed samples. Pooled cecal material was created from layer chicks to establish the bioreactors. After a steady-state was reached the plankton components were sampled repetitively for three weeks and was then characterized by phenotypic, biochemical and ribotype analysis.

No notable differences were found in the bacterial concentrations that came from the same bioreactor. Differences were found in bioreactors initiated from the same stock material. BIBLIOGRAPHY 1. Tawni L Crippen, Cynthia L Sheffield, Kathleen Andrews, Roy Bongaerts, and David J Nisbet, (2008), Bacterial Concentration and Diversity within Repetitive Aliquots Collected from Replicate Continuous-Flow Bioreactor Culture, Open Microbiol J. 2008; 2: 60–65, published online 2008 May 23.