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Research paper

Statistical versus artificial intelligence -based modeling for the optimization of antifungal activity against *Fusarium oxysporum* using *Streptomyces* sp. strain TN71

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ABSTRACT

A *Streptomyces* sp. strain TN71 was isolated from Tunisian Saharan soil and selected for its antimicrobial activity against phytopathogenic fungi. In an attempt to increase its anti–*Fusarium oxysporum* activity, GYM + S (glucose, yeast extract, malt extract and starch) culture medium was selected out of five different production media. Plackett–Burman design (PBD) was used to select yeast extract, malt extract and calcium carbonate ($CaCO_3$) as parameters having significant effects on antifungal activity, and a Box–Behnken design was applied for further optimization. The analysis revealed that the optimum concentrations for the anti–*F. oxysporum* activity of the tested variables were yeast extract 5.03 g/L, malt extract 8.05 g/L and $CaCO_3$ 4.51 g/L. Artificial Neural Networks (ANNs): the Multilayer perceptron (MLP) and the Radial basis function (RBF) were created to predict the anti–*F. oxysporum* activity. The comparison between experimental and predicted outputs from ANN and Response Surface Methodology (RSM) were studied. The ANN model presents an improvement of 14.73%. To our knowledge, this is the first work reporting the statistical versus artificial intelligence -based modeling for the optimization of bioactive molecules against mycotoxigenic and phytopathogenic fungi.

1. Introduction

In agriculture, plant diseases need to be controlled to maintain the quality and abundance of food and feed [1,2]. Moreover, the excessive use of synthetic pesticides for the management of plant pathogens has given rise to problems due to the development of resistance in phytopathogenic fungi against traditional fungicides [3]. Therefore, the biological management of pathogens has gained interest as an alternative approach that is sustainable and safe both economically and environmentally [4]. Consequently, there has been an increase in research aiming not only to find new effective safe plant agents, but also to limit the use of chemical pesticides [1,5].

The objective of modern agricultural systems is to ensure sustainable crop production by the search of new natural antifungal resources [6]. Microorganisms have provided abundant sources of bioactive metabolites that have been developed as commercial products for plant crop protection [6–8]. Furthermore, from the 22,500 biologically active compounds that have been obtained so far from microbes, 45% are produced by actinomycetes, with approximately 75% of metabolites being produced by species of the genus *Streptomyces* [9]. *Streptomyces* species provide a rich source of natural products that may have potential agricultural uses. Their activity has been evaluated against different plant pathogens, and found to exhibit great potential in suppressing plant diseases caused specifically by fungal pathogens [10,11].

To improve the production of antifungal secondary metabolites in actinomycetes, different approaches were used to optimize culture media using statistical methods to reduce time and expense [12]. Response surface methodology (RSM) is a very beneficial tool to optimize numerous parameters of trails and to find relativeness among the factors, as well as the best combination of parameters and response prediction [13]. This method was extensively used for the optimization of *Streptomyces* antifungal production [1,14]. In recent years, artificial neural network (ANN) has arisen as an efficient and attractive approach for non-linear multifactor modeling due to its generic structure and ability to learn from historical data [15]. The multilayer perceptron (MLP) and radial basis function (RBF) neural network architectures are

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likely to be the most used ANNs [16,17]. MLP and the RBF neural network structures have been employed in making predictions [17]. Due to the non-linear efficiencies of these networks, they are considered good estimators providing very accurate results.

During the screening program to search antimicrobial compounds active against phytopathogens, we have previously described the taxonomy of a *Streptomyces* sp. TN 71 isolated from a Tunisian Saharan soil having remarkable antimicrobial activities [18]. In our previous study, the effect of critical medium components on the production of anti-*Agrobacterium tumefaciens* secondary metabolites was investigated [18]. In this way, RSM and ANN have been used to optimize the medium composition for high anti-*Agrobcaterium tumefaciens* activity from *Streptomyces* sp. TN71.

As part of further research work pertaining to the exploration of the biological control against phytopathogens, in this work, the culture medium of *Streptomyces* sp. TN 71 was optimized using RSM and ANN techniques to enhance the antifungal potential against *Fusarium oxysporum*. Equally, the optimized conditions predicted by both RSM and ANN were compared and experimentally verified. To the best of our knowledge, no data on the comparison of RSM and ANN techniques for culture medium optimization for anti–*F. oxysporum* activity by *Streptomyces* are not available in the literature. It is in this context that the present research work was conducted to resume the investigation about the *Streptomyces* sp. TN 71 strain to increase current knowledge in terms of modeling-based optimization approaches.

2. Materials and methods

2.1. Microorganism and growth medium

Streptomyces sp. TN71 strain was isolated from Tunisian Saharan soil and identified in a previous research work as a bacterium belonging to *Streptomyces* genus [18]. The culture of *Streptomyces* sp. TN71 was grown and maintained on ISP2 broth. The antifungal activity of *Streptomyces* metabolites was tested on *Fusarium oxysporum* (CTM10402). *Fusarium oxysporum* procured from the local culture Collection of Tunisian Microorganisms "CTM" of the Centre of Biotechnology of Sfax (CBS) was used in this study. The following fungal strains were also tested: *Aspergillus niger* (CTM 10099), *Fusarium graminearum* (ISPaVe 271), *Fusarium culmorum* (ISPaVe 21w) and *Alternaria alternata* (CTM 10230). Fungal strains were maintained on potato dextrose agar (PDA) medium preserved at 4 °C and sub-cultured once in every three weeks. All fungal strains were maintained in 50% glycerol, 50% in Potato Dextrose Broth (*v*/*v*) at −20 °C.

2.2. Production, extraction of bioactive compounds and anti–F. oxysporum activity determination

All the antifungal production experiments were carried out in 1000 mL Erlenmeyer flasks with 100 mL of production medium prepared with different nutrient concentrations according to the selected factorial design. The flasks were inoculated with 10 mL of seed culture (spores at 10^7/mL) and incubated on orbital shakers (250 rpm) for 4 days at 30 °C. The cell-free supernatant was extracted twice with ethyl acetate and the obtained organic extract was concentrated in vacuum to dryness. The crude extract was assayed in triplicates for their antifungal activity against all fungal strains by disc diffusion assay [19].

Fungal spores, grown in PDA for 7 days at 25 °C, were collected in sterile distilled water and then adjusted to a spore density of approximately10^4 spores/mL. Antifungal activity was determined by the agar diffusion test [19]. The quantity used for each pure active compound was 50 μg per disk. Plates were examined for evidence of antifungal activity represented by a zone of growth inhibition around the paper disk.

2.3. Selection of basic medium

Antifungal activity against *Fusarium oxysporum* was determined upon the growing of the *Streptomyces* sp. TN71 strain on five different basal production media as follows: (BM1) Bennett's medium: glucose 10 g/L, pancreatic digest of casein 2 g/L, yeast extract 1 g/L and beef extract 1 g/L, pH 7.0; (BM2) Czapek medium: sucrose 30 g/L, $NaNO_3$ 3 g/L, K_2HPO_4 1 g/L, KCl 0.5 g/L, $MgSO_4 \bullet 7H_2O$ 0.5 g/L and $FeSO4 \bullet 7H_2O$ 0.01 g/L pH 7.3; (BM3) GYM + S medium: starch 20 g/L, malt extract 10 g/L, $CaCO_3$ 4 g/L, glucose 4 g/L, and yeast extract 4 g/L, pH 7.2; (BM4) ISP4 medium: starch 10 g/L, $CaCO_3$ 2 g/L, $(NH4)_2SO_4$ 2 g/L, K2HPO4 1 g/L, $MgSO_4 \bullet 7H_2O$ 1 g/L, NaCl 1 g/L, $FeSO_4 \bullet 7H2O$ 1 mg, $MnCl_2 \bullet 7H2O$ 1 mg and $ZnSO_4 \bullet 7H_2O$ 1 mg, pH 7.2 and (BM5) Waksman's Glucose medium: glucose 10 g/L, peptone 5 g/L, beef extract 5 g/L and NaCl 5 g/L, pH 7.5 [20]. All the media (100 mL) were inoculated with 10 mL of the seed culture and incubated at 30 °C under agitation (250 rpm) for 4 days. After incubation, the crude extract from the culture broth was assayed against *Fusarium oxysporum*. The medium that showed high antifungal activity was selected for subsequent statistical optimization.

2.4. RSM based modelling

2.4.1. Screening of essential medium components using the Plackett–Burman design

The Plackett–Burman design was used to analyze important factors. Twelve experiments were conducted in triplicate to evaluate five factors. Five components: A: starch; B: malt extract; C: $CaCO_3$; D: glucose; E: yeast extract, were selected for the study, with each variable being represented at two levels, high (+) and low (−), as well as two dummy variables in twelve assays.

The effect of each variable on the anti–*F. oxysporum* activity was calculated and their significance was determined via Student's *t*-test using Minitab 15.0 version (Minitab Inc. PA, USA). The variables with confidence levels above 95% were considered to have a significant effect on antifungal compound production, and thus chosen for further optimization.

2.4.2. Box–Behnken design and optimization by RSM

The significant variables were optimized for enhanced antimicrobial activity by employing a Box–Behnken design [21]. Three variables: B: malt extract; C: $CaCO_3$ and E: yeast extract were selected for studying the effect and significance on anti–*F. oxysporum* activity. These selected variables were analyzed at three levels low, medium, and high coded as −1, 0, and +1, respectively, in twelve runs. The dummy variables were used to calculate the standard error. Each run was carried out with three replicates. The behavior of the system was explained by a second-order polynomial equation [Eq (1)].

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ij} X_i X_j + \Sigma\beta_{ii} X^2{}_i \quad (1)$$

where Y is the predicted response, β_0 is offset term β_i is linear effect, β_{ii} is squared effect, β_{ij} is interaction effect, and X_i is coded value of independent variables under study.

This design was used to evaluate the main effects, interaction effects and quadratic effects. It is also used to optimize the levels of parameters for enhancing antifungal activity. The statistical software (Minitab 15.0 version) was used for the experimental design and data analysis. Three-dimensional response surface plots were drawn to illustrate the relationship between the

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