Institut Pasteur

Research in Microbiology 165 (2014) 803–812

www.elsevier.com/locate/resmic

Original article

Characterization of virus-like particles associated with the human faecal and caecal microbiota

Lesley Hoyles [a], Anne L. McCartney [b], Horst Neve [c], Glenn R. Gibson [b], Jeremy D. Sanderson [d], Knut J. Heller [c], Douwe van Sinderen [a,e,*]

[a] School of Microbiology, University College Cork, Western Road, Cork, Ireland
[b] Food Microbial Sciences Unit, Department of Food and Nutritional Sciences, The University of Reading, Whiteknights, Reading RG6 6UR, UK
[c] Institute of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany
[d] Department of Gastroenterology, Guy's and St Thomas' NHS Foundation Trust and King's College London, London, UK
[e] Alimentary Pharmabiotic Centre, Biosciences Institute, National University of Ireland, Western Road, Cork, Ireland

Received 20 July 2014; accepted 12 October 2014
Available online 22 October 2014

Abstract

This work represents an investigation into the presence, abundance and diversity of virus-like particles (VLPs) associated with human faecal and caecal samples. Various methodologies for the recovery of VLPs from faeces were tested and optimized, including successful down-stream processing of such samples for the purpose of an in-depth electron microscopic analysis, pulsed-field gel electrophoresis and efficient DNA recovery. The applicability of the developed VLP characterization method beyond the use of faecal samples was then verified using samples obtained from human caecal fluid.
© 2014 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Bacteriophages; Microbial ecology; Gastrointestinal tract; Transmission electron microscopy; Caecum; Faeces

1. Introduction

Viruses are the most numerous biological entities within the biosphere of our planet (being present at an estimated number of ~10^{31}), with bacteriophages representing the most abundant group of environmental viruses [1,2]. Bacteriophages are ubiquitous viruses that infect bacterial cells and disrupt their metabolism. Multiple bacteriophage types can infect a specific microbial isolate, with most bacteriophages infecting only certain species or even strains of bacteria [3].

Although they are abundant and potentially important to microbial populations indigenous to different ecological niches within the human gastrointestinal tract and to host health, little attention has been paid to bacterial virus-like particle (VLP) assemblages and their interactions with the gastrointestinal microbiota and/or human host until recently. Various publications have highlighted the potential importance of bacteriophages in inflammation states, including Crohn's disease [4–6], and as therapeutic agents [7,8]. Breitbart et al. [9] conducted the first metagenomic study on dsDNA-containing VLPs associated with the human faecal virome using a fresh sample from a healthy adult male. On the basis of previous estimates of gut microbial diversity, Breitbart et al. [9] predicted that there are two to five times more viral genotypes (~1200 viral genotypes) present in the human gastrointestinal microbiota than the number of bacterial species, with the vast majority of these VLPs representing

* Corresponding author. School of Microbiology, University College Cork, Western Road, Cork, Ireland.
E-mail addresses: lesley.hoyles@reading.ac.uk (L. Hoyles), a.l.mccartney@reading.ac.uk (A.L. McCartney), horst.neve@mri.bund.de (H. Neve), g.r.gibson@reading.ac.uk (G.R. Gibson), jeremy.sanderson@kcl.ac.uk (J.D. Sanderson), knut.heller@t-online.de (K.J. Heller), d.vansinderen@ucc.ie (D. van Sinderen).

http://dx.doi.org/10.1016/j.resmic.2014.10.006
0923-2508/© 2014 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

bacteriophages and prophages. RNA viruses present in human faeces have been found to be mostly associated with plant viruses, such as Pepper Mild Mottle Virus, with RNA bacteriophages making a minimal contribution to the diversity of the intestinal virome [10]. Using frozen samples from four adult female monozygotic twins and their mothers, Reyes et al. [11] demonstrated that each individual harbours a unique virome (ssDNA and dsDNA) regardless of their genetic relatedness to another individual, and that intrapersonal virome diversity is very low, with >95% of virotypes retained over a one-year period (between 71 and 2773 viral genotypes identified among the samples). More recently, Minot et al. [12] confirmed inter-individual variation in the virome and, similar to modulation of the faecal bacteriome by foodstuffs, demonstrated that diet influences host faecal virome structure. Minot et al. [13] also confirmed the relative stability of an individual's virome, monitoring 24 faecal samples from one individual over a 2.5-year period.

The murine virome has been used to demonstrate enrichment of bacteriophage-encoded, antibiotic-resistance genes (related and unrelated to the drug used) after antibiotic therapy, with the adaptive capacity of the virome (specifically its bacteriophage component) suggested to protect gut bacteria, thereby preserving the microbiota's robustness during antibiotic stress [14].

It is clear from the aforementioned studies that the virome–bacteriome community in the gut is governed by complex and dynamic interactions in health, and that its balance may be disturbed when under stress, e.g. during antibiotic intervention [14]. Although metagenomic studies have greatly improved our understanding of the virome associated with the human and murine gut microbiomes, it has been notable how little bacteriophage material, and consequently DNA, has been isolated from samples when this information was included in a publication. Reyes et al. [11] reported the isolation of ~500 ng DNA from 2 to 5 g of frozen faeces, whereas Thurber et al. [15] stated that between 500 and 3000 ng of DNA could be isolated from 500 g of human faeces, though the publication they cite [9] provides no information regarding the amount of DNA isolated from the 500 g faecal sample examined in the original study. In addition, to the best of our knowledge, no attempts have been made to enumerate bacteriophages in faecal filtrates prior to CsCl purification. The aims of this study were to develop reliable and effective methods for the recovery and characterization of VLPs in human samples, and to apply these methods to human faecal and caecal samples to demonstrate the methods' efficiency.

2. Materials and methods

2.1. Processing of and isolation of VLPs from faecal and caecal samples

Faecal samples were obtained from six healthy adult (2 male, 4 female) donors of between 23 and 52 years of age, all of whom were members of the Department of Food and Nutritional Sciences, University of Reading. None of the donors had taken antibiotics, prebiotics and/or probiotics in the 3 months prior to sampling, and none had any history of gastrointestinal disorder. All donors gave informed oral consent for their faecal samples to be used for microbiological analyses. Ethical approval for the collection of caecal effluent from patients was obtained from St Thomas' Hospital Research Ethics Committee (06/Q0702/74) covering Guy's and St Thomas' Hospitals and transferred by agreement to London Bridge Hospital. Where available, clinical information for the colonoscopy patients is given in the text. Samples were collected at Reading (faeces) or transported from St Thomas' Hospital (caecum), and maintained under anaerobic conditions (faeces, MACS1000 anaerobic workstation, Don Whitley Scientific, gas composition 80% N_2, 10% H_2, 10% CO_2; caecum, on ice in a gas jar with an anaerobic gas-generating pack; Oxoid Ltd) for a maximum of 2 h before processing. Caecal samples were collected during routine colonoscopy following preparation of the bowel with sodium picosulphate and a reduced fibre diet for 3 days. Liquid residue in the caecum was aspirated via the colonoscope suction channel into a standard 30 ml trap specimen container and immediately transferred to a gas jar.

Faecal homogenates (20%, w/v, from 25 g of faecal material) were prepared in sterile TBT [0.1 μm filtered prior to autoclaving; 100 mM Tris/HCl, pH 8.0; 100 mM NaCl; 10 mM $MgCl_2.6H_2O$] or sterile 0.5% 'Lab-Lemco'/6% NaCl (LL [16]). Caecal homogenates (20%, v/v, from 10 to 30 ml of caecal effluent) were prepared in LL. Faecal and caecal samples were placed into a filter stomacher bag and homogenized in a Stomacher 400 Lab System (Seward) for 120 s at low speed. The bag was removed from the stomacher and massaged manually to further disperse any large particles remaining in the sample; the sample was then stomached for a further 120 s. The homogenate was kept on ice for 2 h to allow desorption of VLPs from solid material, then centrifuged at 11,180 *g* for 30 min at 10 °C. The supernatant was transferred to a fresh tube and centrifuged again at 11,180 *g* for 30 min at 10 °C. Supernatant [herein referred to as faecal filtrate (FF) for both faecal and caecal samples] was passed through sterile 0.45 μm cellulose acetate filters (Millipore) and the FF was then collected in a sterile container. An aliquot (10 μl) of each of the FFs was examined by epifluorescence microscopy (EFM) after viral particles had been stained with SYBR Gold (see below) to confirm that the samples were free of bacteria.

FF prepared in LL was used for enumeration of VLPs in faeces via EFM (see below) and 1 ml aliquots of FF prepared in TBT were for examination by transmission electron microscopy (TEM; see below).

Aliquots of FF and LL (two sets of three aliquots of 100 μl each: one for aerobic cultivation, one for anaerobic cultivation) were spread onto Columbia blood agar containing 5% laked horse blood (Oxoid), and incubated aerobically and anaerobically. Sterility of the filtrates was confirmed by the absence of microbial growth on plates following incubation for 2 (aerobic) and 5 (anaerobic) days. Sterile brain–heart infusion broth was inoculated with 100 μl of FF and LL, and incubated aerobically for 2 days: aliquots (100 μl) were spread

Download English Version:

https://daneshyari.com/en/article/4358567

Download Persian Version:

https://daneshyari.com/article/4358567

Daneshyari.com