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Characterization of fecal indicator bacteria in sediments cores from the largest freshwater lake of Western Europe (Lake Geneva, Switzerland)

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ABSTRACT

This study characterized the fecal indicator bacteria (FIB), including *Escherichia coli* (*E. coli*) and *Enteroccocus* (ENT), disseminated over time in the Bay of Vidy, which is the most contaminated area of Lake Geneva. Sediments were collected from a site located at ~500 m from the present waste water treatment plant (WWTP) outlet pipe, in front of the former WWTP outlet pipe, which was located at only 300 m from the coastal recreational area (before 2001). *E. coli* and ENT were enumerated in sediment suspension using the membrane filter method. The FIB characterization was performed for human *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) and human specific bacteroides by PCR using specific primers and a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Bacterial cultures revealed that maximum values of 35.2×10^8 and 6.6×10^6 CFU g^{-1} dry sediment for *E. coli* and ENT, respectively, were found in the sediments deposited following eutrophication of Lake Geneva in the 1970s, whereas the WWTP started operating in 1964. The same tendency was observed for the presence of human fecal pollution: the percentage of PCR amplification with primers ESP-1/ESP-2 for *E. faecalis* and *E. faecium* indicated that more than 90% of these bacteria were from human origin. Interestingly, the PCR assays for specific-human *bacteroides* HF183/HF134 were positive for DNA extracted from all isolated strains of sediment surrounding WWPT outlet pipe discharge. The MALDI-TOF MS confirmed the presence of general *E. coli* and predominance *E. faecium* in isolated strains. Our results demonstrated that human fecal bacteria highly increased in the sediments contaminated with WWTP effluent following the eutrophication of Lake Geneva. Additionally, other FIB cultivable strains from animals or adapted environmental strains were detected in the sediment of the bay. The approaches used in this research are valuable to assess the temporal distribution and the source of the human fecal pollution in aquatic environments.

1. Introduction

Pathogenic organisms and micropollutants contamination of freshwater drinking resources is a major problem in many parts of the world. Diarrheal diseases, mainly due to the consumption of microbiologically contaminated drinking water, cause about one billion illnesses and 2.2 million deaths per year (Montgomery and Elimelech, 2007). Although most infections occur in developing countries, waterborne diseases are a worldwide problem that also concern industrialized societies where highly populated centers draw their supply of drinking water. Although fecal pollution originates from a variety of human and non-human sources, fecal indicator bacteria (FIB) including *Escherichia coli* (*E. coli*) and *Enterococcus* (ENT) contamination from animals and human fecal material is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens (WHO, 2004).

Several studies have demonstrated that sediments may constitute an important reservoir of FIB in freshwater environments (LaLiberte and Grimes, 1982; An et al., 2002; Haller et al., 2009a). Accumulation of FIB and pathogenic organisms in sediments has been attributed to the sorption of the microorganisms to particles suspended in water, whereas the desorption of the microorganisms from sediment can occur under changing physicochemical conditions (e.g., pH, oxygen availability, redox conditions). Sediments may contain 100–1000 times as many FIB as the overlying

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water (Davies et al., 1995). FIB can survive longer in sediments than in the water column since sediments are enriched in organic compounds and thereby provide favorable nutrient conditions (Gerba and McLeod, 1976; LaLiberte and Grimes, 1982; Poté et al., 2009), and also a protection from sunlight inactivation (Sinton et al., 1999) and protozoan grazing (Davies and Bavor, 2000). The resuspension of FIB and pathogens from the sediments to the water column due to human activities or natural processes may contribute to potential human health risk (An et al., 2002; Craig et al., 2004). Moreover, the evaluation of FIB in current sediments may represent a more stable index of overall or long-term water quality than in the overlying water (LaLiberte and Grimes, 1982; Ferguson et al., 2005; Haller et al., 2009b; Pachepsky and Shelton, 2011). The presence of FIB including *E. coli* and ENT, which affects water quality and safety, can originate from a variety of human and non-human sources also indicates the potential for the presence of pathogenic organisms (An et al., 2002; Poté et al., 2009). It has been further suggested that human fecal material is generally considered to be of greater risk to human health as it is more likely to contain human pathogens (Scott et al., 2003).

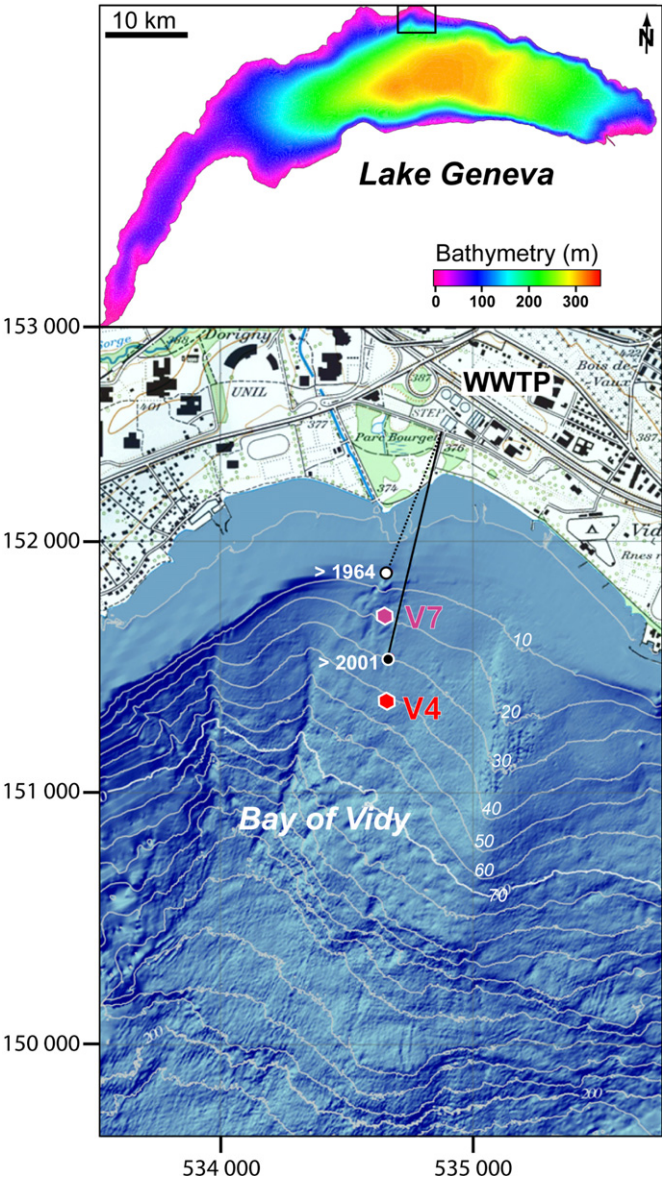

Fig. 1. (Upper panel) Bathymetric map of Lake Geneva showing the studied area (square on the north shore). (Lower panel) Bathymetric and topographic maps of the Vidy Bay based on single- and multi-beam echosounder data (details in Sastre et al., 2010) showing the location of the two sediment cores studied: core V4, located at ~500 m from the current WWTP outlet, and core V7 located in between the present WWTP outlet and the former one used from 1964 to 2001.

Lake Geneva is the largest freshwater reservoir in Western Europe with a surface area of 580 km^2, a volume of 89 km^3 and a maximum depth of 309 m. Approximately 700,000 people are served by this freshwater resource. Lausanne, a city of 127,000 inhabitants on the northern lake shore, pumps 58% of its water supply from the lake, while the city is also discharging the largest volumes of treated domestic and industrial wastewaters into the nearby Bay of Vidy (Fig. 1). Accumulation of FIB, organic and inorganic contaminants in this bay and its related ecological impacts and health risks have been already demonstrated by our previous studies (Poté et al., 2008; Haller et al., 2009a, b). The results indicated that the Bay of Vidy is the most contaminated area of the Lake Geneva with great values of various organic and inorganic contaminants including heavy metals, PCBs, PAHs and antibiotics in surface sediments (Poté et al., 2008). Dramatic values, more than 10^8 CFU g^{-1} dry sediment of *E. coli* and ENT, have been detected in the surface sediments close to the WWTP outlet discharge (Haller et al., 2009a). However, the extent and the nature of FIB in sediments of the bay have not been yet characterized. Thus, little information is available concerning the quantification, and the specific distribution and characterization of FIB in sediment profiles of the bay.

This study aims to assess and characterize the FIB contents and their temporal distribution (i) in sediments deposited before and after WWTP outlet pipe extension in the bay of Vidy, and (ii) in sediments accumulated at ~500 m from the present-day outlet. The sediment cores were collected from two sites and the extracted FIB were isolated and quantified using the membrane filter methods. PCR assays were performed on both the isolate colonies and DNA extracted from membrane filters (with colonies) for deciphering the absence/presence of human-specific FIB and MALDI-TOF MS for general FIB characterization. MALDI-TOF MS is presently widely used in bacteriology labs for the identification and characterization of clinically important microorganisms. The currently available identification databases target primarily the human microbial pathogens (Benagli et al., 2011), but environmental microorganisms can also be identified if phylogenetically defined isolates and reference strains are introduced in the database and validated (Gaia et al., 2011).

2. Materials and methods

2.1. Sediment collection

The boat "La Licorne" of the Institute F.-A. Forel was used for sediment coring in the Bay of Vidy, near and on both sites of WWTP outlet pipe discharge (Fig. 1): core V4 (40 m water-depth, 62 cm-length, Swiss coordinates *X*: 534,682, *Y*=151,410, distance to coast ~715 m), and core V7 (35 m water-depth, 38 cm-length, Swiss coordinates *X*: 534,670, *Y*=151,570). After their collection, the cores were brought to the laboratory, opened and sliced into 2 cm thick sections. The sediment samples were placed in sterile plastic containers (stored in an icebox) and treated in the laboratory within 24 h for microbiological analysis. For chemical analysis, the sediment samples were frozen, freeze-dried and ground into a fine homogenized powder (Poté et al., 2008).

2.2. Organic content

Total nitrogen concentration (%N) was analyzed in a CHN Elemental Analyzer (Carlo Erba Flash EA 1112 CHNS/MAS200) on about 10 mg of dry powdered sediment (Thevenon et al., 2004). Total organic (C_{org}) carbon content was determined using Rock-Eval pyrolysis (Espitalié et al., 1985; Lafargue et al., 1996) with a Model 6 device (Vinci Technologies) and the standard IFP 160000. The analyses were carried out on 50–100 mg of powdered dry sediment under standard conditions. During Rock-Eval pyrolysis ca. 150–100 mg of ground and homogenized sample was subject to a pyrolysis step. A flame ionization detector

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(FID) measured the hydrocarbon released during pyrolysis, while CO_2 and CO were detected by infrared absorbance. Pyrolysis started isothermally at 300 °C for 3 min, after which the sample was heated to 650 °C. The oxidation step started isothermally at 400 °C (3 min) and then heated up to 850 °C. Peak of CO_2 released during pyrolysis was used to calculate the amount of total organic carbon (C_{org}). Mean error measurements are about 5%.

2.3. Sediment chronostratigraphy

The chronostratigraphy of core V4 is based on a continuous high-resolution (every cm) sedimentary record of cesium (^{137}Cs) obtained from a former core taken at the same location than V4 (core Vs 14; details in Glass-Haller, 2010). Fig. 2 presents the related Cs profile, which evidences (i) the maximum atmospheric radionuclide fallout of 1963/64 (coinciding with the WWTP operating) at 44 cm core depth, and (ii) the absence of the Chernobyl ^{137}Cs peak of 1986 certainly due to the dilution by the anthropogenic sediment discharged into the bay. One radiocarbon (^{14}C) date (ETH-41999) performed on a small piece of wood found at 60 cm in core V4 (62 cm core length) yields a radiocarbon age of 175 ± 40 years BP (before present = 1950). Calibration using OxCal v3.10 program (Bronk Ramsey, 2001) gives a calendar age of 1765 ± 45, so that the lowermost sediments of core V4 may have deposited before the impact of the industrial revolution of the late 19th century. Anthropogenic ^{137}Cs was not measured on the 38 cm long core taken in the Vidy Bay (core V7) because this dark and organic enriched sequence does not recover the pre-WWTP sediments that deposited before 1964.

2.4. Isolation and quantification of FIB

The FIB including EC and ENT were isolated from sediment samples using the method previously described by Balkwill and Ghiorse (1985) and modified by Haller et al. (2009a). Briefly, the sediments were resuspended by adding 100 g (wet weight) of sediment to 500 mL of 0.2% $Na_6(PO_3)_6$ in sterile 1 L plastic bottles and mixed for 30 min using an agitator rotary printing press Watson–Marlow 601 controller (Skan, Switzerland). The mixture was centrifuged at 4000 rpm for 15 min at 15 °C. FIB in the supernatant were then quantified according to the Swiss standard methods for water quality determination using the membrane filtration method (Ordonnance sur l'hygiène (OHyg), 2005). For each sample, triplicates of 100 mL of supernatant were passed through a 0.45-mm filter (Sartorius stedim, biotech, Germany), which was placed on different culture media (Biolife Italiana) supplemented with the anti-fungal compound Nystatin (100 mg mL^{-1} final concentration). For quantification of *E. coli* the filter was placed on tryptone soy agar (TSA) medium for 4 h at 37 °C, then transferred to TBX medium and incubated at 44 °C for 24 h. For ENT the filter was placed on Slanetz–Bartley agar medium (SBA), incubated at 44 °C for 48 h and transferred into bile aesculin agar medium at 44 °C for 4 h. The results are expressed as colony forming units per 100 g of dry sediments (CFU 100 g^{-1}). The reproducibility of the whole analytical procedure was tested by triplicates of selected sediment samples, which revealed a mean variation coefficient of 13% for *E. coli* and 8% for ENT.

2.5. PCR assays for detection of general FIB, E. faecalis, E. faecium and human bacteroides

The genomic profiles of general origin of *E. coli* and ENT were performed by PCR assays (presence/absence) using specific primers and operational conditions as summarized in Table 1 (Ke et al., 1999; Bernhard and Field, 2000; Sabat et al., 2000; Hammerun and Jensen, 2002; Scott et al., 2005; Ahmed et al., 2007; Morrison et al., 2008). A total of 306 isolated colonies from V4 for each FIB (*E. coli* and ENT) (at different depths) were selected. PCR amplifications were performed directly on the colonies picked from selective-media plates and resuspended in 20 µL of sterile water.

PCR assays for human *E. faecalis* and *E. faecium* and for the human-specific *bacteroides* were performed on selected colonies and on the DNA extracted from isolated colonies (Scott et al., 2003). DNA was extracted from the bacteria on the membrane filter using Ultraclean soil DNA Kit (Mo Bio Labs, Solana Beach, CA) according to the manufacturer's recommendations. The concentration of extracted DNA was measured spectrophotometrically (OD_{260}) and DNA quality was assessed by electrophoresis on 0.8% agarose gels stained with 1 × SYBR Safe DNA gel stain (invitrogen). The purified DNA was kept at −20 °C until used. The human specific *bacteroides* were analyzed by PCR assays (presence/absence) using specific primers and operational conditions according to the published methods as summarized in

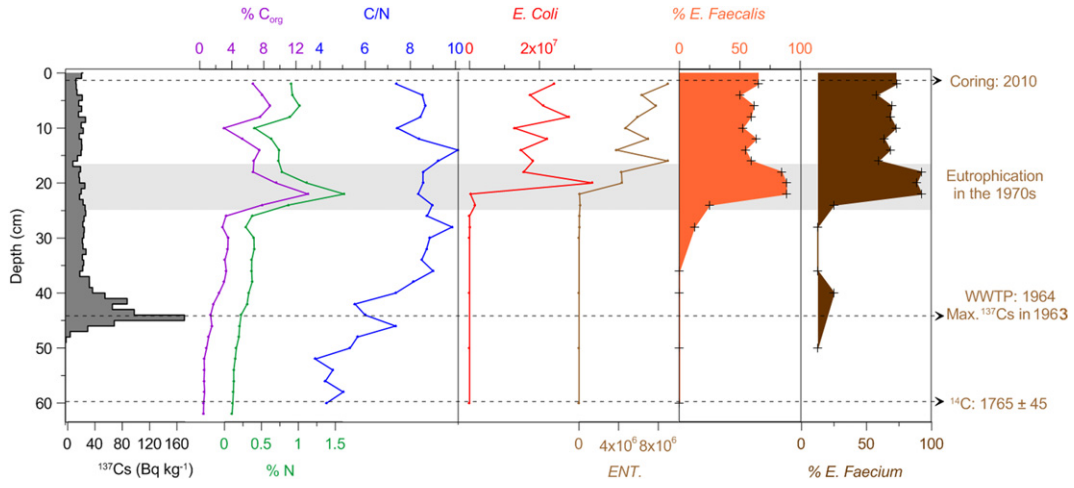

Fig. 2. ^{137}Cs profile from core Vs14 (data from Glass-Haller, 2010) and the physicochemial parameters results of core V7: organic carbon content (%C_{org}), nitrogen concentration (% N), and C/N ratio expressed as a function of depth in cm. Isolated *Escherichia coli* (*E. coli*) and Enterococcus (ENT) strains (CFU g^{-1} dry sediments), and the percentage of *E. faecalis* and *E. faecium*.

Table 1
Primer for human *Enterococcus faecalis* and *Enterococcus faecium* and human-specific *bacteroides* used in this study*.

Primers	Target	Size of PCR prod.	Sequence (5′ to 3′)	Anealing Temp. (°C)	Reference
ECA75F ECA619R	General *E. Coli*	544	GGAAGAAGCTTGCTTCTTTGCTGAC AGCCCGGGGATTTCACATCTGACTTA	60	Sabat et al. (2000)
Ent1 Ent2	General *Enterococci*	112	TACTGACAAACCATTCATGATG AACTTCGTCACCAACGCGAAC	55/49	Ke et al. (1999)/Morrison et al. (2008)
ESP-1 (F) ESP-2 (R)	*E. faecium/faecalis*	680	GGT CAC AAA GCC CAA CTT GT ACG TCG AAA GTT CGA TTT CC	60	Hammerun and Jensen (2002)/Scott et al. (2005)
HF183/134 Bac708R	Human HF183 Human HF134	520 570	ATCATGAGTTCACATGTCCG ATCARGTCACATGTCCCG CAATCGGAGTTCTTCGTG	59	Bernhard and Field (2000)/Ahmed et al. (2007)

* The operational conditions for PCR amplification were carried out according to the published methods (references in this table with minor modification).

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Table 1, sometimes with few modifications. Briefly, for human *Enterococcus faecalis* and *Enterococcus faecium* (Hammerun and Jensen, 2002), the primers used for PCR amplification were F-(5′-GGT CAC AAA GCC CAA CTT GT-3′) and R-(5′-ACG TCG AAA GTT CGA TTT CC-3′). For human-specific *bacteroides HF183/Bac708* (Bernhard and Field, 2000; Ahmed et al., 2007), the extracted DNA was amplified with F-(5′-ATCATGAGTTCACATGTCCG-3′) and R-(5′-CAATCGGAGTTCTTCGTG-3′). PCR reactions of 25 μl total volume consisted of 0.025 U/μl Takara Ex Taq HS DNA polymerase (Takara Bio Europe, Saint-Germain-en-Laye, France), 1 × PCR buffer (Takara, containing 2 mM of Mg^{2+}), 800 μM of dNTP, 0.2 μM of each forward and reverse primers (Invitrogen), 50 ng/μl of BSA and 7.5 ng of DNA. PCR was performed in a Biometra thermocycler (BIOLABO) with the following conditions: initial denaturation at 95 °C for 15 min; 35 cycles of denaturation (94 °C for 30 s), annealing (60 °C for 1 min) and extension (72 °C for 1 min), followed by a final extension at 72 °C for 10 min. PCR products were visualized after electrophoresis on 0.8% agarose gels containing 1 × SYBR Safe DNA gel stain (invitrogen) in 1 × TAE buffer.

The experiment was conducted in triplicate in each set of conditions. The negative (without DNA) and positive controls (e.g. the expected 520 bp length (for HF183/Bac708) from sewage (Poté et al., 2009)) were used for each PCR assays.

2.6. Characterization of FIB by MALDI-TOF MS

After isolation from sediment samples, the colonies for each FIB (*E. coli* and ENT) were taken at 22 and 50 cm depth of V4. The colonies were re-inoculated and incubated in selected medium (24 h in TBX medium for *E. coli* and 48 h in SBA for ENT). The grown colonies were used for FIB characterization using MALDI-TOF MS. The methodology is based on the detection and identification of proteins by molecular weight determination of individual specific fragments (Benagli et al., 2011). Sample preparation for MALDI is carried out by mixing the sample with a matrix present in large excess (matrix/sample ratio: about 10 000:1). The composition of the matrix varies depending on the analyte and the type of laser used (Tonolla et al., 2009). A small amount of bacterial colony material was spotted onto wells of 48-position stainless steel Fleximass™ target plates (Shimadzu Biotech, Kyoto, Japan), and then overlaid with 1 μl of alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix.

Plates were analyzed in positive linear mode with a MALDI-TOF MS Axima Confidence (Shimadzu Biotech) mass spectrometer. Spectrum profiles obtained were compared with the SARAMIS (Spectral ARchive And Microbial Identification System) database application from AnagnosTec GmbH (Potsdam, Germany).

The reference strain *E. coli* K12 (GM48 genotype) was used as a standard for calibration and as reference measurement for quality control. Sample information such as medium and growth conditions was imported into the software Shimadzu Biotech Launchpad™, v.2.8 (Shimadzu-Biotech Corp., Kyoto, Japan). Protein mass profiles were obtained with detection in the linear positive mode at a laser frequency of 50 Hz and within a mass range from 2000 to 20,000 Da. Acceleration voltage was 20 kV, and the extraction delay time was 200 ns. A minimum of 20 laser shots per sample was used to generate each ion spectrum. For each bacterial sample, 50 protein mass fingerprints were averaged and processed. Spectra were analyzed using SARAMIS™ at default settings. Dendrograms were based on the peak patterns of all analyzed strains submitted to single-link clustering analysis using SARAMIS™ (0.08% error, range from *m/z* 2000 to 20,000).

3. Results

3.1. Sedimentary record of organic matter (OM) deposition

The organic compounds (C_{org} and N) showed a strikingly similar pattern of variation, with (i) the lowest concentrations for the lowermost sediments deposited during the pre-industrial period, (ii) increasing values after the beginning of the WWTP discharges in 1964, and (iii) values that doubled between 24 and 18 cm in the 1970s (Fig. 2).

Pre-industrial sediments deposited at 60 cm core depth in the Vidy Bay (core V4, Fig. 2) exhibited C/N ratio of 4, indicating that phytoplankton (which is the major source of autochthonous OM in lakes) dominated before the waste sediment discharge in the Vidy Bay. Subsequently, increasing C/N ratio (> 6) above 52 cm likely indicated a relatively higher contribution of terrestrial inputs. By contrast, the abrupt raise (C/N ratio > 8) above 42 cm (following the 1963 ^{137}Cs peak; Fig. 2) certainly indicated a change of OM source due to the release of anthropogenic sediments of different composition into the bay after 1964.

Table 2
Distribution of *Escherichia coli* (*E. Coli*) and *Enterococcus* (ENT) isolated strains (colony forming units per 100 g of dry sediments (CFU 100 g^{-1})) in the sediments from the site V4*.

Depth (cm)	V4			
	E. coli (CFU × 10^6) 100 g^{-1}	± (SD × 10^6)	ENT (CFU × 10^6) 100 g^{-1}	± (SD × 10^6)
2	24.1	4.78	8.98	1.98
4	17.3	6.11	6.34	3.25
6	21.2	5.04	7.77	2.59
8	28.4	6.98	5.92	1.94
10	12.8	2.01	4.68	1.61
12	22.2	3.69	6.94	1.46
14	14.7	4.79	3.77	2.98
16	18.2	3.34	8.98	1.91
18	15.5	2.29	4.29	1.73
20	35.2	6.58	4.35	6.16
22	0.24	0.22	0.045	0.050
24	1.61	0.19	0.097	0.011
26	0	0	0.027	0.017
28	0.0089	0.0042	0.032	0.048
40	0.0015	0.00011	0.00032	0.00022
50	0.0002	0.000019	0.0011	0.00051
60	0.0002	0	0.00079	0

* The reproducibility of the whole analytical procedure was tested by triplicates of selected sediment samples, which revealed a mean variation coefficient of 13% for *E. coli* and 8% for ENT.

3.2. Time distribution of FIB

Similar to the changes in OM sedimentation, the highest amount of FIB were observed after 1970 (0–20 cm) for both sites (V4 and V7) (Table 2, Fig. 2). For core V4, the FIB levels significantly varied ($P < 0.05$) before ca. 1970 (ca. 20 cm), ranging between 14.7 and 35.2 × 10^6 and 3.8 and 9.0 × 10^6 CFU 100 g^{-1} for *E. coli* and ENT, respectively. Concerning the sediment deposited before 1970 (22–60 cm), the FIB concentration levels varied from 0 to 1.6 × 10^6 and 0.8 to 97 × 10^3 CFU 100 g^{-1} for *E. coli* and ENT, respectively. The ENT was observed throughout the sediment samples of core V4 (until 60 cm core depth), i.e., also before the WWTP implantation, but very low values were recovered before 1970 (0–22 cm) (Table 2). Concerning core V7, which was more influenced by the WWTP discharge, especially before the outlet extension in 2001, the FIB level varied from 3.1 to 6.2 × 10^5 and 3.5 to 21 × 10^4 CFU 100 g^{-1} for *E. coli* and ENT, respectively (Table 2).

3.3. Identification of general FIB, E. faecalis, E. faecium and human-specific bacteroides

We applied qualitative PCR assays for large-scale screening of the colonies isolated from core V4 to detect presence/absence of general FIB (*E. coli* and ENT) as well as human-specific *E. faecalis*, *E. faecium* and bacteroides. General FIB could be detected throughout the whole cores V4 and V7, whereas human-specific positive PCR (for *E. faecalis*, *E. faecium* and human-specific *bacteroides)* was observed in all DNA extracted from isolated colonies on membrane filters at V4. No PCR amplification for human-specific *bacteroides* was observed in the lowermost sediments of V4 (40–60 cm; Table 3) that deposited before the WWTP implementation. The percentage of FIB of human origin significantly varied with time ($P < 0.05$). It is meaningful to note that percentage of both *E. faecalis* and *E. faecium* was very low (e.g. No PCR amplification was observed for *E. faecalis*) before 1970, while sewage sediment accumulated in the bay since 1964. The percentage of both *E. faecalis* and *E. faecium* varied from 50% to more than 90% after ca. 1970. The maximum value occurred

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Table 3
PCR presence/absence assays for detection of *Enterococcus faecalis*, *Enterococcus faecium* and human-specific *bacteroides* in sediment profiles of sites V4 and V7.

Depth (cm)	V4					V7
	E. faecalis		*E. faecium*		Human *bacteroides*	Human *bacteroides*
	NT/NP	PCR on extracted DNA	NT/NP	PCR on total DNA	PCR on extracted DNA	PCR on extracted DNA
2	26/17	+	26/19	+	+	+
4	26/13	+	26/15	+	+	*
6	26/16	+	26/18	+	+	*
8	22/13	+	22/15	+	+	+
10	22/11	+	22/16	+	+	+
12	22/14	+	22/14	+	+	*
14	22/12	+	22/15	+	+	*
16	22/13	+	22/13	+	+	+
18	26/22	+	26/24	+	+	*
20	26/23	+	26/23	+	+	+
22	26/23	+	26/24	+	+	*
24	8/2	+	8/2	+	+	*
28	8/1	+	8/1	+	+	+
36	8/0	+	8/1	+	+	+
40	8/0	+	8/2	+	+	−
50	8/0	+	8/1	+	+	*

NT: number of tested colonies, NP: number of PCR positive, +: positive PCR, −: negative PCR, *: analysis not performed.

at 18–22 cm depth, when organic matter deposited strongly following the eutrophication of the lake. The peak of the presence of both *E. faecalis* and *E. faecium* was observed at 18–22 cm sediment core depth, i.e. synchronously with the abrupt rise in nutrient content (Fig. 2) that highly favored bacterial activity in Lake Geneva sediments (Thevenon et al., 2011a) and the dissemination of antibiotic resistant bacteria/genes in the Vidy Bay (Thevenon et al., in press).

3.4. Characterization of FIB by MALDI-TOF MS

The whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) method was performed to identify 4 FIB isolated strains. According to the results above (see Sections 3.1–3.3), two depths (22 and 50 cm) were chosen to characterize *E. coli* and ENT by MALDI-TOF MS. MALDI-TOF MS sequences from selected bacterial isolates indicated the predominance of strains belonging to the species *E. coli* and *E. faecium* (Fig. 3).

4. Discussion

The sediments of the Bay of Vidy are subject to extensive microbial contamination due to three identified sources including WWTP outlet discharge, the Chamberonne and Flon rivers (Goldscheider et al., 2007; Haller et al., 2009a, b; Poté et al., 2009). The inferred age of the sediment samples allows to assess the effect of WWPT effluent water on time distribution of *E. coli* and ENT in proximity points and also to identify the processes (such as accumulation of organic matter and lake eutrophication) that influenced the spatiotemporal variability of FIB in lake sediments (Thevenon et al., 2011a).

In order to better estimate the human health risk and design appropriate best remediation strategies for managing the pathogens risk in aquatic environment, *E. faecalis* and *E. faecium* and several *Bacteroides* spp. have been suggested as potential alternative indicators of human fecal material because human material is generally considered to be of greater risk to human health (Converse et al., 2009; Scott et al., 2005, 2007). In this study, positive PCR assays for general *E. coli* and ENT recovered strains, which were observed for all sediment samples, indicated the accumulation of FIB in the sediments deposited in Vidy Bay during the past several decades. These results were confirmed by MALDI-TOF MS, which indicated that the greater part of recovered ENT strains was *E. faecium*. The MALDI-TOF MS is a rapid and validated method for identification of strains belonging to *Escherichia* and *Enterococcus*, this is reflected in the cluster analysis showing clear groupings of *Escherichia coli* and *Enterococcus faecium* strains together with each ATCC reference strain.

According to the sediment physico-chemical characteristics, the FIB can accumulate and develop in the aquatic environmental compartment (Haller et al., 2009b; Poté et al., 2009). The relative amount of OM and nutrients in sediments have a strong effect on the persistence and growth of FIB and may result in different degrees of survival (LaLiberte and Grimes, 1982; Craig et al., 2004; Poté et al., 2009; Haller et al., 2009b). Alm et al. (2003) detected *E. coli* and ENT up to a depth of 20 cm in coarse sediments deposited in Lake Michigan (US), at levels between 200 and 400 CFU $100\ g^{-1}$. In the Bay of Vidy, the high organic matter content of the sediments surrounding the WWTP outlet may lead to a higher rate of FIB adsorption (Haller et al., 2009a). In this study, the maximum levels of *E. coli* and ENT in sediments were recovered between 18 and 24 cm in core V4 (Fig. 2), which may be explained by the high organic matter preservation in the sediments at that time. It has been further demonstrated that the vertical distribution of bacterial abundance in the sediments of Lake Geneva highly increased following the eutrophication that affected Lake Geneva in the 1970s and early 1980s (Thevenon et al., 2011a). The accumulation and persistence of FIB in the upper part of core V4 may be explained by the continuous contamination of the sediments by the WWTP effluent water that is highly enriched in organic compounds.

It is well known that *E. coli* and ENT can be encountered throughout the environment, from human, animal and food sources (Ke et al., 1999). These bacteria are used worldwide as indicators of human health risk in drinking and recreational waters (WHO, 2004). In aquatic environment, the culturable ENT can persist more than *E. coli*. Therefore, it has been indicated that ENT can be a better useful indicator of the efficacy of WWTP for purpose of reclamation (Scott et al., 2003; Poté et al., 2009). On the other hand, several studies demonstrated that the majority of both *E. coli* and ENT (*E. faecium*) detected in WWTP and septic tanks are more than 95% from human origin. In this study, more than 300 FIB isolated colonies were screened to assess the effect of WWTP discharge on the distribution of human fecal pollution in sediment depth. The PCR amplification performed for fecal human pollution showed high specific-human strains in younger sediment (deposited after ca. 1970), with the values ranging from 50% to more than 90%. In general, the human fecal bacteria increased dramatically in sediment deposited after 1970 in the Vidy Bay. Surprisingly, it seems that the vertical distribution of *E. coli* and ENT as well as *E. faecalis*, *E. faecium* and specific-human *Bacteroides* in V4 (Tables 2 and 3, Fig. 2) did not clearly evidenced the WWTP implementation after 1964, but rather the impact of the eutrophication that affected Lake Geneva in the 1970s and early 1980s. Interestingly, we detected FIB of human origin (e.g. specific-human *Bacteroides*) at several depths such as 50 cm (i.e., before WWTP implementation in 1964), indicating the accumulation of human pollution since the late 19th century in the bay or the possible effect of bioturbation in the sediment of the bay (Haller et al., 2009a). Additionally, the PCR essays with primers ESP-1/ESP-2 (Table 1) for *E. faecalis* and *E. faecium* were not positive for all tested strains, indicating that some *Enterococcus*

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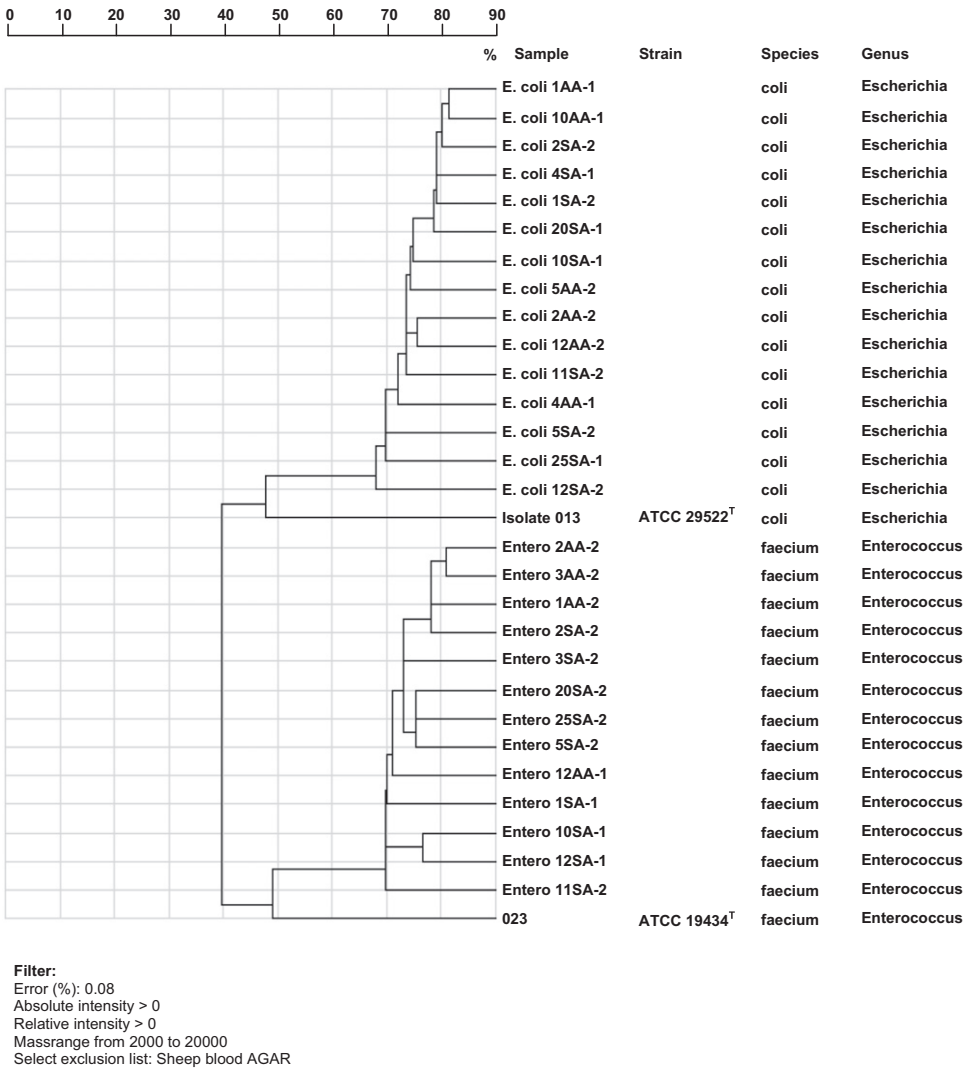

Fig. 3. Cluster analysis resulting from single-link clustering analysis (SARAMIS^{TM} database software) of MALDI-TOF MS data on isolated strains of *Escherichia coli* and Enterococcus. Error 0.08%; range of m/z from 2000 to 20,000 Da.

isolated strain from the sediment of the bay may have other origins such as animals or environmental adapted strains.

5. Conclusion

The main objective of this study was to investigate the accumulation and characterization of fecal indicator bacteria (and specific-human bacteria pollution) in sediment profiles from different parts of Lake Geneva (Switzerland) over the last decades. Several studies have been performed to assess the presence and distribution of organic and inorganic pollutants in the sediments of Vidy Bay (Poté et al., 2008; Thevenon et al., 2011b), as well as the distribution, persistence and activities of bacteria in the bay (Haller et al., 2009 a, b; Thevenon et al., 2011a). The former results confirmed the effects of WWTP effluent water on the contamination of sediment of the bay and the effects of eutrophication on the bacteria distribution and activities. Our present findings reveal the presence of *E. faecalis*, *E. faecium* and specific-human *Bacteroides* at several depths of sediment cores from the bay of Vidy, indicating the presence of human pollution in the lake before and after the WWTP input in the lake. The percentage of *E. faecalis* and *E. faecium* showed a good agreement with the high values of OM content, which is related to the lake eutrophication (Table 3, Fig. 2). The results of this study will serve as a reference point for future research in polluted aquatic environments affected by wastewater inputs and lake trophic changes, resulting from anthropogenic nutrient release and/or warmer climate impact on oxygen depletion of lake's bottom waters. However, given the long history of pollution in the Bay of Vidy, additional researches are needed for better understanding of the production, the quantification and the emergence of *E. faecalis*, *E. faecium* and specific-human *Bacteroides* in freshwater sediments since the last decades to century.

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