IMPROVEMENT OF WATER QUALITY IN KUMAMOTO USING LACTIC ACID BACTERIA

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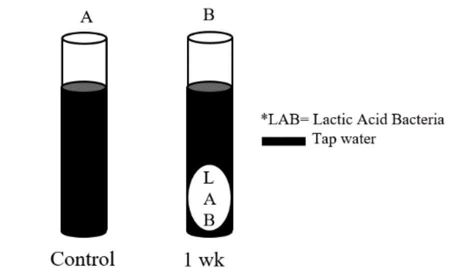
1. INTRODUCTION
Kumamoto is located on the Kyushu island in the southwest region of Japan and is renowned for its pristine groundwater. However, recent studies have raised the concern of increasing concentration of nitrate-nitrogen (NO_3-N) in Kumamoto's water, possibly due to intensive agriculture (Hirohata et al, 1999; Kumazawa, 2002; Tomiie et el, 2011). Use of nitrogen-based fertilizers in agricultural work and the excess NO_3-N from cattle manure can easily percolate to the groundwater if not taken up by plant materials (WHO, 2011).

2. OBJECTIVES
Lactic acid bacteria (LAB) are an order of gram-positive, acid-tolerant bacteria that are generally found in milk products (e.g. yogurt). They produce lactic acid as the major metabolic end-product of carbohydrate fermentation (Sonomoto et al, 2011). The cellular composition of some LAB enables them to absorb some metal ions naturally (Bhakta et al, 2012; Chang et al, 2012; Yi et al, 2017). They have especially shown affinity to absorbing heavy metal ions including cadmium, cesium and strontium (Kinoshita et al, 2013, 2015 and 2016). Selective LAB strains can provide a fast, metabolism-independent surface process for removal of toxic heavy metals from food and water (Halttunen et al, 2007; Ibrahim et al, 2006). The aim of this research was the selection of useful LAB strains effective in decreasing the concentration of nitrate-nitrogen in Kumamoto's water by utilizing their natural biosorption capabilities.

3. METHODOLOGY
3.1 Sample preparation
To carry out the experiments for selection of useful LAB strains capable in decreasing NO_3-N in water, tap water samples have been prepared in Tokai University's (Kumamoto campus) 'Laboratory of Bioscience'. The reasons for selecting tap water for the analyses were: (1) High levels of NO_3-N in Kumamoto's water had already been proven; (2) Tap water was easy to collect at any time and in any required amount so the number of samples was not limited.

Legend	Incubation condition
A	Control volumes: samples were put in freezer immediately after sterilizing.
B	One week after adding LAB, samples were centrifuged, filtered and frozen.



3.2 Incubation experiment
Successful incubation experiments were carried out in July and September of 2018. The incubation period was one-week and warming temperature was 37 °C. In July, 40 mL culture medium was used whereas in September the amount was changed to 14 mL. For the incubation experiment, tap water and distilled water were autoclaved at 121 °C for 15 minutes. Bacteria were cultured in culture medium and centrifuged washing was carried out with sterilized distilled water (5800×g, 5 min, 4 °C). This process was executed two times and the supernatant was discarded. Sterilized tap water (40 mL or 14 mL) was added to the washed bacterial cells and was well suspended (vortex) before incubating it at 37 °C. Filtered distilled water and tap water were set as controls. After centrifugation (5800×g, 5 min, 4 °C), the supernatant was frozen through a 0.45 µm PTFE filter (Millipore). Number of samples per type of LAB was set to 5 (n=5).

3.3 Analyses for NO_3-N
After preparing the samples and freezing them, they were transported to Tokai University's Shonan Campus for analyses and were analyzed for NO_3-N in the 'Water Environment Lab'. Dissolved inorganic nutrient (NO_3-N) was determined spectrophotometrically following HACH's principle using portable colorimeters (HACH DR/890): cadmium metal reduces nitrates present in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt which couples to chromotropic acid to form a pink-colored product (HACH Procedures Manual; pp 327).

Keywords: Nitrate, Nitrogen, Lactic Acid Bacteria, Kumamoto, Biosorption
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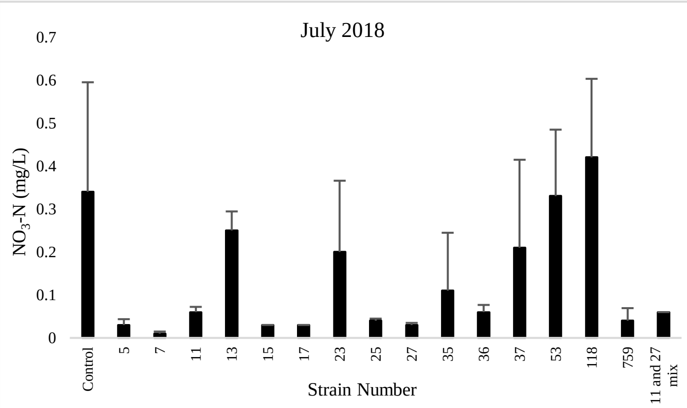
4. RESULTS

Incubated samples including 15 types of TOKAI LAB strains (5, 7, 11, 13, 15, 17, 23, 25, 27, 35, 36, 37, 53, 118 and 759) and one mix of two types of LABs (mix of 11 and 27) were analyzed for NO_3-N in July. Among these samples, tap water control volume samples showed 0.342 mg/L of NO_3-N on average. Samples containing LAB strains of 5, 7, 11, 13, 15, 17, 23, 25, 27, 35, 36, 37, 53, 118 and 759 showed 0.034, 0.014, 0.060, 0.250, 0.030, 0.030, 0.196, 0.036, 0.034, 0.112, 0.060, 0.212, 0.328, 0.424 and 0.042 mg/L of NO_3-N on average respectively. The mix of 11 and 27 showed 0.060 mg/L NO_3-N which was equal to the amount of NO_3-N showed by 11 independently. The analyses results are expressed graphically in Fig. 1. Samples incubated in September included 13 LAB strains (7, 17, 52, 53, 54, 59, 60, 61, 62, 63, 65, 74 and 759). The tap water control sample of September showed 0.308 mg/L of NO_3-N on average. LAB strains 7, 17, 52, 53, 54, 59, 60, 61, 62, 63, 65, 74 and 759 showed 0.108, 0.308, 0.530, 0.400, 0.612, 0.108, 0.105, 0.143, 0.065, 0.125, 0.181, 0.118 and 0.035 mg/L of NO_3-N on average respectively. Fig. 1 shows the average analyses results of the samples prepared in September 2018.



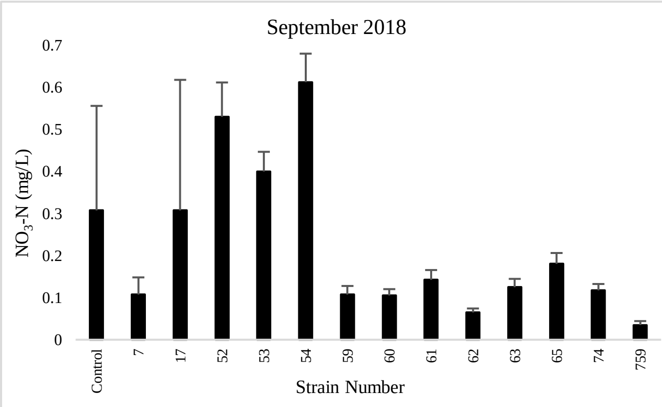


Fig. 1: (Left) NO_3-N analyses results (average) of July (37 °C, one-week incubation in 40 mL medium).
(Right) NO_3-N analyses results (average) of September (37 °C, one-week incubation in 14 mL medium).

5. DISCUSSIONS

Comparing the results of July and September, it can be noticed that several LAB strains have shown affinity in lowering the level of NO_3-N. In July, strain number 5, 7, 11, 15, 17, 25, 27, 36, 759 and the mix of 11 and 27 had shown satisfactory results. In September, strain number 7, 59, 60, 61, 62, 63, 74 and 759 had demonstrated promising results. Even though the incubation conditions in July and September were almost same, some LAB strains had shown different reactions in these months. Strain number 7, 17, 53 and 759 were used in both months, but, all of them showed better response in July compared to September. This could indicate that the amount of culture medium could be a key factor. Overall the incubation conditions of July (37 °C warming temperature, one-week incubation in 40 mL culture medium) seemed to be suitable for the desired outcome. However, both the experiments carried out in July and September established the possibility of some LAB strains being able to effectively lower the concentration of NO_3-N in Kumamoto's water. From our observation, LAB strain of 759 has shown the most promising and stable result lowering the level of NO_3-N 8~9 times compared to the control volume.

6. CONCLUSIONS

The upward tendency of NO_3-N in Kumamoto's water is becoming a pressing issue as presence of excessive NO_3-N in water can pose various adverse health, ecological and environmental effects. Using the natural biosorption properties of LAB to lower the concentration of NO_3-N in Kumamoto's water could be a feasible and environment-friendly solution to the problem. Multiple LAB strains have been found in this research that could be effective in decreasing the amount of NO_3-N in water. Given the research becomes successful in local level, it could be implemented to help the developing countries as a reasonable option for water quality improvement.

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