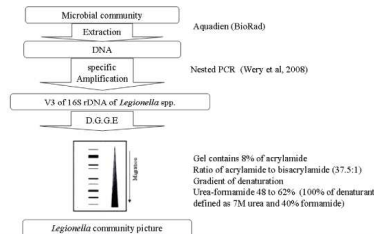


## Introduction

- *Legionella* species is ubiquitous in aquatic environment. Under specific environmental conditions the density of these organisms can increase, causing outbreaks of disease. Various systems (cooling towers, spa, hydrotherapeutic establishment, water supplies) provide ideal growth conditions and then represent a worrying source of exposure for humans. Controlling the *Legionella* risk in these systems is necessary to protect the population.
- In 2006, a French norm (AFNOR XPT 90-471) concerning the "detection and quantification of *Legionella* and/or *Legionella pneumophila* by concentration and gene amplification by using polymerase chain reaction (PCR)" has been published. It establishes in particular the requirements of PCR methods performances. Moreover, detection of *Legionella* spp has been adopted for cooling tower systems prevention.
- Contrary to cultural methods, molecular techniques are able to estimate rapidly the density (Q PCR) and the composition (DGGE) of a microbial community. The development of these techniques allowed new perspectives in the control of *Legionella* community in environmental samples. As example, other genomes than *L. pneumophila* are now much more frequently detected.
- In this work, a specific DGGE method has been developed to monitor *Legionella* community in environmental samples. It consists of a reference profile based on pathogenic *Legionella* strains. The ecology of *Legionella* spp. was investigated in the water networks of two different facilities (hydrotherapeutic establishment and cooling tower)

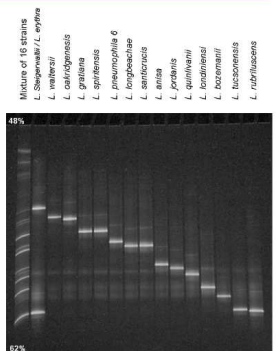
## Methodology

DGGE (Denaturing Gradient Electrophoresis) is a technique used to profile and identify dominant members of the microbial community based on their genetic fingerprint. The separation of DNA product is based on sequence composition that can be assessed by GC% and Tm values.



To validate the *Legionella* analysis, a reference profile has been developed. The migration of this marker allowed to:

- Confirm the analysis quality for the different gel
- Compare several gels
- Pre-identify the bands of sample which co-migrated with bands of marker

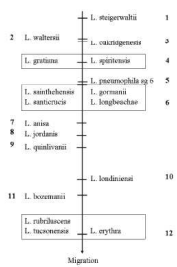


DGGE banding pattern of V3-16S rDNA PCR of *Legionella* strains

## Results

### DGGE system for *Legionella* ecology analysis

#### Migration of *Legionella* strain by DGGE analysis



#### Values of GC% and Tm for *Legionella* species used in this work

<i>Legionella</i>	ATCC	GC (%)	Tm
<i>steigerwaltii</i>	52 551	86	86
<i>walterii</i>	33 061	86.1	86.1
<i>sainthelensis</i>	33 846	82.8	82.8
<i>oakridgensis</i>	700 515	54 359	87.3
<i>gratiana</i>	40 413	54 592	86.3
<i>santicrovici</i>	35 301	54 592	86.3
<i>bozemani</i>	33 217	54 592	86.4
<i>longbeachae</i>	33 462	54 592	86.4
<i>pneumophila</i> sp 6	31 002	54 872	86.6
<i>gormanii</i>	33 297	55 102	87
<i>spiritisensis</i>	35 249	55 102	87
<i>anisus</i> CH47		55 102	87
<i>jordanis</i>	33 623	55 330	87.2
<i>quinlivanii</i>	43 830	56 122	87.5
<i>londiniensis</i>	49 505	56 122	87.3
<i>rubrilucens</i>	35 304	56 633	87.9
<i>erythra</i>	35 303	56 633	88
<i>tucsonensis</i>	49 180	56 633	88

► Two of the eighteen strains showed multiple bands, indicating an intraspecies heterogeneity  
*L. sainthelensis* : 2 bands ; *L. gormanii* : 3 bands

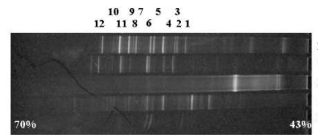
► Some strains co-migrated:

- *gratiana*, *spiritisensis*
- *sainthelensis*, *gormanii*, *santicrovici*, *longbeachae*
- *rubrilucens*, *tucsonensis*, *erythra*

► The migration pattern of *Legionella* strains are not completely correlated with GC% or Tm. For example, 18 strains have been tested with 9 different GC% values and 12 distinct bands have been obtained after DGGE analysis. *L. quinlivanii* and *L. londiniensis* have the same GC% values and 2 bands are obtained.

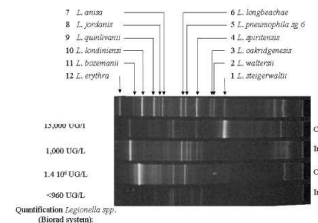
### DGGE analysis of facility samples

#### A/ Based on bacteria community



Marker  
Hydrotherapeutic establishment  
Output  
Input  
Cooling Tower  
Output  
Input

#### B/ Based on *Legionella* community



12,000 UG/L  
1,000 UG/L  
1.4 10<sup>6</sup> UG/L  
~360 UG/L  
Output  
Hydrotherapeutic establishment  
Input  
Cooling Tower  
Output  
Input  
Quantification *Legionella* spp. (Biorad system)

► A set of 12 *Legionella* strains was used to construct a standard marker for DGGE analysis.

► Composition of bacteria and *Legionella* community have been monitored by DGGE in water networks of two different facilities (hydrotherapeutic establishment and cooling tower)

► One to eleven bands are counted bacteria community profile. Community structures change during the facility processes.

► Three to six DGGE dominant bands were obtained for the both facility samples in *Legionella* analysis. The composition of *Legionella* communities varied during the process. No correlation between *Legionella* density and diversity has been established.

► For cooling tower, only 1 band are present in both samples 3 bands present in input water disappeared and 3 appeared. One band of the output sample co-migrated with *L. pneumophila*. This band represent 20% of the PCR product intensity, the band located under the *L. bozemani*'s band represent 45%.

► On hydrotherapeutic establishment sample profiles two bands are common, four bands disappeared and two appeared in the water network. One band co-migrate with *L. spiritensis* in both samples and 1 band co-migrate with *L. steigerwaltii* in output sample. For each profile, one band dominated. However it was not the same. The most important band in input profile (28% of the PCR product intensity) represented 16% on the output sample pattern. A not detected band on input profile dominated on output profile (48% of the PCR product intensity).

## Conclusion

- A DGGE method has been developed to study the *Legionella* community including a reference profile with 12 pathogenic strains.
- The analysis of structure of the *Legionella* community in two facilities shows the modification of the V3 pattern during process.
- Marker can be improved by the addition of some other pathogenic *Legionella* strains as *L. dumoffii*, *L. micdadei* more often retrieve in clinical sample.
- This method can be applied to monitor the colonization of water network after a biocide treatment or to evaluate the pathogenic risk correlated with a high *Legionella* spp. density.