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Subject: Project of abroad sampling campaigns

Our reference: NR/V9/exchanges/UPE

My Paris-Est University has granted an international mobility fellowship to me in order to complete my work about sources of nontuberculous mycobacteria (NTM) in watersheds. In agreement with my Ph.D co-supervisors and with the director of host laboratory (see below: host Laboratory), who works on subjects which are closely related to my thesis goals (see below: Thesis subject), sampling campaigns were planned in Virginia watersheds in order to study potential sources of NTM which were not taken into account during the sampling campaigns carried out in the watershed of the Seine River (see below: scientific project).

Host laboratory: Pr. Falkinham's laboratory

- University of VirginiaTech: Blacksburg, VA 24061-0346 (<http://www.vt.edu/>), Department of Biological Sciences (MC 0406): 2125 Derring Hall (<http://www.biol.vt.edu/faculty/falkinham.html>), Laboratory: 5026 Derring Hall (<http://www.biol.vt.edu/faculty/falkinham/>).
- Research area: Microbiology and epidemiology of *Mycobacterium avium*; metal oxidation and reduction in biofilms; predatory bacteria ecology; antibiotic discovery and development.

Thesis subject

Surface water (river, lake, pond, hot spring) is considered as a likely source of NTM infections [1, 2]. Among human infections caused by water-borne NTM, pulmonary infections [3-12] and cutaneous infections are often described [13-19]. However, lack of knowledge about their life cycle in the environment requires analytical tools, which are not currently adapted to environmental samples. Within the framework of a European directive (2000/60/CE) which establishes objectives on preservation and restoration of surface water and ground water by the year 2015, my Ph.D project was undertaken to develop quantification tools and to determine sources of NTM from surface water.

In order to develop quantification methods that will be adapted to surface water samples, two axes of research were explored these last two years. First, comparison of culture methods has allowed the isolation of different species of potentially harmful NTM from surface water used by drinking water treatment plants of Paris, but has not allowed the enumeration of NTM accurately (Radomski *et al.* 2010, Applied and Environmental Microbiology, 76(11), 3514-3520). Second, several primers were screened to select one primer pair that could be used to develop a new qPCR method targeting NTM at the genus level. Sensitivity and specificity of this method were compared to two other qPCR methods already published without exhaustive specificity study. Sensitivity and specificity were tested against a reference NTM strain collection and a strain collection of non-NTM isolates from the Seine River (identified by 16S rDNA sequencing). Results show that the new qPCR is totally specific, which is not the case for the two qPCR already published (paper in review).

During the development of the cultural and molecular methods, several sampling campaigns in Ile de France were performed in order to determine sources of NTM in the watershed of the Seine River (WWTP and combined sewage overflow, storm water runoff). Water samples were collected to study NTM presence and their impact on River quality from wastewater treatment plants and treatment processes as decantation and biofiltration (source 1), from combined sewage overflow during storm events (source 2), and from runoff in rural areas (source 3) and urban areas (source 4) during storm events. A study of zoological garden as a potential source was also conducted in the beginning of the year 2010 (source 5), and all these samples are currently analyzed by qPCR.

Scientific project

In order to study potential sources of NTM and in agreement with my thesis co-supervisors (Françoise Lucas^[a], Régis Moilleron^[a], Laurent Moulin^[b], Sophie Haenn^[b] and Emmanuelle Cambau^[c]), we plan to apply our new qPCR method to surface water samples which were not taken into account during the sampling campaigns carried out in the watershed of the Seine River (see above Thesis subject: sources noted from 1 to 5). This project is supervised in France by Dr. Françoise Lucas and in the USA by Pr Falkinham.

After consultation, we have chosen a section of the Virginia New River between Radford and Peterstown, because it passes through three different kinds of potential NTM sources which could be studied during dry weather. Going downstream, this river section presents the Wastewater Treatment Plant (WWTP) of Fairlawn (source 6), the Radford Army Ammunition site (source 7), and the Jefferson National Forest (source 8), which are potential sources corresponding to human waste, industrial activity and natural presence respectively (Fig. 1A).

- Source 6: Because NTM occurrence seems to be correlated with high organic matter level and biofilm formation [20, 21], treatment processes of WWTP has already been the subject of the sampling campaign in France, however the impact on the River of WWTP effluent has not been studied in France during dry weather.
- Source 7: Industrial activities such as those of Radford Army Ammunition site could impact levels of NTM of the Virginia New River. Indeed, NTM infections of industrial metal-grinding machinists have frequently been associated with high levels of mycobacteria in the water-based metalworking fluids [22].
- Source 8: Based on his experience, Pr. Falkinham suggests quantifying NTM from surface water sampling sites which are known as rich in NTM because of their low pH values and their high level of humic acid [23-27]. Consequently the Jefferson National Forest has been selected in this sampling campaign.

During this three-month stay (date of arrival: 1st May 2010, date of departure date: 31st July 2010); we plan to study another natural source with a low level of pH and a high level of humic acid [23-27]. We plan to study the impact of Lake Drummond (source 9), which is surrounded by the Great Dismal Swamp National Wildlife Refuge, on a canal which reached urban areas of Norfolk and Chesapeake (Fig. 1B). Pr. Falkinham's laboratory has sampled this swamp before and found high numbers of NTM.

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^[b] Eau de Paris - Research department about expertise and control of water from Paris

^[c] CNR mycobacteries - French National Center of mycobacteria and resistant to anti-tuberculous antibiotics

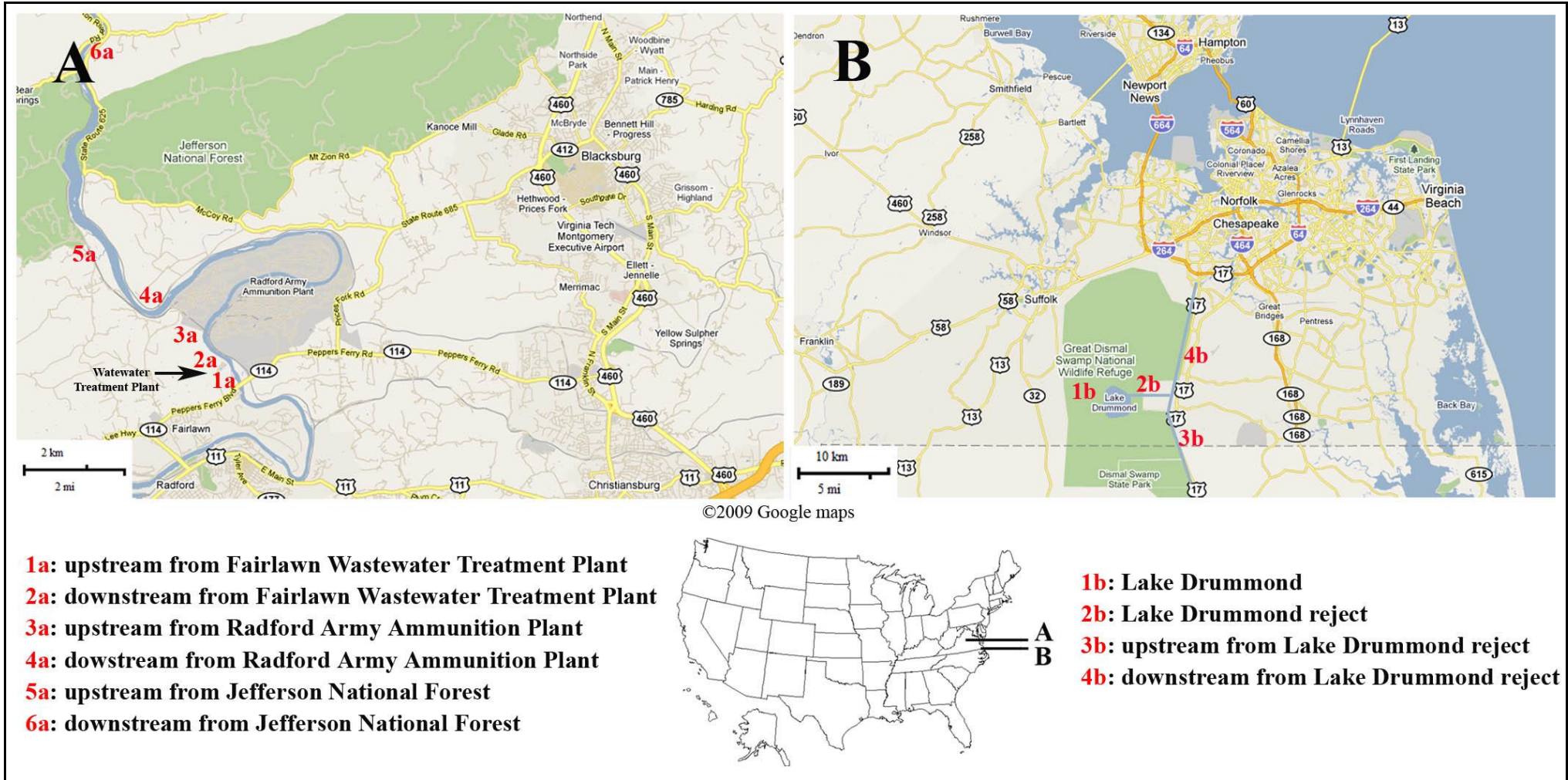


Figure 1: Sampling campaigns of the Virginia New River (A) and the canal of the Great Dismal Swamp National Wildlife Refuge (B), reiterated six times respectively

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