RESPONSE SURFACE METHODOLOGY AND BOX-BEHNKEN DESIGN FOR THE OPTIMIZATION OF ANTIMICROBIAL ACTIVITY FROM THE THERMOHALOPHILIC ACTINOMYCETE SMBG3

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Abstract
Actinomycetes are the most economically and biotechnologically prokaryotes for their ability to produce novel second metabolites [1]. The halophilic actinomycetes are noteworthy for their potential to produce novel bioactive compounds [2]. Among them, the production of antimicrobial compounds is greatly influenced by various fermentation parameters such as available nutriment, mineral salts, pH and temperature [3]. In the present study, we report the statistical optimization of antibacterial activity of Thermoactinomycete strain SMBg3 isolated from sediment solar saltern of Sfax in Tunisia.

Keywords: Antibiotics, Tunisian Plateau

Introduction
Actinomycetes are the most economically and biotechnologically prokaryotes for their ability to produce novel secondary metabolites [1]. The halophilic actinomycetes are noteworthy for their potential to produce novel bioactive compounds [2]. Among them, the production of antimicrobial compounds is greatly influenced by various fermentation parameters such as available nutriment, mineral salts, pH and temperature [3]. In the present study, we report the statistical optimization of antibacterial activity of Thermoactinomycete strain SMBg3 isolated from sediment solar saltern of Sfax in Tunisia.

Materials and Methods Strain isolation
SMBg3 was isolated from sediments solar saltern of Sfax in Tunisia and selected among a group of actinomycetes that has the capability of producing antibiotic against a range of pathogenic bacteria. The strain SMBg3 was isolated on steptomyces agar medium and was maintained on Bennett's medium supplemented with 10% NaCl.

Optimization of nutritional and cultural conditions by Taguchi and Box-Behnken
First, the screening for essential medium components of suitable medium using Taguchi Design was employed for screening the most eight significant medium components for growth and antimicrobial compound production by SMBg3. Second, the optimization of selected ingredients was effected by Response Surface Methodology (RSM). RSM was used with Box-Behnken design to optimize the selected media constituent and conditions (% of MgSO4, pH, and temperature) for enhanced growth and antibiotic production in SMBg3.

Results and Discussion
SMBg3 strain was isolated from sediments solar saltern of Sfax in Tunisia on pond M1. The phenotypic, chemotaxonomic and molecular characteristic based on the basis of 16S rRNA gene sequencing and % G+C showed that the SMBg3 strain is a new member of the *Thermoactinomycetaceae* family. SMBg3 strain exhibited a very strong activity against tested Gram-positive and Gram-negative bacteria in Bennett medium which was selected as suitable medium for optimization of growth and antimicrobial activity. Taguchi design was performed to evaluate the effects of eight variables according to the orthogonal matrix L18 [4]. Statistical analyses showed that temperature, pH and % of salt solution were the most influential and significantly parameters affected the antibacterial compound production. The RSM was applied with Box-Behnken and were found to be very effective in selecting and optimizing the medium components. These optimized values of the three factors were validated in a tow runs maximize to 47% for the growth, 44% for the antimicrobial activity against *E. coli* strain. The coefficient of determination (R^2) were found to be 0.8 for the growth and the antimicrobial activity models, value closer to 1.00 indicates the goodness of the models in accurate prediction of the response.

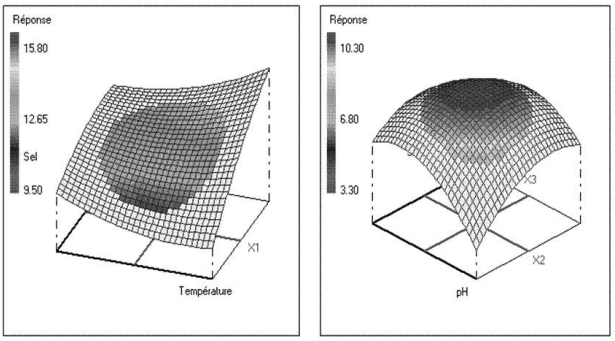

Fig. 1. Isoresponse curve of biomass (on the right) and antitibacterial activity against *E.coli*. (on the left).

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