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Design and Development of Fluoride Riboswitch Based Biosensor to Detect the Fluoride Level in Drinking Water

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Abstract

Fluoride plays a major role in human health as it is categorized as an essential micronutrient. Fluoride may cause adverse effects or beneficial effects depending on the concentration present in the human body. Since drinking water plays an important role in the fluoride diet, it is crucial to determine the fluoride concentration in the drinking water to reduce the vulnerability of communities to fluoride - dependent diseases. However, there are many drawbacks associated with the conventional methods for fluoride detection, such as interference with other anions, which greatly limits the scope of their use. Bacterial riboswitches are well known for their high selectivity towards the ligand. The detection method utilizes an *Escherichia coli* mutant carrying a plasmid cloned with fluoride responsive riboswitch, coupled to the lacZ reporter gene. LacZ, which is under the regulation of fluoride-riboswitch, encodes β-galactosidase in response to the intracellular fluoride levels. The activity of β-galactosidase was determined by the turn-over of its substrate ONPG, which forms a yellow color product ONP which absorbs light at 420 nm. The ONP absorbance was used to quantify the fluoride level in the aqueous samples. The calibration curve showed a linear relationship (R^2=0.9842) for the concentrations between 5-100 μM. Next, the biosensor was used to measure the groundwater samples collected from different regions of Sri Lanka. The highest level of groundwater fluoride was found in Ampara, Batticaloa, Kurunegala (>2 ppm), and the lowest level was found in Colombo and Matara (<0.5 ppm). The experimentally determined fluoride levels are in agreement with the reported values. Our results also demonstrate that the novel biosensor is highly selective for fluoride and can readily discriminate between chloride and hydroxyl ions. This work provides a critical proof-of-principle for utilizing the fluoride riboswitch-based whole-cell biosensor to selectively and accurately detect fluoride levels in drinking water.

Keywords: Fluoride, Riboswitch, LacZ