

Novel *Bacteroides* host strains for detection of human- and animal-specific bacteriophages in water

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ABSTRACT

Bacteriophages active against specific *Bacteroides* host strains were shown to be suitable for detection of human faecal pollution. However, the practical application of this finding is limited because some specific host strains were restricted to certain geographic regions. In this study, novel *Bacteroides* host strains were isolated that discriminate human and animal faecal pollution in Switzerland. Two strains specific for bacteriophages present in human faecal contamination and three strains specific for bacteriophages indicating animal faecal contamination were evaluated. Bacteriophages infecting human strains were exclusively found in human wastewater, whereas animal strains detected bacteriophages only in animal waste. The newly isolated host strains could be used to determine the source of surface and spring water faecal contamination in field situations. Applying the newly isolated host *Bacteroides thetaiotaomicron* ARABA 84 for detection of bacteriophages allowed the detection of human faecal contamination in spring water.

Key words | bacteria, contamination, indicator, pollution, source tracking

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INTRODUCTION

Outbreaks of waterborne diseases as a result of faecal contamination of water resources are caused by various bacteria, viruses and protozoan pathogens. Pathogens which affect human health are more frequently transmitted through faecal pollution of human rather than of animal origin. Human pathogens reaching drinking water resources pose a hazard to public health and may cause economic losses due to temporary closure and decontamination of the water supply. In order to differentiate between human and animal contamination of drinking water and to trace the origin of faecal pollution, effective and easy to handle methods should therefore be established.

Bacteriophages infecting specific *Bacteroides* host strains were shown to be applicable in microbial source tracking (Sinton *et al.* 1998; Scott *et al.* 2002). Several host strains have

been isolated and evaluated for their specificity. *Bacteroides fragilis* HSP40 was described as a host strain for detection of human-specific bacteriophages (Tartera & Jofre 1987; Tartera *et al.* 1989). Owing to the low sensitivity of *B. fragilis* HSP40, additional human host strains, *Bacteroides ovatus* GB-124 and *B. thetaiotaomicron* GA-17, were isolated (Payan *et al.* 2005). However, differences in geographic distribution of these strains were observed. While *B. fragilis* HSP40 and *B. thetaiotaomicron* GA-17 performed well in certain areas, bacteriophages were absent in others or formed turbid plaques which were difficult to interpret (Puig *et al.* 1999; Duran *et al.* 2002; Payan *et al.* 2005). Therefore, the aim of the present study was to identify new host strains suitable to detect either human or animal faecal pollution for the socio-ecological area of Switzerland

doi: 10.2166/wh.2010.165

and to assess the occurrence and concentration of bacteriophages infecting these host strains in wastewater, surface water and springs.

MATERIALS AND METHODS

Isolation of *Bacteroides* host strains

Isolation of novel *Bacteroides* host strains for detection of human- or animal-specific bacteriophages was based on a method for isolation of *Bacteroides* bacteriophage host strains suitable for tracking sources of faecal pollution in water (Payan *et al.* 2005).

In two independent trials, two human wastewater samples were examined for new *Bacteroides* host strains specific for bacteriophages of human origin. Isolation of presumptive *Bacteroides* strains, preparation of phage suspensions and screening of specific strains over three levels (level 1 to level 3) were undertaken according to a previously described method (Payan *et al.* 2005). Gram-negative rods growing under strict anaerobic conditions were classified as level 1 isolates. Level 1 isolates were further classified into level 2 isolates when they proliferated well in BPRM broth ($OD_{620} > 1$) and when they detected bacteriophages in phage suspensions prepared from samples of their origin. Level 2 isolates were further classified as level 3 isolates showing specificity for their origin. For classification of human level 3 isolates, one slaughterhouse wastewater sample was used to prepare the phage suspension.

Animal *Bacteroides* host strains were isolated from wastewater of two slaughterhouses, six samples of pooled cow manure (from one to approximately ten cows) and eight samples of horse faeces. Isolation of animal strains from slaughterhouse wastewater was performed in the same way as for human host strains. Animal faeces were analysed within 2 hours after collection by plating them onto *Bacteroides* bile esculin (BBE) agar (Livingston *et al.* 1978). To prepare phage suspensions, faecal samples were suspended in peptone saline solution in a ratio of 1:10 (wt/vol) and filtered with MillexGP filters (Millipore). The suspensions were frozen with 10% glycerol (85%) at -70°C or else used immediately for analysis. Further screening and classification

over the three levels of the animal *Bacteroides* isolates was done according to Payan *et al.* (2005). For classification of animal level 2 isolates, phage suspensions were prepared from animal samples (slaughterhouse, cow and horse faeces). For classification of animal level 3 isolates the human phage suspension, previously prepared to test human isolates, was used.

Specific isolates of human and animal origin were phenotypically characterized with API rapid ID 32 A (Biomérieux) or by sequencing of the 16sRNA gene (IMD, Zurich, Switzerland) and used for further analysis during the field validation.

During the whole study, *B. fragilis* RYC 2056 (provided by the Department of Microbiology, University of Barcelona, Spain) was used as reference strain. Enumeration of plaque forming units (PFU) was done following the standard protocol for detection and enumeration of bacteriophages (ISO 10705-4, 2001b). According to the standard method, *Bacteroides* host strains which are used for detection of bacteriophages were utilised in the exponential growth phase after inoculation in a ratio of 1:10 (wt/vol) in *Bacteroides* phage recovery medium (BPRM) (ISO 10705-4, 2001b). *Bacteroides fragilis* RYC 2056 was incubated for 3 h and all novel *Bacteroides* host strains for 5 h under anaerobic condition. For this purpose, plates were incubated in an anaerobic jar and anaerobic condition was generated with AnaeroGen (Oxoid).

Field validation of new *Bacteroides* host strains

Human wastewater

Ten wastewater treatment plants were randomly selected from all Swiss wastewater treatment plants, each processing sewage from more than 100,000 inhabitants. The selected plants were distributed all over the country. Wastewater samples from these plants were filtered through a hydrophilic polyethersulfone membrane with $0.22\ \mu\text{m}$ pore size (MillexGP, Millipore) to remove bacteria and analysed within 24 h or frozen with 10% Glycerol (85%) at -70°C . From each sample, serial dilutions were analysed in pairs. All samples were analysed with the finally selected new host strains (*B. thetaiotaomicron* ARABA 84, *B. fragilis* ARABA 19, *B. fragilis* KBA 60, *Bacteroides caccae* RBA 63 and

B. caccae RBA 64). Enumeration of PFU was carried out according to a standard method for detection and enumeration of bacteriophages (ISO 10705-4, 2001b).

Samples of animal origin

Liquid manure from four farms and three wastewater samples obtained from two slaughterhouses were analysed. The farms with 20 to 30 cows or horses were located in different regions of Switzerland. Slaughterhouses represented wastewater from cows, calves, bulls and swine. Animals were delivered daily from all over the country. Animal phage suspensions were prepared as described above. The standard method for detection and enumeration of bacteriophages was used for enumeration of PFU in dilution series of each sample (ISO 10705-4, 2001b).

Study area

Two study areas in the north-western part of Switzerland were chosen for field studies. They are located in karst areas which are vulnerable to contamination and where the land is used for agricultural purposes. In both study areas, a wastewater treatment plant is located along the stream to be investigated. Because the effluents are being discharged into the stream, sampling sites were chosen upstream and downstream of the effluent discharge point.

In the Röschenz study area, two karst springs, termed 'Lützelquelle' (LQ) and 'Kächbrunnenquelle' (KQ), were investigated. Both springs are very vulnerable to contamination (Auckenthaler 2004). They are located close to the Luetzel stream and downstream of the wastewater treatment plant. Previous hydrogeological studies have shown that the Luetzel stream infiltrates to some extent into the KQ spring. The LQ spring is fed by infiltrating rainwater from a plateau south of the spring on which the hamlet of Huggerwald is located (Auckenthaler 2004).

In the second study area, Oberdorf, the karst spring 'Z'Hof' (ZQ) was also studied; it is vulnerable to contamination by being infiltrated by the 'Weigistbach' stream (Schudel *et al.* 2000). The wastewater treatment plant discharges sewage upstream of the spring. During dry periods all the water from the stream infiltrates the ground about 500 m upstream from the ZQ spring.

Surface and spring water

During a period of 6 months, from March to August 2009, four samples from all seven sampling sites were analysed during dry weather conditions.

During the period of investigation, additional spring water samples were taken after three rainfall events. Such a rainfall event was defined as rain of more than 10–20 mm h⁻¹ according to local weather radar. Samples were collected approximately 72 h after rainfall. After the second rainfall event, on 14 July 2009, samples were taken not only on 17 July but also on 21 July because additional rainfall (<10 mm h⁻¹) was recorded on 18 July.

One litre of spring water and 0.5 litre of river water were filtered through a membrane filter of 0.22 µm pore size and 47 mm diameter (GSWP04700, Millipore) to concentrate the water samples before analysis. Filtration and re-suspension were done following a method previously described (Mendez *et al.* 2004). Samples were analysed within 48 h according to the standard method ISO 10705-4 (2001b) with the following modification: 5 ml elution buffer containing bacteriophages was mixed with 5 ml of the host strain and 12.5 ml semisolid BPRM agar. The mixture was poured onto a BPRM agar plate with a diameter of 145 mm. Plaque forming units were counted after 21 h of anaerobic incubation.

To determine non-specific faecal contamination, somatic coliphages were quantified using the bacterial host strain *Escherichia coli* WG5 (provided by the Department of Microbiology, University of Barcelona, Spain). Analysis was according to the standard procedure for enumeration of somatic coliphages (ISO 10705-2, 2001a).

Statistical analysis

Statistical tests were performed with the software package, SPSS 14.0.

RESULTS

Isolation of *Bacteroides* host strains

Presumptive *Bacteroides* strains were isolated from wastewater and animal faeces. *Bacteroides* strains grow as typical black colonies on BBE agar. The occurrence of such presumptive

isolates was much higher in human wastewater than in horse or cow faeces. While both human wastewater samples were positive for *Bacteroides* strains, five of ten cow samples (manure and slaughterhouse waste water) were negative for *Bacteroides* strains after analysis on selective BBE agar. Therefore, more animal samples than human samples were analysed to get an appropriate number of isolates for further examination (Table 1).

During the screening of specific host strains, isolates were classified into three levels (Table 1). From the isolates obtained by analysis of human wastewater, 81% were identified as strictly anaerobic, Gram-negative rods and therefore classified as level 1 isolates; 90% of isolates from bovine samples and 76% of isolates from horse faeces could be classified as level 1 isolates.

Twenty-four (25%) isolates obtained from human wastewater, 16 (23%) isolates from bovine samples and 16 (18%) isolates from horse samples had an optical density at 620 nm of > 1 after incubation under anaerobic conditions for 18–23 h in BPRM broth (ISO 10705–4). These isolates were classified as level 2 isolates.

Two human host strains (termed ARABA 19 and ARABA 84) were classified as level 3 isolates because they detected no bacteriophages in animal phage suspension (0 PFU per ml). One isolate from cow faeces (KBA 60) and two isolates from horse faeces (RBA 63 and RBA 64) did not detect bacteriophages in human phage solution. Hence they were specific for bacteriophages of animal origin.

The five host strains specific for detection of bacteriophages of either human or animal origin were confirmed to belong to the *Bacteroides* species by either API or DNA sequencing. The two human host strains, showing specificity

for bacteriophages of human origin, were identified as *B. thetaiotaomicron* (ARABA 84) and *B. fragilis* (ARABA 19). KBA 60, isolated from cow faeces, belongs to the species *B. fragilis* and the host strains RBA 63 and RBA 64, isolated from horse faeces, were identified as *B. caccae*.

Field validation of new *Bacteroides* host strains

Human wastewater

The new *Bacteroides* isolates were used as host strains to test for phage suspensions from human wastewater collected from ten different treatment plants. The human host strain ARABA 84 detected bacteriophages in 100% of human wastewater samples tested. When the host ARABA 19 was used for detection of phages, only one out of ten samples was negative. The median concentration of bacteriophages detected by the host strain ARABA 84 and ARABA 19 was 39 and 9.5 PFU per ml, respectively. *Bacteroides fragilis* RYC 2056 which was used as reference strain detected a median of 85 PFU per ml in the same human wastewater samples.

Bacteriophages infecting the animal host strains KBA 60, RBA 63 or RBA 64 were absent in human wastewater.

Samples of animal origin

Bacteriophages detected by the reference strain RYC 2056 were not found in liquid manure. Therefore, only one out of four liquid manure samples was analysed with host strains ARABA 19, ARABA 84, KBA 60, RBA 63 and RBA 64. The sample tested negative with all host strains.

Table 1 | *Bacteroides* isolates obtained from wastewater of human, bovine and horse origin in Switzerland

Host origin	No. of samples	No. of isolates	Level 1 isolates	Level 2 isolates	Level 3 isolates	Strain designation
Human	2	96	78 (81%)	24 (25%)	2 (2%)	<i>B. fragilis</i> ARABA 19 <i>B. thetaiotaomicron</i> ARABA 84
Bovine	10	69	62 (90%)	16 (23%)	1 (2%)	<i>B. fragilis</i> KBA 60
Horse	8	89	68 (76%)	16 (18%)	2 (2%)	<i>B. caccae</i> RBA 63 <i>B. caccae</i> RBA 64

Level 1: Gram-negative rods growing under strict anaerobic conditions

Level 2: level 1 isolates with good growth in BPRM broth (OD₆₂₀ > 1) and positive phage counts testing phage suspension of the same origin as the isolate

Level 3: level 2 isolates showing specificity for their origin

The percentages of positive isolates given in parentheses were calculated from the initial number of isolates

Bacteriophages which were active against the animal host strains KBA 60, RBA 63 and RBA 64 were present in 67% of the slaughterhouse wastewater samples. The median concentration of bacteriophages detected was one PFU per ml for phages infecting RBA 63 and two PFU per ml for bacteriophages active against RBA 64 and KBA 60. The median of bacteriophages infecting the host RYC 2056 was one PFU per ml and therefore comparable to the concentrations detected by animal hosts.

Bacteriophages active against human strains ARABA 84 and ARABA 19 were not detected in any of the animal samples.

Surface water samples

For field validation, 16 surface water samples were analysed. The occurrence and median concentration of bacteriophages active against novel host strains are summarized in Table 2. In both study areas, bacteriophages reacting with the human host strain ARABA 84 were present in all samples taken downstream from the wastewater treatment plants. There was a significant difference between bacteriophages detected by host ARABA 84 in samples collected upstream and downstream of the effluent discharge point in the Weigistbach, Oberdorf (Mann–Whitney $U = 0$, $n_1 = n_2 = 4$, $p = 0.017$ two-tailed). The concentration of bacteriophages infecting any other host strain did not increase in samples collected downstream of the wastewater treatment plant (Mann–Whitney U test, $n_1 = n_2 = 4$; $p > 0.05$). In addition, no significant differences in phage counts were observed between samples col-

lected upstream and downstream of the effluent discharge point in the Röschenz study area (Mann–Whitney U test, $n = 4$; $p > 0.05$).

Spring water

The concentration of bacteriophages detected by the novel host strains ARABA 19, ARABA 84, KBA 60, RBA 63 and RBA 64 was determined during different weather conditions. The median values of bacteriophages detected in the three springs during dry weather conditions and after rainfall are shown in Table 3. As shown, bacteriophages detected by the host strains ARABA 19, ARABA 84, KBA 60 and RBA 63 were present in the investigated spring water samples after rainfall. However, the new host strains never detected bacteriophages during dry weather conditions.

In total, 24 spring water samples were analysed: 12 samples were taken during dry weather, representing low water flow conditions, and an additional 12 samples were taken after rainfall. The general faecal contamination was measured with somatic coliphages detected by *E. coli* WG5. The median concentration of somatic coliphages in spring water increased significantly from 4 PFU per 100 ml during dry weather conditions to 675 PFU per 100 ml after rainfall (Mann–Whitney $U = 11$, $n_1 = n_2 = 12$, $p < 0.05$ two-tailed). Beside the demonstrated increase of general faecal contamination, an increase of human faecal contamination was detected. The concentration of bacteriophages detected by ARABA 84 increased significantly after rainfall

Table 2 | Detection of bacteriophages active against novel *Bacteroides* host strains in water samples from two streams in Switzerland

Sampling site	Phages of ARABA 84		Phages of ARABA 19		Phages of RBA 63		Phages of RBA 64		Phages of KBA 60	
	% positive	Median ^a	% positive	Median ^a	% positive	Median ^a	% positive	Median ^a	% positive	Median ^a
Luetzel upstream WTP	100	102	25	0	0	0	0	0	50	1
Luetzel downstream WTP	100	140	33 ^b	0 ^b	0	0	25	0	50	0.5
Weigistbach upstream WTP	25	0	0	0	25	0	25	0	75	1
Weigistbach downstream WTP	100	9.5	0	0	25	0	50	0.5	25	0

WTP = wastewater treatment plant

^aMedian values are in PFU per 0.5 litre

Four samples per sampling site were analysed

^bOnly three samples were analysed

'Luetzel' and 'Weigistbach' refer to names of the streams in the study areas of Röschenz and Oberdorf, respectively

Table 3 | Bacteriophage concentrations detected by novel host strains in spring water analysed before and after rainfall events in the study area in Switzerland

Isolate	Weather	Spring water sampling sites					
		LQ		KQ		ZQ	
		Median ^a	% positive	Median ^a	% positive	Median ^a	% positive
ARABA 84	dry	0	0	0	0	0	0
	rainfall	6.5	100	4 ^b	67 ^b	2.5	75
ARABA 19	dry	0	0	0	0	0	0
	rainfall	1.5	50	0	25	0	0
RBA 63	dry	0	0	0	0	0	0
	rainfall	0	25	0	25	0	25
RBA 64	dry	0	0	0	0	0	0
	rainfall	0	0	0	0	0	0
KBA 60	dry	0	0	0	0	0	0
	rainfall	0 ^b	0 ^b	0	0	0	25

^aMedian value of bacteriophages in PFU per litre

Four samples were analysed collected at four different time points for each type of weather condition

^bonly three samples analysed

LQ: Spring Lützelquelle, KQ: spring Kächbrunnenquelle, ZQ: spring Z'Hof

(Mann–Whitney $U = 12$, $n_1 = 12$, $n_2 = 11$, $p < 0.05$ two-tailed). The concentration of bacteriophages reacting with any other host strain did not increase after rainfall (Mann–Whitney U test, $n_1 = n_2 = 12$; $p > 0.05$).

The load of faecal contamination was different after the three rainfall events. The highest concentration of somatic coliphages was found after the second rainfall event (median 25,600 PFU and 1,020 PFU per 100 ml). A lower median of 14 PFU per 100 ml and 330 PFU per 100 ml was found after the first and third rainfall event. As shown in Table 4, novel host strains indicating the highest faecal contamination were obtained following the second rainfall event.

After the first rainfall event, bacteriophages active against the human host strain ARABA 84 were present in two springs (LQ and ZQ). The result for the third spring (KQ) is missing because the agar plate which was used for analysis did not solidify. Specific bacteriophages active against other host strains were not detected in the three springs after the first rainfall event.

Higher phage counts were present after the second rainfall event in both samples taken from each spring. Bacteriophages active against ARABA 84 and RBA 63 were detected in all three springs. Moreover, one sample from the LQ springs tested positive for bacteriophages detected by

ARABA 19. Specific bacteriophages infecting other host strains were not detected after the second rainfall event.

Using the two human host strains, ARABA 84 and ARABA 19, for detection of bacteriophages after the third rainfall event, all water samples from the LQ springs and KQ were positive. In the ZQ spring, bacteriophages reacting with the *Bacteroides* hosts ARABA 84 and KBA 60 were present. However, bacteriophages detected by any other host strain could not be found in analysed spring water samples after this rainfall event.

Table 4 | Presence of bacteriophages detected by novel *Bacteroides* host strains in spring water analysed after rainfall in the study area in Switzerland

Isolate	Rainfall event 1			Rainfall event 2			Rainfall event 3		
	LQ	KQ	ZQ	LQ	KQ	ZQ	LQ	KQ	ZQ
ARABA 84	+	n.d.	+	+	+	+	+	+	+
ARABA 19	–	–	–	+	–	–	+	+	–
RBA 63	–	–	–	+	+	+	–	–	–
RBA 64	–	–	–	–	–	–	–	–	–
KBA 60	–	–	–	–	–	–	–	–	+

LQ: Spring Lützelquelle, KQ: Spring Kächbrunnenquelle, ZQ: Spring Z'Hof

n.d. = not determined

While somatic coliphages detected by *E. coli* WG5 were present after all three rainfall events, bacteriophages infecting the reference strain RYC 2056 could be detected in only one spring water sample after the second rainfall event.

DISCUSSION

Previous studies documented the variation in detection of *Bacteroides* host strains in different geographic regions. Therefore, different host strains need to be tested for use in particular regions. Moreover, the isolation of new specific host strains and their validation in the region of interest is recommended (Payan et al. 2005).

In this study, five *Bacteroides* host strains with specificity for bacteriophages of either human or animal origin were isolated. With human host strains higher numbers of bacteriophages were detected than with animal host strains. However, the five strains successfully differentiated between human and animal wastewater. The occurrence and concentration of bacteriophages infecting the five host strains was similar to the values obtained using the reference host *B. fragilis* RYC 2056 in wastewater, surface water and spring water. The suitability of individual strains varied according to whether they would be applied in testing for contamination in wastewater, surface and spring water (see below).

Human host strains

There was a clear difference between the two human strains ARABA 84 and ARABA 19. In human wastewater and surface water samples, plaque counts were always higher when using the host ARABA 84 compared with the host ARABA 19. Higher sensitivity of the host strain ARABA 84 was also observed in spring water samples. When bacteriophages active against ARABA 19 were present in spring water samples, ARABA 84 always detected bacteriophages in the same sample as well. Moreover, bacteriophages active against ARABA 84 were present in both study areas while ARABA 19 never detected bacteriophages in the Oberdorf study area.

Treating wastewater reduces its bacterial population. Nevertheless, treated sewage is still heavily loaded with faecal bacteria (Vilanova et al. 2004; Vilanova & Blanch 2005). Therefore, human faecal contamination might be present in

surface water downstream of the effluent discharge point from wastewater treatment plants. The increase of bacteriophages detected by ARABA 84 downstream from the treatment plant in the Weigstbach confirmed this hypothesis. However, a similar increase was not detected in the Röschenz study area, which might be due to additional wastewater effluent discharge further upstream.

Animal host strains

There is a difference between *Bacteroides* isolates found in human and animal intestines. *Bacteroides* isolates are found in very low numbers in animal faeces, which made the isolation of new animal host strains difficult. Bacteriophages active against *B. fragilis* RYC 2056 were not detected in liquid manure. Therefore, only one out of four liquid manure samples was analysed for bacteriophages active against novel host strains. No bacteriophages were detected in the specimen. *B. fragilis* RYC 2056 is recommended as a reference strain to determine the concentrations of phage suspensions (Payan et al. 2005). In this study, it was shown that phages infecting host *B. fragilis* RYC 2056 were absent or found in low concentrations in animal waste. Other studies, in which wastewater of animal origin was analysed, showed the same trend (Puig et al. 1999; Blanch et al. 2004; Payan et al. 2005).

For the first time we describe here host strains with specificity for phages of animal origin. The occurrence and detection of bacteriophages was comparable for the three animal host strains in wastewater. In surface and spring water, a comparison of the three strains was difficult. The occurrence of bacteriophages detected by KBA 60 was higher compared with bacteriophages infecting the two other hosts in surface water, while in spring water the most phages were detected by RBA 63. The different patterns of occurrence of bacteriophages in surface and spring water samples might be due to differences in persistence of the host strains. Such a phenomenon was recently shown for F-specific RNA phage subgroups (Muniesa et al. 2009).

Bacteriophages were present in very low numbers in liquid manure and slaughterhouse wastewater. The field validation was thus undertaken to get a better knowledge of the potential of the newly isolated animal host strains for use in microbial source tracking. Bacteriophages detected by all three animal host strains were present in surface water.

Moreover, RBA 63 and KBA 60 detected bacteriophages in spring water after rainfall events. However, in most of the analysed water samples bacteriophages active against animal host strains were not detected or present only in low concentrations. The practicability of using animal host strains for microbial source tracking should be evaluated in further studies by including additional indicators of animal pollution.

Increased faecal contamination after rainfall

In Switzerland, spring water from karst aquifers are important sources of drinking water but are vulnerable to contamination including by faecal bacteria (Auckenthaler *et al.* 2003). In this study, somatic coliphages were used to test the microbiological quality of spring water and revealed considerable faecal contamination after rainfall. This finding was in accordance with previous studies which detected an increase of faecal indicator organisms, *E. coli* and enterococci after rainfall (Schudel *et al.* 2000; Auckenthaler 2004). In both study areas, previous hydrogeological analysis indicated that faecal contamination derives mainly from agriculture although human contamination is probable (Schudel *et al.* 2000; Auckenthaler 2004). To evaluate the potential of the newly isolated host strains, these karst aquifers were considered to be ideal study areas.

The differentiation of the origin of various types of contamination in spring water is crucial for public health. In this study, specific *Bacteroides* host strains were tested to assess spring water contamination. A significant increase of human faecal contamination after rainfall was detected when using the human host strain ARABA 84. Detection of somatic coliphages confirmed an increase of faecal pollution after rainfall. Therefore, it can be concluded that human faecal contamination could successfully be detected using the host strain ARABA 84, especially after rainfall events where high faecal contamination is present.

Prior to this study, no human-specific faecal indicator has been used for field investigations in Switzerland. Bacteriophages were detected by human host strain ARABA 84, indicating the presence of human faecal pollution in samples collected from all three springs after rainfall events. Besides the human contamination detected in spring water, additional contamination from agriculture was expected (Schudel *et al.* 2000; Auckenthaler 2004). However, the abundance of

bacteriophages indicating animal faecal pollution in water samples was rather low and animal faecal contamination was detected in only a small number of environmental water samples.

Geographical distribution and host restriction

A good indicator of human or animal contamination would be easy detectable, geographically widespread, restricted to the host organism and would be prevalent and abundant in the host organism as well as in environmental samples.

The suitability of previously isolated *Bacteroides* host strains such as *B. thetaiotaomicron* GA-17 varied in different geographical areas (Payan *et al.* 2005). Such variations might be related to regional distinctions in diets which lead to different species composition of the faecal flora. Leser *et al.* (2000) showed that the bacterial species composition in the large intestine of pigs changed with different experimental diets; some profiles obtained by genetic fingerprinting techniques were stable while others varied in distribution and were related to specific diets. Geographical differences might also be due to spatial and temporal differences as previously reported for the composition of *E. coli* isolates within and between host species (human versus individual animals) (Gordon 2000; Stoeckel *et al.* 2004). In this study, bacteriophages active against human host strains were abundant in human wastewater obtained from plants distributed all over Switzerland. In addition, animal host strains detected bacteriophages in samples from slaughterhouses representing wastewater from animals from all over the country. This suggests that these bacteriophages of novel host strains are widespread in Switzerland. In addition, the field studies in two areas in the north-western part of Switzerland showed that the host strains detected bacteriophages in Swiss surface and spring water samples. Therefore, we conclude that novel *Bacteroides* host strains were useful to indicate human and animal faecal contamination in Switzerland. Further international comparative studies would be desirable in order to evaluate the applicability and performance of these novel indicators in other geographic regions.

In this study, the analysis of human wastewater, liquid manure and slaughterhouse wastewater indicated host restriction of bacteriophages of the isolated human and animal host strains. Although bacteria of the genus *Bacteroides* and corre-

sponding phages are present in the human and animal faecal flora, it was shown that bacteriophages of particular strains such as *B. fragilis* HSP40, *B. ovatus* GB-124 and *B. thetaiotaomicron* GA-17 were almost restricted to samples from human origin (Tartera & Jofre 1987; Tartera et al. 1989; Payan et al. 2005). This might be due to the co-evolution of phages, bacteria and the host (human or animal).

CONCLUSIONS

The human host strain ARABA 84 was shown to perform well in detecting wastewater, surface water and even spring water faecal contamination. The high specificity of the strain detecting bacteriophages of human origin could be shown in wastewater analysis. No plaque-forming units were detected in animal samples. Moreover, the sensitivity of the strain was demonstrated through spring water analysis. After rainfall, human contamination could be identified by use of ARABA 84 host strain in three springs used as a source for drinking water. In conclusion, *Bacteroides thetaiotaomicron* ARABA 84 is a promising new tool for microbial source tracking.

ACKNOWLEDGEMENTS

The authors would like to thank Maite Muniesa and Anicet R. Blanch from the Department of Microbiology, University of Barcelona, Spain, for scientific advice, technical instruction and for providing reference material. The present study was conducted in the laboratories of the Consumer Protection Directorate of the Federal Office of Public Health (FOPH) and financed by research grant FOPH/07.006623.

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First received 23 November 2009; accepted in revised form 28 June 2010. Available online 27 October 2010