

## **A bacterial whole-cell biosensor for explosives' detection: strain optimization and the development of a spraying option**

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Over the past century, an extensive use of explosives for military and civil purposes has led not only for direct risk to human safety, but also to significant soil and water pollution. Since no current technology allows the remote detection of such devices, the mapping of minefields necessitates the on-site presence of personnel, along with the obvious risks involved.

A possible remote solution for this problem could be the use of biosensors. Biosensors are often composed of three elements: a bioreceptor, a transducer, and a signal-processing unit; when the analyte interacts with the bioreceptor, a quantifiable signal is generated. Whole-cell biosensors are live cells that generate a quantifiable signal upon interacting with the analyte. By using genetic engineering tools, such cells can be engineered to detect diverse analytes and provide information on their biological effects.

A bacterial biosensor for sensitive detection of 2,4,6-trinitrotoluene (TNT) and its derivative 2,4-dinitrotoluene (DNT) has been previously developed in our lab. The envisaged utilization of this sensor involved immobilization in alginate beads before application on the inspected area. In the present study, I have investigated an alternative application method, focusing on spraying non-immobilized bacterial sensor cells. Following the optimization of growth conditions and cell density in the sprayed suspension, improvement in signal intensity and duration was accomplished by (a) manipulation of medium composition and (b) overexpression of the Curli protein, thus enhancing biofilm formation. The latter was achieved by expressing the regulatory gene *OmpR* under an inducible gene promoter (*araC*).

The combination of the optimized sensor strain and its suspension medium allowed the sensitive detection of low amounts of DNT buried under sand (a detection limit of 12.75  $\mu\text{g}$  DNT), and the maintenance of a visible bioluminescence signal up to 11 hours.

The results of this study may open the way for much greater flexibility in the use of whole-cell sensors for the detection of buried explosives, as well as for diverse additional applications.