Abstract

This study will perform the comparison between different pre-treatment and different composition of isolation medium for the isolation rate of *Legionella pnemophila*. Results indicated that there are no differences regarding to the appearance of *L. pneumophila* colonies in BCYEα agar w/L-cysteine, BCYEα with L-cysteine/BCP+BTB and BCYEα agar w/L-cysteine/PAV plates. The color of colonies in the above-mentioned media are bluish-white, light green and light bluish-white needle-shaped colonies in order. Traditionally, the water specimen was filtered through a filter membrane, then mixed with tri-angels glass-rod, then inoculated to isolation media and incubated at a suitable conditions. To investigate the efficiency of removing the bacteria from filter membrane, thus affecting the isolation rate of *L. pneumophila*, we firstly employed the glass rod to mix the membrane contents, and then cut into several pieces for further mixing. After that, the sample was treated with ultra-sonifier for 15 minutes. The results of ultrasonifier treated and un-treated sample were compared. Results showed there are no difference among the number of isolated bacteria (the latter treatment had 1-1.2 colonies more than the former). We further applied both treatment methods to 100 environmental water specimen, and found that the isolation rate for the ultra-sonifier un-treatment was 18.3%, and the treatment 20.6%. We also employed 100 clinical sputum specimens to isolate *L. pneumophila*. After acid-digestion, the sputum was inoculated into BCYEα agar w/L-cysteine/CCVC, BCYEα agar w/L-cysteine/BCP/BTB and BCYEα agar with L-cysteine/PAVplates. Results indicated there was no *L. pneumophila* was found. Regarding to PCR rapid identification for *L. pneumophila*, we used tLeg primer for the first PCR cycle, a 633 bp DNA fragment appeared, and for the second cycle, 410 bp DNA fragment appeared. However, using mLeg primer, a 200 bp DNA fragment appeared. The sensitivity test for PCR primer's tests, we found (i) tLeg primer will detect the amplified 410 bp DNA fragment from $l0^8$ to 10^1 CFU of L. pneumophila; (ii) mLeg primer can also detect the amplified 200 bp DNA fragment when the bacterial mnumber was more than 10^2 CFU. The detection rate was 59% when applied the tLeg primer to detect 100 environmental water specimens. Whereas, the detection rate was 34% for the mLeg primer. Furthermore, the detection rate was only 6% for mLeg primer to 100 clinical sputum specimens. However, there was no *L. pneumophila* isolated from the specimens mentioned in the above experiments. Therefore, we can not differentiate whether the higher detection sensitivity or false positive occurred for the PCR methods. For the comparison between the environmental isolates from our laboratory and clinical isolates from CDC, Taiwan, we employed the multilocus sequence typing , MLST and based on the gene sequences of *flaA*, *pile*, *asd*, *mip*, *momps* and *proA* published in the EWGL I website to analyze the 39 environmental strains, we found 23 strains for the gene codes of 1,4,3,1,1,1, four strains for the gene codes of 11,14,16,1,15,13, and 3 strains for gene codes of 11,14,16,16,15,13. There were only three new gene codes sequence appeared among those 39 strains. However, regarding to the 15 environmental strains, we found 4 strains for the gene codes of 1,4,3,1,1,1, two strains for the gene codes of 3,4,1,1,14,9, and all others with different types of gene codes. There were 4 strains with new gene codes sequence appeared among those 15 clinical strains. From the above, we can conclude that there will be more stable for gene codes in

the environment strains and the gene variation is not so great as clinical strains. We further proceed the pulse field gel electrophoresis (PFGE) with Sfi restriction enzyme. This enzyme will cut the genome of 21 clinical strains and 27 of environmental strains into several fragments with different sizes. Among them the genome of 14 clinical strains and 26 environmental strains were cut into 9~19 DNA fragments with different sizes. For the 4 strains of *L. dumiffii*, its genome will be cut into 1~7 DNA fragment with different sizes. This will be act as control. Based on the PFGE map, we can classify the DNA fragment into four groups. Group 1 contained most of the environmental strains in which 12~16 DNA fragments were cut. There will be approximately 70% similarity with the standard strains of L. pneumphila. Group 2 contain most of clinical strains in which 9~19 DNA fragments were cut. There will be approximately 40% similarity with standard strains of the organism in this group. Most of the Group 3 was environmental strains in which 12~15 DNA fragments were cut. There will be 64% similarity. Group 4 were L. dumoffii in which 1~7 DNA fragments were cut. The similarity of this organism with standard strains was only 20%. Based on the PFGE map, the variation of DNA fragments among environmental strains was not great. The most number of DNA fragments cut was only 4 fragments differences. However, there were 10 DNA fragment differences among clinical strains. This demonstrates that the variation of DNA fragments in clinical strains was greater than those of environmental strains. The results were similar those obtained from MLST typing method. We concluded that ultra-sonification will increase slightly the isolation rate of *L. pneumophila* from environmental sources. Whether PCR technology will promote the detection rate of *L. pneumophila* or create false positive rate still unjustified. Combined the MLST and PFGE typing technologists will be no doubt to differentiate the types of L. pneumophila, assist in tracing the infection source(s) and help to prevent the spread of this organism.

Keyword: *Legionella pneumophila*, ultra-sonification treatment, PCR, MLST, PFGE