

水資源と水環境に関する研究

Researches on Water Resources and Environments



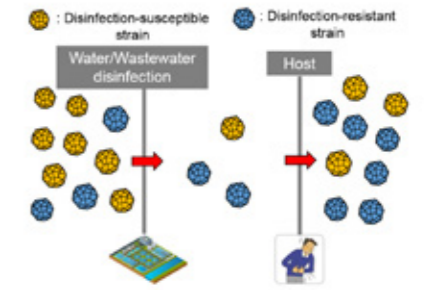
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水資源システム学分野では、世界の水問題を解決することを目指し、以下の研究に取り組んでいる。

- 1) 気候変動と土地利用変化が自然環境に与える影響評価手法の開発
- 2) 地球温暖化と林業の衰退など森林の荒廃による流木発生メカニズムの解明
- 3) 下水処理場における温室効果ガス発生と最適な浄化機能の解析
- 4) 嫌気性消化槽にかかる排水処理と微生物群集動態の解明
- 5) 水インフラへの病原ウイルスの遺伝的適応評価

Our research topics in 2018 were

- 1) The development of evaluation approaches for the effect of climate change and land utilization change on natural environments
- 2) Mechanisms of flood wood generation caused by global warming and forest industry declination
- 3) The optimization of wastewater treatment and greenhouse gas emission
- 4) Bacterial community structure in wastewater treatment and anaerobic digestion reactors
- 5) Water infrastructure and enteric virus evolution

胃腸炎ウイルスの進化と水インフラ

国連の持続可能な開発目標の6番目 (Sustainable Development Goal 6: SDG6) として、2030年までに世界中の人々に安全な飲料水と安全なトイレを普及することが謳われている。このSDG6を達成するためには、導入・運転コストが低く、維持管理が容易な水インフラを新たに創出することが必要不可欠であるが、同時に、新しい水インフラを用いる人々の水系感染症リスクが本当に守られているのかについても慎重に評価することが求められる。しかしながら、新しい水インフラ導入下における微生物曝露に関する情報は限られているので、水系感染症を引き起こす病原体がどのように新しい水インフラに適応するかを予測することは容易ではない。特に、ある種のRNAウイルス (世界中で感染症リスクをもたらしているノロウイルスやロタウイルスを含む) は、高い変異速度を駆動力とした高い適応能力を有していることから、特に注意が必要である。

本研究は、ヒトノロウイルスの代替としてのマウスノロウイルス (murine norovirus: MNV) が、消毒剤として世界中で広く用いら

Water Infrastructure and Enteric Virus Evolution

The United Nations' Sustainable Development Goal 6 (SDG6) is to supply all people on the globe with improved drinking water and sanitation systems by 2030. The development of sustainable water infrastructures that are easily installable and maneuverable is essential to achieve the SDG6, but at the same time, it must be carefully determined whether the newly installed water infrastructure's users are really protected from microbial risks. However, it is not a simple task to determine how to adapt water pathogens to the human community using a brand-new water infrastructure because there must be no accumulated datasets about water pathogens' genetic responses to the water infrastructure in the early years of its installation. The precise understanding of water-related pathogens' adaptation mechanisms is very important to protect the health of the newly developed water infrastructures' users. Particularly, some types of RNA viruses require special attention because they have a relatively higher adaptation capability among water-related pathogens due to their higher mutation rate.

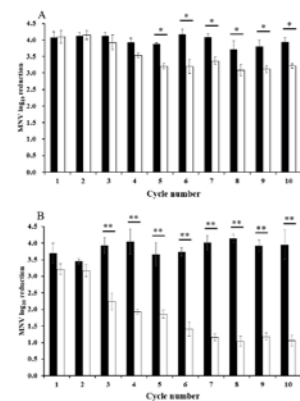


Fig.1 Log₁₀ reduction of chlorine-treated population (white bar) and control population (black bar) during the 1st trial (A) and 2nd trial (B) of cycle experiments. *, statistically different at $\alpha < 0.05$ (Wilcoxon signed-rank test); **, statistically different at $\alpha < 0.01$ (Wilcoxon signed-rank test)

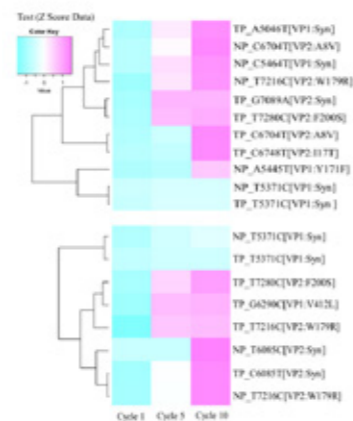


Fig.2 Changes in nucleotides in the 1st, 5th, and 10th cycles in the chlorine-treated and control populations in the first (upper) and second trials (lower).

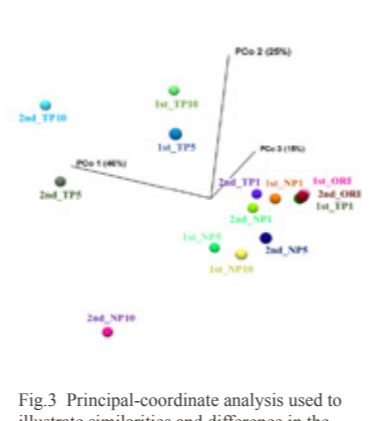


Fig.3 Principal-coordinate analysis used to illustrate similarities and difference in the substitution rate using single-nucleotide polymorphism composition as distance matrices of two populations in the 1st, 5th, and 10th cycles of chlorine-treated (TP) and control populations (NP) in the 1st and 2nd trials

れている遊離塩素への繰返し曝露により耐性を得ることを示したものである。遊離塩素への繰返し曝露により、遊離塩素耐性は徐々に増加し、希釈のみを施した対照集団と比較して曝露集団は10倍から100倍の耐性を示した (Fig.1)。遊離塩素への繰返し実験は独立に二回行ったが、二回ともに遊離塩素耐性集団が得られた。

MNVに遊離塩素耐性をもたらした原因を探るため、ウイルス粒子の外殻タンパク質遺伝子の配列を次世代シーケンス技術により解析した。その結果、2つの非同義置換および1つの同義置換が遊離塩素曝露実験で得られた2つの集団において共通して観察された (Fig.2)。2つの非同義置換のうちの1つは7280番目の遺伝子変異によるフェニルアラニンからセリンへの変異であり、このアミノ酸変異の存在割合は遊離塩素曝露集団において当初の1%から10回目の曝露後に99%まで増加した。

次に、MNV集団間の遺伝的距離を評価するために Principal-Coordinate Analysis を行った (Fig.3)。その結果、遊離塩素曝露1回 (対照集団では希釈一培養サイクル1回目) 後は遊離塩素曝露前の集団に近い位置でクラスターを形成したのに対し、遊離塩素曝露5回及び10回後の集団は、遊離塩素曝露前集団及び対照集団とは異なる位置でクラスターを形成した。これらの結果から、遊離塩素曝露がMNV集団に対して選択の効果をもたらしていると言える。

Fig.2に示したアミノ酸変異が遊離塩素耐性をもたらしているか否かを確認するために、遊離塩素曝露集団及び対照集団から6株ずつのMNVを精製し、それらの遊離塩素感受性を評価した。遊離塩素曝露集団から得られた6株全てが当該変異を有しており、対照集団に由来する株は有していないことを確認した。遊離塩素感受性試験の結果、遊離塩素曝露集団から得られた株は対照集団から得られた株よりも有意に遊離塩素耐性が高いこと確認された (Fig.4)。

本研究の見解により、進化により胃腸炎ウイルスが示しうる最大の遊離塩素耐性を知ることが可能であることから、水の消毒の立場から考えれば、安全な水を創出するうえで必要最低限の消毒剤投入量を算出することが可能となる。このことは、トリハロメタンなどの消毒副生成物の発生を最小限に抑えた環境にやさしい消毒法の確立につながるものと考えられる。

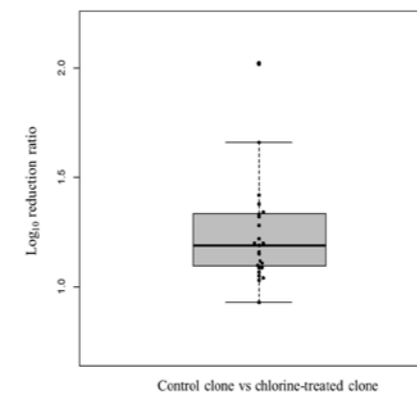


Fig.4 Log₁₀ reduction ratio of clones from the control population to those from the chlorine-treated population

In this study, we proved that a population of murine norovirus (MNV), a surrogate of human noroviruses, can be resistant to free chlorine after repeated exposure to a disinfectant. Free chlorine is the most widely used disinfectant around the world. Populations' susceptibility to free chlorine gradually decreased compared to the control populations after repeated exposure (Fig.1). A next-generation sequencing (NGS) technique was used to investigate the genomic basis for MNV's lower chlorine susceptibility. PCR products of capsid genes were obtained from the MNV populations (chlorine-treated and control populations from the first and second trials) in the first, fifth, and tenth cycles and analyzed by NGS. Both trials resulted in two nonsynonymous and one synonymous mutations. One of the two shared nonsynonymous mutations was located at nt7280, in which phenylalanine was substituted for serine, and appeared only in the chlorine-treated populations from both trials. The relative quantity of this mutation gradually increased from 1%, 90%, and 99% through cycles 1, 5, and 10, respectively (Fig.2). The principal-coordinate analysis was employed to examine the differences in the substitution rates among the populations using single nucleotide polymorphisms as distance matrices from the original population (before free-chlorine exposure) and the chlorine-treated and control populations in the first, fifth, and tenth cycles (Fig.3). After the first exposure, the chlorine-treated (TP) and control populations (NP) were clustered together close to the original populations. Meanwhile, the chlorine-treated populations in the fifth (TP5) and tenth cycles (TP10) were clustered separately from the original (ORI) and control populations (NP). These results indicate the presence of a selection pressure on MNV populations after free-chlorine exposure. To determine whether the mutations found in the capsid region are associated with the lower susceptibility to free chlorine, a chlorine sensitivity test was carried out. Six plaque-purified clones were acquired from each population in the tenth trial. All clones from the chlorine-treated population had the nonsynonymous mutation at nt7280. The plaque-purified clones' free-chlorine sensitivity was evaluated to determine whether the nonsynonymous mutation (T7280C[VP2:F200S]) would affect MNV's susceptibility to free chlorine. As a result, the plaque-purified clones from the chlorine-treated population had significantly lower susceptibility to free chlorine than those from control populations (Fig.4).

In conclusion, we proved that the free-chlorine treatment can work as a selection pressure on MNV's evolution, and a single mutation in the capsid protein may change its susceptibility to free chlorine. Based on this knowledge, it is possible to expect the highest resistance of viral mutants to the disinfection processes. In the other words, we can identify the minimum disinfectant doses to reduce pathogenic viruses significantly, leading to the establishment of environmentally friendly water disinfection practices because the excess usage of disinfectants can be prevented and the formation of toxic disinfection byproducts, such as trihalomethane, is minimized.