## Development of a Fluoride Riboswitch-based Whole-cell Biosensor for Detecting Fluoride in Drinking Water

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Drinking water is the main dietary source of fluoride for humans. Despite the benefits, high ingestion of fluoride causes adverse health concerns. Interference with other ions is a major drawback associated with conventional methods available for detection of fluoride concentration in drinking water. Bacterial riboswitches are well known for their high selectivity towards the ligand. The novel method of fluoride detection utilizes an *Escherichia coli* mutant carrying a plasmid cloned with fluoride responsive riboswitch (FRS), coupled to the *lacZ* reporter gene. Fluoride binding into the aptamer region of FRS induces the expression of $\beta$- galactosidase which then hydrolyzes the colorless Ortho-Nitrophenyl-$\beta$-galactoside (ONPG) into yellow color product, Ortho nitrophenol (ONP) which can be detected at 420 nm. The ONP absorbance was used to quantify the fluoride level in the aqueous samples. The calibration curve for the absorbance of ONP showed a linear relationship for the concentrations between 5 $\mu M$ - 40 $\mu M$ with $R^2$>0.9. The groundwater samples from ten different regions of Sri Lanka were analyzed for fluoride concentration using the constructed biosensor. The highest and lowest level of groundwater fluoride was found in Polonnaruwa (23 $\mu M$) and Kurunegala (14 $\mu M$) regions respectively. These values were compared with fluoride concentrations obtained using a fluoride ion selective electrode (FISE). Eight samples showed significant discrepancies between the FISE and the FRS-based biosensor, except for the water sample obtained from Polonnaruwa. Further, the biosensor performance was evaluated in the presence of common interfering ions ($Na^+$, $K^+$, $Ca^{2+}$, $CO_3^{2-}$, $HCO_3^-$ and $NO_3^-$). The results showed low interference from these ions, indicating the biosensor's high sensitivity towards the analyte. The FRS-based whole-cell biosensor must be further optimized for on-site detection of fluoride levels in drinking water to ensure its practical application and reliability.

***Keywords***: *Riboswitch, Fluoride Ions, Drinking Water, Portable Biosensor, E. coli*