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Bacterial Regrowth in Water Distribution Systems and Its Relationship to the Water Quality: Case Study of Two Distribution Systems in Korea

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Abstract This study was done to observe the occurrence of heterotrophic bacteria in terms of free chlorine residuals in two different water distribution systems, which belongs to both K and Y water treatment plant of S city in Korea. The data analyzed in the distribution systems show that the free chlorine residuals decreased from 0.10 to 0.56 mg/l for K, and 0.51 to 0.78 mg/l for Y. The decay of free chlorine is clearly higher in both March and August than in January. The HPC in the distribution systems are ranged from 0 to 40 cfu/ml for K, 0 to 270 cfu/ml for Y, on R_2A medium. In particular, its level is relatively high at the consumer's ground storage tanks, taps, and the point-of-end area of Y. The predominant genera that were studied in the distribution systems were *Acinetobacter*, *Sphingomonas* (branch of *Pseudomonas*), *Micrococcus*, *Bacillus*, *Staphylococcus*. The diversity of heterotrophic bacteria increases in the end-point area. Most of them are either encapsulated cells or of Gram-positve cocci. In conclusion, the point-of-end area in distribution systems shows the longer flow distance from the water treatment plants, along with a greater diversity and a higher level of heterotrophic bacteria, due to the significant decay of free chlorine residuals.

Key words: Chlorine residuals, distribution systems, heterotrophic bacteria

It is not possible to supply safe tap water in the case of a problem of pipe corrosion and contamination by microorganisms, while the purified water flows through the distribution systems. Therefore, the systematic control of any distribution system includes not only the process of water treatment and quality control of source water but the process from the water treatment plant to the consumer's tap water to supply an excellent quality of water [8]. The contamination source in distribution systems causes the water quality to become much worse. Moreover, its main inflow is located in the open finished water reservoir and damaged bad-junction pipe [4]. According to many reports, the excessive bacteria shown in tap water were not detected at the water treatment plant, and the reason for this is due to the regrowth of bacteria [2, 5, 8, 10].

The sudden increase in the number of heterotrophic organisms, which come from tap water through the distribution systems through the water treatment plants, is called 'regrowth' or 'after-growth' of microorganism. Brazos and O'Conner [3] define that the regrowth is a recovery process of the damaged microorganism by disinfectants in water supply pipes, and after-growth is a proliferation of settled microorganism on the surface of the pipe. The regrowth of microorganisms occurred by specific favorable growth conditions in distribution systems such as, direct inflow from the outside, and forming biofilms in the inner wall of the water supply pipe which is not inactivated by disinfection processes [4, 11].

It is common to denote that the disinfection of the water treatment process includes inactivation of microorganisms that prevents the waterborne diseases, and maintenance of the concentrations of free chlorine residuals in distribution systems to control local water pollution and to minimize the regrowth of microorganisms. In addition, Korean distribution system including a lot of storage tanks, valves, reservoir, and pressure plants cause a regrowth of microorganisms due to the difficulties in maintaining free chlorine residuals. The stationary water area in a large water reservoir has the by structural defects, and corrosions and coating materials cause internal pollution as well.

There are some opportunistic pathogens of heterotrophic bacteria in distribution systems, which are known to causes waterborne diseases [12, 13]. Mostly, they strongly affect the weak immunity groups such as infants, hospitalized patients, and the elderly. They also causes secondary

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infection. However, not enough reports have been presented in regard to the distribution of microorganisms and growth conditions or even its characteristics in distribution systems. They are needed in order to define the method of optimum controls and prevent the regrowth of microorganisms.

The main focus of this study was to provide a method of stable control of the quality of water in distribution systems of tap water by means of evaluating of heterotrophic bacteria in terms of free chlorine residuals for the K and Y water distribution systems, which belong to S city in Korea. Another goal of this study was to investigate the predominant heterotrophic bacteria and the distribution of microorganisms by elements of facilities in the above mentioned distribution systems.

MATERIALS AND METHODS

Structural Characteristics of Distribution Systems

The distribution systems of K water treatments plants consist of finished water, reservoir, and tap water or finished water, reservoir, water storage tank, and tap water, while those of Y consist of finished water, reservoir, small reservoir, and tap water or finished water, reservoir, small reservoir, water storage tank, and tap water.

The characteristics of both K and Y distribution systems are shown in Table 1. The total capacity level of distribution system K and Y is 10,000, and 50,000 tons, respectively, therefore the total capacity level of the distribution system Y is 5-fold greater than that of K. The retention time of the water reservoir and small water reservoir of the Y distribution system are 496 and 743 min, respectively, and the retention time of water reservoir of the K distribution system is 166 min. That is, the retention time of Y is 3-fold longer than that of K, and it is about 7.5-fold longer than that of small reservoir. The total lengths of pipes K and Y distribution system are 14.2 and 14.5 km, respectively, and the water supply pipes are 3.7 and 7.0 km respectively. The total length of both distribution systems is nearly the same as shown above, but the total water supply pipe of Y is about 2-fold longer than K.

Sample Collection

The samples of this study were collected from water reservoir, underground water reservoir, water tank, and the tap as shown in Table 1 for the K and Y distribution systems and these systems differ in each structure. The sample bottle includes 3% of the sodium thiosulfate ($Na_2S_2O_3$) in order to neutralize chlorine residuals, and it was sterilized by high pressure steam. In order to prevent the infection from the outside, the sample was prepared by using flames and sterilized without filters, and rubber hose, and the internal pipe deposits were drained for 2 to 3 min.

Analytical Procedure

The tests of free chlorine residuals, pH, temperature, and turbidity were done at the site, and DO and NH_3-N were transferred to the laboratory and tested. Free chlorine levels were determined by the DPD colorimetric method with a chemical chlorine kit (Hach chemical) [1]. The quality of water in this test is shown in Table 2.

The heterotrophic bacteria were inoculated by the spread plate method on R_2A media [1]. The sterilized media was distributed to the 147 mm Petri-dish and dried for one day. One ml of sample was inoculated on the media, and spread by a glass stick. The inoculated sample was cultivated at 21±1°C for 723 h, and the colonies were counted. In order to identify the formed colonies of microorganism, the single colony on R_2A media was treated by a quadrant streak with a flame sterilized loop, and cultivated at 21±1°C for 723 h. The colony of microorganisms was inoculated in tryptic soy broth agar and R_2A broth, and cultivated at 28±1°C for 24 h. The pure cultured microbe was treated by a quadrant streak in TSBA (Tryptic Soy Broth Agar), and was cultivated at 28±1°C for 24±2 h. The sample of 2 µl fatty acid was

Table 1. Sampling sites in distribution systems.

	Sites	Distance from water works plant (km)	Retention time (min)	Characteristics
K distribution system	Finished water			K water works plant
	Reservoir	10.5	166	10,000 ton
	Tap water without storage tank	14.0	203	
	Storage tank	14.1	143	1,100 ton
	Tap water with storage tank	14.2	510	
Y distribution system	Finished water			Y water works plant
	Reservoir	7.5	496	50,000 ton
	Small Reservoir	14.2	743	500 ton
	Tap water without storage tank	14.4	748	
	Storage tank	14.5	839	2 ton
	Tap water with storage tank	14.5	840	

Table 2. Water quality in distribution systems.

Item	January		March		August	
	K distribution system	Y distribution system	K distribution system	Y distribution system	K distribution system	Y distribution system
Temp. (℃)	2.9	2.7	8.6	9.2	26.4	25.9
pH	7.20	7.09	7.33	7.33	7.13	7.18
Turbidity (NTU)	0.16	0.14	0.28	0.19	0.23	0.26
DO (mg/l)	14.49	14.60	11.81	12.01	8.35	8.42
Cl^- (mg/l)	9.2	13.7	20.0	25.2	10.5	11.4
NH_3-N (mg/l)	0.02	0.05	0	0	0.03	0.03
TOC (mg/l)	1.53	1.59	2.35	2.82	1.62	1.56
Fe (mg/l)	0.02	0.01	0.04	0.02	0.05	0.03

analyzed by the GC/FID (HP 6890 Series, Hewlett Packard) with MIDI (Microbial Identification Systems), and the results were compared with the standard library. The operation conditions for the gas chromatography is shown in Table 3. Using MIDI, the heterotrophic bacteria were Gram-stained and then observed under the 1,000th scale of the optical microscope for detecting the types of bacteria and Gram positivity or negative. The observed types and structures were analyzed by the image analyzer.

RESULTS AND DISCUSSION

Heterotrophic Bacteria

The seasonal variations of heterotrophic bacteria for the target area of K and Y distribution systems are shown in Fig. 1 and Fig. 2. The variations of K distribution systems in January, March, and August were 0-4, 1-43 and 0-40 cfu/ml, respectively, while those of Y distribution systems in the above mentioned months were 0-270, 1-86, and 0-12 cfu/ml, respectively.

Therefore, the density of heterotrophic bacteria of the Y distribution systems is higher than that of the K distribution systems during each season, and the distribution of heterotrophic bacteria is contrary to the density variation of free chlorine residuals in relation to the temperature condition for both cases. This increase is most likely due to relatively high concentrations of nutrition components, such as organic matters and nitrogen, etc., in water of the Y distribution system.

The maximum values of heterotrophic bacteria of the K and Y distribution systems are observed in March and, January, respectively. The relationship between heterotrophic bacteria and organic carbon is presented at the point of the maximum values of heterotrophic bacteria. In the K distribution system, the DOC of storage tank in March and that of tap water in January were 1.95 and 2.38 mg/l, respectively, whereas that of storage tank in January and that of tap water in August were 0.75 and 1.40 mg/l, respectively. These results suggest that the biological treatment or oxidation of DOC control in water treatment affects heterotrophic bacteria in the distribution system. Therefore, it seems that the lower the DOC levels become, the lower presence of heterotrophic bacteria are in the distribution system.

Table 3. Conditions of gas chromatography for microbial identification.

Item	Condition
Detector	FID
Initial temp. (℃)	170
Final temp. (℃)	310
Detector temp. (℃)	300
Injection port temp. (℃)	250
Column flow rate (℃)	0.3
Split ratio	100
Hydrogen gas (ml/min)	30
Air gas (ml/min)	400
Nitrogen gas (ml/min)	30

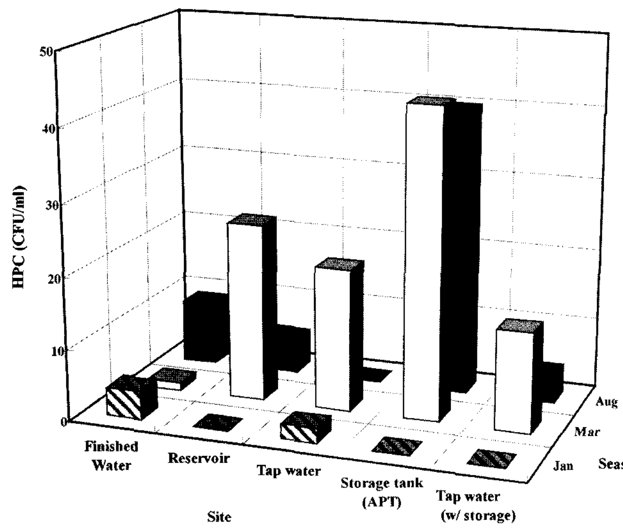

Fig. 1. HPC in K distribution systems.
January (▧), March (□), and August (■).

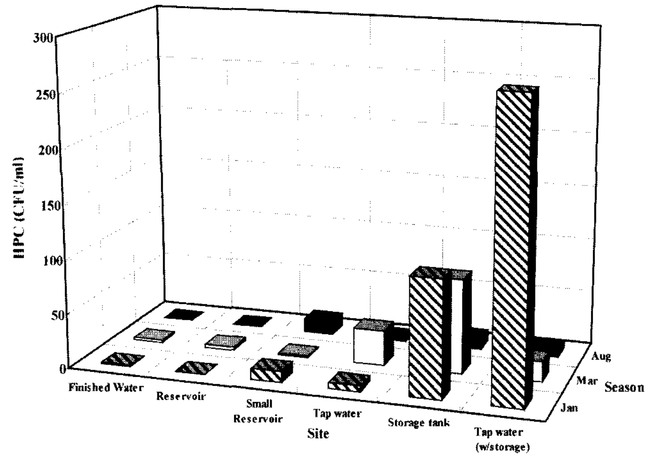

Fig. 2. HPC in Y distribution systems.
January (▧), March (□), and August (■).

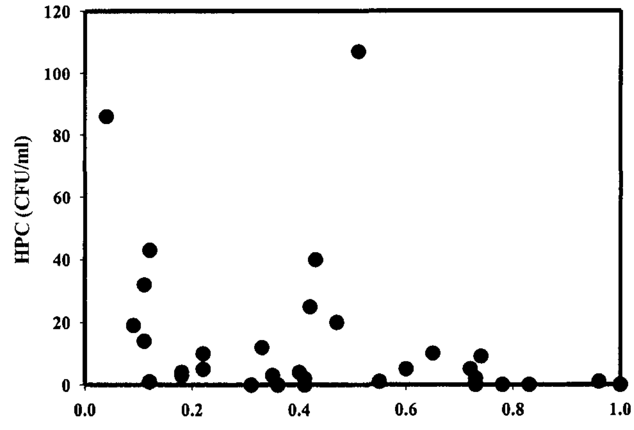

Fig. 3. Relationship between free chlorine residuals and HPC.
Based on the analysis of 31 samples from the K and Y distribution systems.

The average density of heterotrophic bacteria for the facilities is as follows. The average density of heterotrophic bacteria in finished water, reservoir, tap water without storage tank, storage tank, and tap water passed through storage tanks of the K distribution systems are 5, 10, 7, 28, and 6 cfu/ml, respectively, and that of heterotrophic bacteria for finished water, reservoir, tap water without storage tank, storage tank, and tap water passed through storage tanks are 1, 1, 8, 13, 68, and 98 cfu/ml, respectively. According to the results, it can be said that the increase in the density of heterotrophic bacteria is caused by the regrowth of microorganisms. In the case of the Y distribution systems, it clearly shows the increase in the density of heterotrophic bacteria by approaching the point-of-end area. The heterotrophic bacteria of finished water for the storage tank and tap water that passed through storage tanks were 107, and 270 cfu/ml, respectively. In fact, they were different from the 1 cfu/ml of finished water. The density of heterotrophic bacteria is increased by approaching the point-of-end area in March and August.

Y distribution systems show that there is a high decay rate of free chlorine residuals in the reservoir, and they are decreasing near the point-of-end area, in which the increase in heterotrophic bacteria is more apparent than those shown in the K distribution systems. In the case of the Y distribution systems in January and August, the density of heterotrophic bacteria was sharply increased in spite of the equivalent chlorine residuals (0.2 mg/l).

The Relationshop between Free Chlorine Residuals and Heterotrophic Bacteria

The relationship between the free chlorine residuals and the heterotrophic bacteria in the K and Y distribution systems is shown in Fig. 3. According to Reilly and Kippin [15] the number of heterotrophic bacteria apparently decreases with increasing free chlorine residuals. However, this experiment does not clearly show the same results for the relationship between the free chlorine residuals and the heterotrophic bacteria. In some facilities, the number of heterotrophic bacteria increases even though the constant level of free chlorine residuals are maintained. In fact, it is estimated by the influence of water quality, flows, and biofilms of the inside of pipe besides the free chlorine residuals.

The Identification of Heterotrophic Bacteria

The heterotrophic bacteria of the target area in distribution systems by change of seasons are shown in Table 4. *Sphingomonas*, *Micrococcus*, *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Staphylococcus*, *Acidovorax*, *Gordona*, *Corynebacterium*, *Arthrobacter*, *Cellulomonas*, *Agrobacterium*, *Deinococcus*, *Methylobacterium*, *Hydrogenophaga*, *Bradyrhizobium*, *Aureobacterium*, *Curtobacterium*, and *Variovorax* were detected and identified as heterotrophic bacteria with MIDI after the extraction of fatty acid in the cell. *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Micrococcus*, *Streptococcus*, *Alkaligens*, *Kingella*, and *Bordetella* were previously extracted by Park *et al.* [12] in the Han river as the heterotrophic bacteria. *Sphingomonas* and *Acinetobacter* were identified in the distribution systems and they are known to be resistant to disinfectant [8]. These microorganisms are not inactivated by adhesion onto surfaces, encapsulation to the outside of cell, and protection effect by coagulants, etc, and survived in distribution systems [16].

The identified heterotrophic bacteria in the K an Y distribution systems are the genus 11 and 14. *Micrococcus* and *Pseudomonas* were shown in finished water of the K distribution systems in January, and *Pseudomonas* and *Acidovorax*, which were not previously detected in the reservoir and tap water, and they were also detected in the reservoir and tap water passed through the reservoir. In particular, *Pseudomonas* was detected in

Table 4. Identified heterotrophic bacteria in sites.

	Sites	January	March	August
K distribution systems	Finished water	*Micrococcus (2)* *Pseudomonas (1)*	-	*Cellulomonas (1)* *Deinococcus (1)*
	Reservoir	-	*Spingomonas (4)* *Bacillus (1)*	-
	Tap water	-	*Micrococcus (1)* *Acidovorax (1)*	-
	Storage tank	*Pseudomonas (1)* *Acidovorax (1)*	*Spingomonas (5)* *Bacillus (1)* *Hydrogenphaga (1)*	*Spingomonas (1)* *Arthrobacter (1)* *Aureobacterium (1)*
	Tap water (with storage tank)	*Pseudomonas (1)*	*Staphylocuccus (1)* *Spingomonas (1)*	*Micrococcus(1)*
Y distribution systems	Finished water	-	-	-
	Reservoir	-	-	-
	Small reservoir	*Staphylocuccus (1)* *Acinetobacter (1)*	-	*Agrobacterium (2)* *Curtobacterium (1)*
	Tap water	-	*Cellulomonas (1)*	-
	Storage tank	*Acinetobacter (1)* *Pseudomonas (1)*	*Bacillus (3)*	*Variovorax (3)*
	Tap water (with storage tank)	*Micrococcus (1)* *Bacillus (1)* *Corynebacterium (2)* *Arthrobacter (1)*	*Micrococcus (1)* *Bacillus (1)* *Methylobacterium (1)* *Gordona (1)* *Brandyrhizobium (1)*	-

both finished water and tap water passed through the reservoir. *Spingomonas*, *Acidovorax*, *Hydrogenophaga*, and *Staphylococcus*, which were not previously detected in finished water in March, were detected in the reservoir, finished water without the storage tank, reservoir and its tap water. *Cellomonas* and *Deinococcus* were detected in finished water, whereas *Arthrobacter*, *Sphingomonas*, and *Aureobacterium* were detected in the reservoir.

On the other hand, the heterotrophic bacteria were not detected in finished water and the reservoir of the Y distribution systems, but they were detected in the area after the small reservoir. In January, *Staphylococcus* and *Acinetobacter* were detected in a small reservoir, but they were not detected in finished water. Moreover, *Acinetobacter* and *Pseudomonas* were identified in the storage tank, and *Micrococcus*, *Bacillus*, *Corynebacterium*, and *Arthrobacter* are in tap water. In particular, *Acinetobacter* was detected in the small reservoir, water tank, and tap water. In March, *Micrococcus*, *Bacillus*, *Gordona*, *Methylobacterium*, and *Bradyrhizobium*, which are not previously detected in finished water, reservoir, and small reservoir, were detected in finished water and tap water passed through the water tank. In August, *Agrobacterium* and *Curtobacterium* were detected in a small reservoir, and *Variovorax* was detected in the water storage tank. Thease results show that the number of identified heterotrophic bacteria was smaller than that of January and March.

Micrococcus, *Pseudomonas*, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Arthrobacter*, and *Cellulomonas* were all present in the two distribution systems as the heterotrophic bacteria.

Microscopic Examination of Heterotrophic Bacteria

The optical microscopic structure of the heterotrophic bacteria are shown in Fig. 4 and Fig. 5. The heterotrophic bacteria have suitable structure with an ability to grow in distribution systems, and contained 50% of the Gram-positive bacteria which have a strong tolerance level to the disinfectant. In particular, the bacteria in finished water and the reservoir were almost in the form of bacillus, but

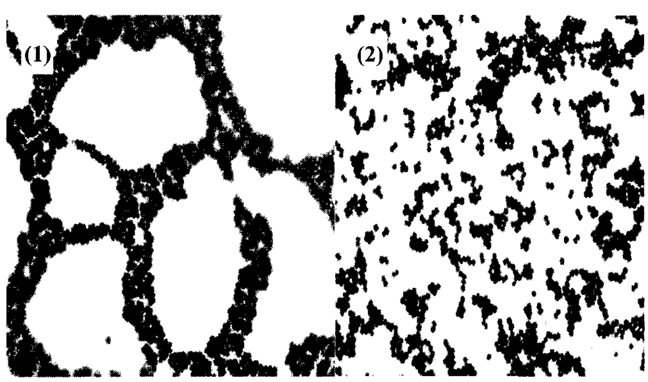

Fig. 4. Photograph of identified coccus.
(1) *Staphylocuccus*; (2) *Micrococcus*.

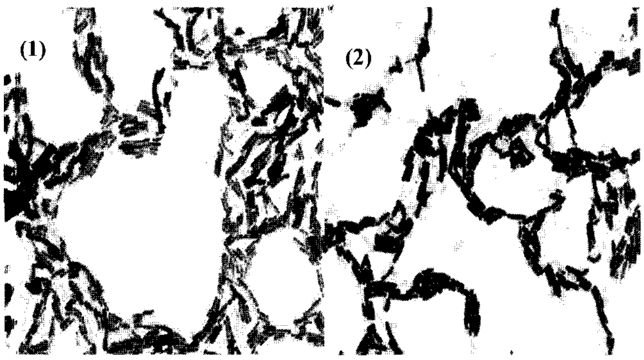

Fig. 5. Photograph of identified bacteria containing capsule.
(1) *Acinetobacter*; (2) *Bacillus*.

Staphylococcus and *Micrococcus* were largely detected near the point-of-end area, and as for the micrococcus, it has a strong tolerance level to disinfectants.

Acinetobacter is a Gram-negative bacillus capsule, and this capsule seems to act as a protection membrane although *Bacillus* is a Gram-positive bacterium with a capsule structure. On the other hand, *Staphylococcus* is the Gram-positive, whereas *Sphingomonas* and *Flavobacterium* are the Gram-negative bacteria.

Almost all of the heterotrophic bacteria in the distribution systems of tap water have the capsule structure. In the case of the Gram-positive bacteria without capsule, they have the distinct layer of peptidoglycan which provides strong tolerance level.

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