TITLE: OPTIMIZATION OF THE PARAMETERS TO MAXIMIZE THE GROWTH OF *Escherichia coli* (HT-115) FOR dsRNA PRODUCTION

AUTHORS: CERQUEIRA, L.R.S[1]; SANTOS, T.J[2]; ANDRADE, E.C[3].

INSTITUTION: [1]UNIVERSIDADE FEDERAL DO RECÔNCAVO DA BAHIA (Rua Rui Barbosa, 710 - Campus Universitário CEP 44380-000, Cruz Das Almas/BA); [2]FACULDADE MARIA MILZA, GOVERNADOR MANGABEIRA– BA - BRASIL; [3]EMBRAPA MANDIOCA E FRUTICULTURA, CRUZ DAS ALMAS – BA - BRASIL.

ABSTRACT:

The discovery and use of RNA interference (RNAi) technology for pest management has been demonstrated under laboratory and field conditions. This technology opens to an environmentally friendly control strategy, called “Highly Specific Pest Control’ (HiSPeC), which means, control a desired target specie with no effects on non-target species. In this context, dsRNA can be considered as an active ingredient of a new class of biopesticide. The applicability of sprayable dsRNA relies on the development of cost-effective methods for the mass production of dsRNA. One method of production that has been explored is the use of fermentative systems using engineered *Escherichia coli* strains such as HT-115 (DE3). The objective of this work was to determine the best parameters for *E. coli* (HT-115) growth and dsRNA production. Initially a standard protocol was established: a colony of *E. coli* harboring a recombinant plasmid was transferred to 5 ml of culture medium with ampicillin and incubated at 37 °C/ 250 rpm for 16 hours. Then 1 mL was transferred to a tube containing 10 mL of culture medium with ampicillin and incubated at 37 °C / 250 rpm. The bacterial growth was monitored until it reaches an OD_{600} 0.4, and dsRNA production was induced with IPTG to a final concentration of 0.4 mM. The bacterial cells were collected by centrifugation at 5000 rpm/ 5 minutes once reaches an OD_{600} 1.0. DsRNA was extracted with Trizol®, treated to remove DNA and single strand RNA and the concentration estimated by spectrophotometer and electrophoresis. The following parameters were evaluated individually: culture medium (LB, 2YT and SOC); time of induction (OD_{600} 0.4 and 0.7); IPTG concentration (0.4, 0.6 and 0.8 mM). Each experiment consisted of three replicates and the experiment was repeated at least three times. The results showed that bacteria growth in LB medium obtained the highest production of dsRNA compared to the other media, reaching a yield of 748 ng/mL. Inducing the production of dsRNA in OD_{600} 0.4 resulted in a superior yield compared to OD_{600} 0.7 (70% more). Among IPTG concentrations, higher dsRNA production was obtained with IPTG at 0.8 mM (1158 ng/mL). Thus, from the first established parameter (LB medium), the optimization of two other parameters resulted in a 54% increase in production of dsRNA (from 748 ng/mL to 1158 ng/mL). Optimization of other bacterial growth and induction parameters will be conducted in order to improve dsRNA production.

Keywords: RNA interference, fermentation, pest control

Development Agency: Embrapa Mandioca e Fruticultura (CNPMF)