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Enhancement of Bioactive Secondary Metabolites Productivity by Marine *Streptomyces acrimycini* MBS-HRS-EG (MYI1) using Blackett-Burman Design and Scaling up using 7.5 L Bioreactor with In vitro Antifungal, Antioxidant and Anticancer Assessment

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Abstract

MARINE actinomycetes, especially *Streptomyces* sp., are known for producing diverse biologically active compounds like antifungal, antioxidant, and anticancer agents. This study aims to optimize the production of bioactive secondary metabolites by marine *Streptomyces acrimycini* strain MBS-HRS-EG (MYI1) ID: PP477812.1 through submerged fermentation. The study involved screening for antifungal and antioxidant activities using agar well diffusion and DPPH methods. A medium pre-screening identified the most effective medium, which was further optimized using a Plackett-Burman design (PBD) with eleven factors and fourteen runs. Statistical analysis showed significant models with a lack-of-fit of 0.212 and R2 above 0.9768 for all responses. $MgSO_4$, sodium pyruvate, and $(NH_4)_2SO_4$ were key bioactivity variables. The optimized conditions were tested in a 7.5 L bioreactor at different agitation speeds, showing higher productivity at 200 and 400 rpm. Cytotoxicity assessment revealed the efficacy of the bioactive compounds against various cancer cell lines, with IC_{50} values ranging from 75.8 to 108.5 µg/mL.

Keywords: Marine *Streptomyces*, Statistical optimization by Blackett-Burman design, Antifungal, Antioxidant and Anticancer, Scaling up with 7.5 L bioreactor.

Introduction

The biodiversity of marine environment proved to be an important resource for identification of potent microorganisms to produce unique biologically active metabolites [1]. The marine environment is a rich source of both biological and chemical diversity. This diversity has been the source of unique chemical compounds with the potential benefits for pharmaceuticals industries [2].

Marine ecosystems are specified by several unique features. It has composed of different salts and minerals in addition to extreme conditions such as high salinity, temperatures, pressures, lack of nutrients and pH [3]. The extreme conditions are reflected in the variations in genes and thus in metabolites, ensuring the potential ability to produce novel bioactive metabolites [4]. The unique ecosystems in marine environments result in a completely different metabolic pathways and defense system compared to their terrestrial counterparts.

The extreme conditions make stimulate marine microorganisms to produce various bioactive compounds to prevent them against others, more that the microorganisms can be coexist with marine sponges symbiotically by the way, the microorganism introduce some defence metabolites as antifungal agents, antibiotics antiparasitic to prevent them and keep the sponge heal their while, the sponge provide the microorganism with nutrients with symbiotic life.

The majority of marine actinomycetes can produce several of biologically active metabolites especially the *Streptomyces* species for instance, Antifungal agents as natamycin produced by fermentation of *Streptomyces (Str.) natalensis* [5], Strevertene A bioactive metabolites of *Str. olivoreticuli* and *Str. abikoensis* [6], and Oligomycin B which produced by *Streptomyces* strains B8496, B8739, A171 [7, 8]. Antibiotics as Pikromycin produced by the

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fermentation of *Streptomyces sp*. and *Str. venezuelae* [9], Okilactomycin D as secondary metabolites of *Str. scabrisporus* [10], Antivrals bioproducts as Delactonmycin produced as bioactive metabolites of *Streptomyces* sp. [11], moreover metabolites can act as antifungal , anticancer and antibiotic together for example, Landomycin which produced from *Str. globisporus* [12], Bafilomycin D produced from fermentation of *Str. cavourensis*, *Str. bacillaris* [13] and Albocycline by *Streptomyces*. sp. *Str. bruneogriseus* [14, 15, 16].

It is well known that designing a suitable fermentation medium is more significant in the production of marine bioactive metabolites [17]. Developing a suitable basal medium may play a significant role in the further media optimization [18]. Production of secondary metabolites through microbial fermentation is influenced by various nutritional and conditional factors [19, 20]. Furthermore, production of valuable metabolites by *Streptomyces* differs qualitatively and quantitatively depending on the producing strains used in fermentation process as one of the most significant roles as sources of precursors and metabolic pathway for potential bioactive secondary metabolite production [21]. Therefore, influences of medium components and environmental conditions are a primary and important step to enhance metabolite production of the genus *Streptomyces*.

However, the ability of *Streptomyces* cultures to form these bioactive products is not a fixed property but can be greatly increased or completely lost under diversity of fermentation process conditions as nutritional and cultivation requirements. Improvement in the strain growth and potential bioactive metabolites production can be executed by manipulating the nutritional and conditional parameters of the culturing fermentation process [22]. Hence a medium component plays a critical role in the competence and economics of the ultimate fermentation process. Therefore, designing a suitable fermentation medium is critical importance in the fermentation production of bioactive secondary metabolites. Fact reported that any changes in the nature or type of carbon and nitrogen sources must be effect on antibiotic biosynthesis of *Streptomyces*. Also, several cultivation parameters like pH, inoculum size and type, incubation period and temperature play a main role in the bioactive metabolites production [23].

In order to maximize the fermentation production of pharmaceutical bioactive metabolites from marine *streptomyces*, suitable nutritional and cultural conditions are obligatory required [24]. The traditional strategy by one variable-at-a-time optimization is very simple and useful only for screening the independent variables. However, it fails to set the optimum response because the factors interactions are not studied [25, 26]. On the other hand, statistically experimental models considered the powerful design for optimization results in improved bioproducts yield, reduced experimental variability, closer confirmation of the output response to nominal and target requirements, and reduced experiments overall costs [27].

The study aimed to identify factors influencing the production of marine *Streptomyces* bioactive metabolites in submerged fermentation. This was achieved through statistical optimization design (PBD) and scaling up production using optimal cultivation conditions and medium composition identified from PBD in a 7.5 L bioreactor. The study also assessed the strain fermentation broth for its antifungal, antioxidant, and anticancer properties in vitro.

Material and Methods

Marine Streptomyces strain

The strain used in this study was marine *Streptomyces* previously isolated from Hurghada locality, Red Sea sponge, identified and reiterated in gene band with name *Streptomyces acrimycini* strain MBS-HRS-EG (MYI1) under accession no. ID: PP477812.1.

Maintenance medium

Strain MBS-HRS-EG (MYI1) was maintained in ISP2 medium: It composed of (g/L). Yeast extract, 4; Malt extract, 10.0; Glucose, 4 and Agar 20.0. The pH was adjusted at pH 7.2-7.4 before sterilization [28, 29, 30].

Inoculum media

Yeast malt extract medium (g/L). Yeast extract, 3; Malt extract, 3; Peptone 5; Glucose, 10 and Agar, 15. The pH was adjusted to 7.0 [31].

Media screening for secondary metabolites production

Different five producing media that vary in their components were used for screening of fermentation production of bioactive secondary metabolites to select the best one to extend production optimization by the statistical modelling. The medium components were purchased from Merck, Darmstadt, Germany and soybean meal was purchased from agricultural research centre, Giza – Egypt. These media composed of (g/L): Medium 1: Glucose, 2; Meat extract, 0.5; Peptone, 0.5; Dried yeast, 0.3; NaCl, 0.5 and $CaCO_3$, 0.3 [32]. Medium 2: Glucose, 15; Soy meal, 15; Corn steep liquor, 5; $CaCO_3$, 2; and Trace elements solution, 6 ml/L. The trace salts solution was consisting of (mg/mL): $FeSO_4$. $7H_2O$, 5.0; $CuSO_4.5H_2O$, 0.39; $ZnSO_4.7H_2O$, 0.44; $MnSO_4.H_2O$, 0.15; $Na_2MoO_4.2H_2O$, 0.01; $CoCl_2.6H_2O$, 0.02 [33]. Medium 3: Soluble starch, 32.0; Glycerol, 15 (ml/L); Soybean meal, 30.0; KH_2PO_4, 0.1; $FeSO_4.7H_2O$, 0.1 and trace salts solution, 1ml/L. The trace salts

solution was consisting of (g/100mL). $FeSO_4.7H_2O$, 0.1; $MnCl_2.4H_2O$, 0.1; $ZnSO_4.7H_2O$, 0.1 [34]. Medium 4: Starch, 10.0; $CaCO_3$, 3.0; K_2HPO_4, 1.0; $(NH_4)_2$ SO_4,2.0; $MgSO_4$,1.0; NaCl, 1.0. [35]. Medium 5: Sodium pyruvate, 0.3; Peptone, 0.5; Arginine, 1; Soluble starch, 4; Glycerol, 3; Dextrose, 3; $CaCO_3$, 3; K_2HPO_4, 1; $MgSO_4$, 1; $(NH_4)_2SO_4$, 2 [36].

Fermentation Production medium

The best medium from five media screening which used in fermentation production with component (g/L), Sodium pyruvate,0.3; Peptone, 0.5; Arginine,1; Soluble starch,4; Glycerol,3; Dextrose,3; $CaCO_3$,3; K_2HPO_4,1; $MgSO_4$,1; $(NH_4)_2SO_4$,2.

Statistical design and modeling

Pearson's correlation coefficient (r) was determined by Microsoft Office Excel 2010. Plackett-Burman design, modeling, analysis of variance (ANOVA) and equations were achieved using the "Design Expert" software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA) statistical package. Plackett-Burman design of eleven real factors consisting of fourteen runs with two center points was conducted as shown in tables 1 and 2.

Biological activities as antifungals and antioxidants of the fermentation broth

Evaluation of antifungal activity of strain fermentation broth

The susceptibility of the fermentation broth for the strain MBS-HRS-EG (MYI1) was performed according to the CLSI M2-A9 protocol, 2006, [37]. Agar well diffusion technique was used. A 0.1mL aliquot of the 18 hours active inoculum cultures of the tested yeast pathogen indicators (*Candida albicans* ATCC-10231, *Candida tropicalis* ATCC-18807) and fungal pathogen indicators (*Aspergillus niger* NRRL-3, *Fusarium oxisporium* ATCC-76616, *Fusarium solani* ATCC-52628 and *Rhizoctonia solani* ATCC-10186) that had been adjusted to the turbidity equal to 0.5 McFarland standards [38] was dispensed into pre sterile 15 cm glass Petri dishes labelled previously with the indicators. Molten sterile potato dextrose agar (Difco (BD), New Jersey, USA) was aseptically poured into the plates and gently rotated for the bacteria to be homogeneously distributed in the medium. The agar plates were allowed to solidify. After solidification of pathogen inoculated plates, under aseptic conditions, using sterile cork porer with 9 mm diameter make wells on the solidified agar and poured 100 µL of the strain fermentation broth. The plates allowed diffusing for two hours at 4 °C in refrigerator. The experiment was conducted in duplicates. All plates were incubated at 28-30 °C for 48 h. Clearance zones around the wells were noted and measured in millimeter.

Antioxidant evaluation of strain fermentation broth

The determination of the strain MBS-HRS-EG (MYI1) fermentation broth ability to scavenging the DPPH (1- diphenyl-2-picrylhydrazyl) as scavenging activity was assessed by the method of [39]. 500 µL of ethanolic DPPH solution (0.4 mmol) was mixed vigorously with 500 µL of 24 h active cells fermentation broth (standardized to obtain a final OD_{600} of 1), or water (control) and incubated at 37ºC in the dark for 1h. The decrease in the absorbance of the mixture was measured spectrophotometrically at 517 nm. The scavenging activity was calculated as; Scavenging activity (%) = [1-(As – Ab)/Ac] ×100, whereas As, Ab and As are the absorbance of the blank (ethanol and sample), the control (DPPH and deionized water) and the sample (DPPH and sample), respectively. This experiment was conducted in duplicate; all values are expressed as means ± standard deviation and compared to the control. Statistical analysis is done as previously mentioned.

Evaluation of cytotoxicity of strain fermentation broth against mammalian cell lines

Fermentation broth of strain MBS-HRS-EG (MYI1) was screened for cytotoxicity on HCT116 (Colon cell line), MCF-7 (Breast cancer cell line), A431 (Epidermoid carcinoma cell line) and A549 (lung cancer cell line). The cell lines were obtained from Cell Culture Laboratory, National Research Centre, Egypt. Cell viability was determined by measuring the mitochondrial-dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [40]. Procedure: All procedures were conducted in a sterile environment using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in DMEM-F12 medium with 1% antibiotic-antimycotic mixture (10,000 U/mL Potassium Penicillin, 10,000 µg/mL Streptomycin Sulfate, and 25 µg/mL Amphotericin B) and 1% L-glutamine at 37 ºC under 5% CO_2.

Two AH plus (white and purple) were mixed together in a 1:1 weight/weight ratio on smooth paper, then incubated in an oven at 37 ºC until dry. After drying, 10 mg of the mixture was weighed and divided into two vials, one soaked in DMEM media for two weeks and the other for four weeks. The same experiment was repeated with the addition of nano silver in a 1:1:1 ratio.

Cells were cultured for 10 days, then seeded at a concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microliter plastic plates at 37 ºC for 24 h under 5% CO_2 in a water-jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). The medium was replaced with the soaked mixture, either with or without nano silver, and cells were incubated for 48 h. Subsequently, MTT salt (2.5 µg/mL) was added to each well and incubated for an additional four hours at 37 ºC under 5% CO_2. To stop the reaction and

dissolve the crystals, 200 µL of 10% Sodium dodecyl sulfate (SDS) in deionized water was added to each well and incubated overnight at 37 ºC. DOX was used as a positive control at 100 µg/mL, resulting in 100% lethality under the same conditions [41, 42].

The absorbance was measured at 595 nm with a reference wavelength of 620 nm using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA).

Statistical significance was determined between samples and the negative control (cells with vehicle) using an independent t-test in the SPSS program, version 11. Dimethyl sulphoxide (DMSO) was used as the vehicle for dissolving plant extracts, with a final concentration on the cells of less than 0.2%. The percentage change in viability was calculated using the formula:

- (Reading of extract / Reading of negative control) -1) x 100

A probit analysis was carried for IC_{50} determination using SPSS program, version 11.

Results and Discussions

Prescreening of fermentation media

In the current study, prescreening five different formulations submerged media were selected from the previous literature for primary evaluation for bioactive metabolites production by strain MBS-HRS-EG (MYI1) as the first step in optimization processes.

The antifungal evaluation results revealed that there were varied between production media but the best medium was a medium five as shown in Table 3. The medium 5 formulation was more satisfied for the strain growth and metabolites production thus it introduced into statistical design to set which type of contents and its optimal concentrations have a significant effect in the bioactive metabolites by fermentation production to enhance the productivity. These results were in accordance of several studies like Ruiz, [21] and Reddy, [22] who reported that the production of valuable metabolites by marine *Streptomyces* varies depending on the strains used in the fermentation process. Medium components and environmental conditions play a crucial role in enhancing metabolite production in marine *Streptomyces*. These factors influence the quality and quantity of bioactive secondary metabolites produced by the marine *Streptomyces*.

Plackett-Burman design (PBD)

Statistical designs considered the most important optimization tool thus, it widely used to optimize varying factors can effect on the fermentation process. The Plackett-Burman design is an important tool for screening and determining the most significant factors as cultural and nutritional ones, concerning their primary effects [43, 44]. Accordance to PBD, the total number of experiments is equal to n^{+1}, where n indicates the total number of variables. Each independent variable was determined with three levels, high (+1), medium (0), and low (−1), as listed in Table 2.

The statistical design in this study, introduced 14 runs with duplicates including 11 real factors were studied to determine the effect of each alone and within different combination with other factors on the production of bioactive metabolites through fermentation production of strain MBS-HRS-EG (MYI1). The antifungal assessment by agar well diffusion method of the different fermentation broth yield was conducted at the end of the fermentation process against several pathogenic fungal indicators as presented in Table 4. The results gave a strong and broad-spectrum antifungal activity for the mid points of all tested statistical designated runs on all fungal test strains, more that the mid points have the highest antioxidant activity which determined as % scavenging activity (DPPH) than low or high factor levels.

The ability of *Streptomyces* cultures to produce bioactive products can be significantly influenced by various fermentation process conditions, such as nutritional and cultivation requirements. Manipulating these conditions can enhance strain growth and the production of bioactive metabolites. The choice of media components is crucial for the efficiency and cost-effectiveness of the fermentation process [22, 23].

Plackett-Burman model studying the factors affecting antifungal and antioxidant activity of strain MBS-HRS-EG (MYI1)

The outputs of Plackett-Burman design, Plackett-Burman model studying the effect eleven variables of fermentation production medium components (10 variable) and inoculum size on antifungal activity of strain MBS-HRS-EG (MYI1) against *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, *Fusarium oxisporium*, *Fusarium solani*, *Rhizoctonia solani* and antioxidant activity.

There are six factors exerted significant effect on antifungal activity of strain MBS-HRS-EG (MYI1), such factors were sodium pyruvate, peptone, soluble starch, magnesium sulphate, ammonium sulphate and inoculum size. Other factors as arginine, glycerol, dextrose, calcium carbonate and dipotassium phosphate were of non-significant effect. The conventional approach of optimizing one variable at a time is limited to screening independent variables and does not consider factor interactions, leading to suboptimal responses [25, 26].

The Plackett-Burman model studying the factors affecting antifungal activity of strain MBS-HRS-EG (MYI1) against

Candida albicans

According to ANOVA results extended in Table 5 concerning antifungal activity of strain MBS-HRS-EG (MYI1) against *Candida albicans*, significant model (p-value 0.0181) having F value of 18.12 was obtained. The propability that a “Model F-Value” could occur due to noise was only 1.8%. The model had the fitness parameters of: R-Squared, 0.9819; Adj R-Squared, 0.9277 which clearly indicated high fitness of the model.

The tested variables were depicted in Fig. s 1 and 2 showed that, the most factors affecting on the production of strain MBS-HRS-EG (MYI1) metabolites with antifungal activity against *Candida albicans* are magnesium sulphate, sodium pyruvate and ammonium sulphate with percent contributions 20.9, 14.1 and 10 % respecteviely from total effect for all different tested factors. The model showed adequate precision of 13.3. Signal to noise ratio "Adeq Precision" of the model was 13.3, which was considerably greater than 4, and indicated markedly the validity to use the model to navigate the design space.

Candida tropicalis

In addation, the ANOVA results extended in Table 5 concerning antifungal activity of strain MBS-HRS-EG (MYI1) against *Candida tropicalis*, significant model (p-value 0.0195) having F value of 50.76 was obtained. The propability that a “Model F-Value” could occur due to noise was only 1.95%. The model had the fitness parameters of: R-Squared, 0.9961; Adj R-Squared, 0.9765 which clearly indicated high fitness of the model.

The tested variables were depicted in Fig. s 1 and 2 showed that, the factors magnesium sulphate, Sodium pyruvate and inoculum size are the most effective factors between other tested factors by 50.86% of total effect exerted with percent controbutions 26.5, 15 and 9.4 % respectively from the total affecting percet of different tested factors on the production of strain MBS-HRS-EG (MYI1) bioactive metabolites having antifungal potentials on *C. tropicalis* pathogenic strain. The model showed adequate precision of 19.99. Signal to noise ratio "Adeq Precision" of the model wsa 13.3, which was considerably greater than 4, and indicated markedly the validity to use the model to navigate the design space.

Changes in carbon and nitrogen sources can impact antibiotic biosynthesis in *Streptomyces*. Cultivation parameters like pH, inoculum size, incubation period, and temperature also play a key role in the production of bioactive metabolites. Designing a suitable fermentation medium is essential for the production of bioactive secondary metabolites [22, 23]. The decrease in anti-*Candida* activity in the organic crude extracts may be attributed to the improper selection of organic solvents for extracting anti-yeast compounds [45].

Aspergillus niger

But in the case of antifungal activity against *Aspergillus niger*, ANOVA results extended in Table 5 concerning significant model (p-value 0.0125) having F value of 79.09 was obtained. The propability that a “Model F-Value” could occur due to noise was only 1.25%. The model had the fitness parameters of: R-Squared, 0.9975; Adj R-Squared, 0.9849 which clearly indicated high fitness of the model. The model showed adequate precision of 25.83. Signal to noise ratio "Adeq Precision" of the model wsa 13.3, which was considerably greater than 4, and indicated markedly the validity to use the model to navigate the design space.

The tested variables were depicted in Fig. s 1 and 2 showed that, the most factors effect on the production of antifungal compounds of strain MBS-HRS-EG (MYI1) are magnesium sulphate, arginine and glycerol and have 48.87% of total effect exerted with percent contributions 30.9, 9.7 and 8.2 % respectively.

Fusarium oxisporium

According to ANOVA results extended in Table 5 concerning antifungal activity of strain MBS-HRS-EG (MYI1) against *Fusarium oxisporium*, significant model (p-value 0.0094) having F value of 106.05 was obtained. The propability that a “Model F-Value” could occur due to noise was only 0.68%. The model had the fitness parameters of: R-Squared, 0.9981; Adj R-Squared, 0.9887 which clearly indicated high fitness of the model.

The antifungal evaluation of strain MBS-HRS-EG (MYI1) against *F. oxisporium* showed that, magnesium sulphate, sodium pyruvate, arginine and inoculum size are the best factors affecting on the antifunals production between all eleven tested factors by 48.2% of total factors effects with factor percent contributions 23.8, 9.6, 7.4 and 7.4 % respectevily. The model showed adequate precision of 30.628. Signal to noise ratio "Adeq Precision" of the model was 13.3, which was considerably greater than 4, and indicated markedly the validity to use the model to navigate the design space.

Fusarium solani

The Plackett-Burman model outpouts according to ANOVA results extended in Table 5 concerning antifungal activity of strain MBS-HRS-EG (MYI1) against *Fusarium solani*, significant model (p-value 0.0261) having F value of 14.03 was obtained. The propability that a “Model F-Value” could occur due to noise was only 26.1%. The model had the fitness parameters of: R-Squared, 0.9768; Adj R-Squared, 0.9071 which clearly indicated high fitness of the model. The model showed adequate precision of

11.914. Signal to noise ratio "Adeq Precision" of the model was 13.3, which was considerably greater than 4, and indicated markedly the validity to use the model to navigate the design space.

Morever, The tested variables were depicted in Fig. s 1 and 2 showed that,the fermentation medium components as sodium pyruvate, ammonium sulphate, magnesium sulphate and peptone are the most affecting from all medium components on the production of antifungals of strain MBS-HRS-EG (MYI1) which evaluated against *F. solani* by 57.8% of total factors effect exerted with percent contributions 17.9, 17.9, 11.4 and 10.6 % respectively.

Rhizoctonia solani

The ANOVA results of Plackett-Burman design extended in Table 5 concerning antifungal activity of strain MBS-HRS-EG (MYI1) against *Rhizoctonia solani*, significant model (p-value 0.0070) having F value of 142.47 was obtained. The propability that a “Model F-Value” could occur due to noise was only 0.7%. The model had the fitness parameters of: R-Squared, 0.9986; Adj R-Squared, 0.9916 which clearly indicated high fitness of the model.

But The tested variables were depicted in Fig. s 1 and 2 showed that, the medium components, ammonium sulphate, sodium pyruvate, magnesium sulphate and inoculum size are the most factors affecting on the production of strain MBS-HRS-EG (MYI1) metabolites with antifungal activity against *R. solani* by 54.1% of total exerted effect with percent controbutions 15.5, 13.5, 13.5 and 11.6 % respectively. The model showed adequate precision of 39.965. Signal to noise ratio "Adeq Precision" of the model was 13.3, which was considerably greater than 4, and indicated markedly the validity to use the model to navigate the design space.

The current results are consistent with those obtained [46], who reported that the marine strain STR2 exhibited promising antifungal activity against the filamentous fungi *Aspergillus niger, Aspergillus fumigatus,* and *Fusarium oxysporum*.

On the other hand, the obtained results were consistent with [47], who indicated that based on the antifungal properties of actinomycetes, two potential isolates, *S. amritsarensis* V31 and *K. karoonensis* MSCA185, showed high antifungal activity against all six fungal pathogens. The crude extracts of these two isolates were evaluated for their antifungal activity using an agar well diffusion assay.

Evaluation of antioxidant activity of strain MBS-HRS-EG (MYI1)

Antioxidant ANOVA results extended in Table 5 concerning antioxidant activity of strain MBS-HRS-EG (MYI1), significant model (p-value 0.0020) having F value of 80.60 was obtained. The propability that a “Model F-Value” could occur due to noise was only 0.2%. The model had the fitness parameters of: R-Squared, 0.9959; Adj R-Squared, 0.9835 which clearly indicated high fitness of the model.

The evaluation of the antioxidant activity of fermentation broth of strain MBS-HRS-EG (MYI1).The tested variables were depicted in Fig. s 1 and 2 showed that, sodium pyruvate, magnesium sulphate and soluble starch are the most effecting medium components to produce compounds with antioxidant potentials by 49.7% of total medium componets exerts with percent contributions 21, 20.9 and 7.8 % respectively. The model showed adequate precision of 28.657. Signal to noise ratio "Adeq Precision" of the model was 13.3, which was considerably greater than 4, and indicated markedly the validity to use the model to navigate the design space.

Pearson’s correlation coefficient between different biological activities of strain MBS-HRS-EG (MYI1).

According to results of Pearson’s correlation coefficient (r), the correlations between different biological activities of strain MBS-HRS-EG (MYI1) were realized. Nearly in all correlations, the coefficient r was closer to 0.90 which indicated strong correlation between different biological activities and suggests the existence of common principal active metabolite for such activities. Also, regarding the summarized Plackett-Burman data extended in Table 6, the common features between the models supported the strong correlation between bioactivities. Factor J was among the most affecting variables in all bioactivities. The second common affecting factor was A, which appeared among most effective in six of the seven studied bioactivities. The third common was factor K, which was most effective in three of bioactivities.

Significance of curvature in all obtained models implied a significant different potency of studied bioactivities at mid-level of tested variables. So, potencies of different bioactivities were reviewed along all levels of each of studied factors to elucidate significance increase or decrease at mid values and it was clearly observed an impressive superiority of all potencies at mid values of studied factors and of course emphasized the highest performance obtained at runs of center points. Accordingly, there was no need for further statistical studies as the fully optimized conditions were obtained at center points.

Scale-up production of bioactive secondary metabolites

Development of the fermentation process is usually carried out in three steps: (1) flask scale to screen strains and optimize the medium composition; (2) pilot scale to establish optimal fermentation conditions; (3) industrial scale process to produce

desired products economically. So, it is important to study scale-up of the fermentation process and adopt a suitable strategy in order to increase the productivity of the desired product to the industrial level.

The bioactive metabolites production in stirred tank bioreactor at different agitation speeds

The secondary metabolites production and carbohydrate consumption were studied in 7.5 L laboratory scale stirred tank bioreactor (STR) using the same final optimized medium composition and inoculum size as in the statistical experimental shake flask scale. Cells were grown in stirred tank bioreactor in batch mode at different agitation speeds (200, 400 and 600 round per minute (rpm). All other process variables (medium composition, cultivation condition and inoculum size / type) were kept constant. In general, as shown in Fig. s 4, 6 and 8, the secondary metabolites production curve was divided into two phases.

During the first phase, the production of secondary metabolites was started and increased gradually until reaching its maximal concentration which is expressed by antimicrobial bioassay. This carried concomitantly with increased consumption of carbohydrate until no carbon source remains in culture except in 600 rpm agitation speed. During the second phase, significant increasing until reached to the maximum then stability in the bioactive metabolite's concentration was observed according to its antifungal bioassay. Both the metabolites production and stability of the productivity were dependent on the applied agitation speed. However, the duration of the first phase (vegetative cell production phase) was varied and affected by the agitation speed followed by the second phase (antifungal metabolites production).

To enhance the fermentation production of pharmaceutical bioactive metabolites from marine *Streptomyces*, specific nutritional and cultural conditions are essential [24]. The metabolic performance of a microbial culture in a bioreactor is influenced by operating conditions like agitation intensity, microbial species, and nutrient supply. These factors affect bulk rheology and cellular morphology, impacting nutrient supply, oxygen levels, and mixing efficiency. Understanding the relationship between rheology and morphology is crucial in fermentations involving filamentous fungi and bacteria, where factors like strain, culture initiation method, growth medium, and hydrodynamic conditions in the bioreactor determine growth morphology. Excessive shear stresses can damage mycelial hyphae and pellets, while lower shear stresses can still impact growth morphology [47].

Cultivation at low agitation speed (200 rpm)

The performance of strain MBS-HRS-EG (MYI1) during cultivation at low agitation speed of 200 rpm was represented in Fig. s 3 and 4. The secondary metabolites production was started at 12 h and increased gradually.

The dissolved oxygen percentage (DO %) profile change in the fermentation culture was represented in Fig. 3. As shown, the DO % decreased gradually during the first phase of cell cultivation and metabolites production reaching almost its lowest value of 52.3 % at 168 h. Thereafter; it increased gradually during cell death and the stability of metabolites production phase until reached a value of 71.4 %.

On the other hand, the pH of the culture was increased gradually during all cultivation time until reached a value of 7.83 at the end of the fermentation time.

The production phase time duration was about 120 h and the maximal antifungal secondary metabolites production was determined by agar diffusion bioassay about 27, 26, 27, 28, 26 and 25 mm for fungal pathogens *C. albicans, C. tropicalis, A. niger, F. oxisporium, F. solani* and *R. solani* respectively. During this phase, the carbohydrates and proteins in culture were consumed almost completely at 132 and 72 h respectively (as shown in Fig. 4).

Cultivation at medium agitation speed (400 rpm)

In the present experiment, increasing the agitation rate aimed to study its effect on the overall secondary metabolites production process using the newly marine isolate strain MBS-HRS-EG (MYI1). During the fermentation time at agitation speed (400 rpm) as shown in Fig. 5, the secondary metabolites production started at from 24 h and its concentration increased gradually in the cultivation medium reaching maximal production till about 144 h.

During the production phase, higher rate of carbohydrate consumption was observed in fermentation culture until depletion was observed after 96 h of cultivations. It was detected that bioactive metabolites concentration remained constant till 180 h in the production medium. On the other hand, it has been observed that the pH of fermentation culture gradually increased with fermentation time to reach about 8.94 at the end of cultivation period.

The DO % decreased gradually during the first 132 h of cultivation reaching about 48.3 % saturation. Thereafter, the DO % in the fermentation culture increased gradually and reached about 70.1 % saturation at the end of cultivation time.

As listed in Fig. 6, the production phase time duration was about 144 h and the maximal secondary metabolites production was determined by agar

diffusion bioassay about 30, 28, 27, 28, 27 and 26 mm for fungal pathogens *Candida albicans, Candida tropicalis, Aspergillus niger, Fusarium oxisporium, Fusarium solani* and *Rhizoctonia solani* respectively. During this phase, the carbohydrates and proteins in culture were consumed almost completely at 96 and 60 h respectively.

Cultivation at high agitation speed (600 rpm)

In this experiment, inoculum cells were cultivated at high agitation speed to study the influence of high shear on secondary metabolites production, substrate consumption and other media profiles such as pH and DO % as shown in Fig. 7. The production of metabolites in the fermentation medium started during a long lag phase of 24 h until attained its maximal bio assayed production at 120 h cultivation. Thereafter, the metabolites undergo a stability production by full time of fermentation batch. On the other hand, different results were observed in the carbohydrate consumption rate. The carbohydrates were incompletely consumed along the fermentation period but the protein is completely consumed at 144 h from fermentation start. However, the pH in culture was increased significantly during the metabolites production phase reaching about 8.28 at the end of the batch.

The DO % value decreased gradually during the metabolites production phase parallel to the time of cell growth activity reaching its minimal value of about 61.1 % saturation after 156 h. Then, the DO % increased with higher rate compared to previous cultures reaching about 77.8 % saturation at 180 h at the end of cultivation time.

The production phase time as shown in Fig. 8, the duration was about 144 h and the maximal antifungal metabolites production was determined by agar diffusion bioassay about 26, 24, 26, 27, 23 and 26 mm for fungal pathogens *Candida albicans, Candida tropicalis, Aspergillus niger, Fusarium oxisporium, Fusarium solani* and *Rhizoctonia solani* respectively.

Morphological characterization of pellets during submerged cultivation in stirred tank bioreactor under 600 rpm

In case of filamentous microorganisms, as the pellet size increase, the cell activity inside the pellet decreased. This is due to the fact that, if the pellet diameter exceeds a certain value, the active cell growth is usually restricted to the outer growing layer of pellet [49]. As the cultivation time increased, the pellet become more hairy, dense and the pellet diameter increased at 84 to 180 h as shown in Fig. 9.

The current results were in accordance with Porcel, [50] who mentioned that the impact of pellet morphology on broth rheology was studied in pelleted submerged cultures of the lovastatin-producing fungus *Aspergillus terreus* in fluidized bed and stirred tank bioreactors. Agitation intensity influenced pellet diameter and compactness, but did not affect total biomass productivity. These effects were unrelated to oxygen transfer, as biomass concentration remained constant across different agitation intensities in the same reactor.

Variations in power input have a significant impact on the growth morphology and production of secondary metabolites in *Str. Acrimycini*. Higher power levels can lead to filamentous growth and increased production of bioactive compounds, but they can also reduce biomass yields and increase respiration rates due to higher shear stress. Young filamentous forms are the most productive, while intermediate power levels favor small pellet formation as cultivation progresses. These findings are consistent with El Enshasy, [49].

In vitro anticancer activity biological assessment

Four human cancer cell lines; HCT116 (Colon cancer cell line), MCF-7(Breast cancer cell line), A431 (Epidermoid carcinoma cell line) and A549 (lung cancer cell line) were assayed with fermentation broth of strain MBS-HRS-EG (MYI1). The results obtained as shown in Table 7 showed that the broth active against all cancer cell lines were used especially at the concentration 100 µg/mL with IC_{50} 75.8, 85.2, 91.5 and 108.5 µg/mL against MCF-7, A549, HCT116 and A431 cell lines respectively.

Marine *Streptomyces* produce bioactive compounds with cytotoxic properties. For example, Bafilomycin D has shown high cytotoxicity against EGFR-TKI-resistant human lung adenocarcinoma cells, with an IC_{50} value of 6.7 µM in A549 cells [51]. Atramycin B, an isotetracenone type antibiotic, is active against mouse leukemia P388 and was first isolated from *Streptomyces atratus* BY90 [52]. Maculosin, obtained from *Streptomyces sp.* KTM18, exhibits various biological activities such as antifungal, anticancer, antibiotic, and antioxidant properties [53, 54].

Conclusion

The study aimed to enhance the production of bioactive secondary metabolites from marine Streptomyces acrimycini strain MBS-HRS-EG (MYI1) ID: PP477812.1 through submerged fermentation. Various production media were evaluated, and the optimal one was chosen for further optimization using a Plackett-Burman design. The results indicated that $MgSO_4$, sodium pyruvate, and $(NH_4)_2SO_4$ were the most influential factors on production. Upscaling production in a 7.2 L stirred tank bioreactor at 200 and 400 rpm increased productivity and reduced production time, while a decrease was observed at 600 rpm. Cytotoxicity testing demonstrated activity against different cancer cell lines, with IC_{50} values of 75.8, 85.2, 91.5, and

108.5 µg/mL against MCF-7, A549, HCT116, and A431 cell lines, respectively.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

Non-applicable.

Author's Contributions

I.G.S.: Conceptualization, Methodology, Experimental and Data analysis, Software and writing original draft; A.E.A.: Conceptualization, Review and Supervision; H.M.A.: Conceptualization, Methodology, Review, Editing and Supervision; M.M.R.: Conceptualization, Review and Supervision; H.A.E.: Conceptualization, Methodology, Review and Supervision.

TABLE 1. Codes and levels of factors studied by Blackett-Burman design affecting production secondary metabolites by *Streptomyces acrimycini* strain MBS-HRS-EG (MYI1)

Factor code	Name	Low level (-1)	Mean level (0)	High level (+1)	Std. dev.
A	Sodium pyruvate (g/L)	0.20	0.300	0.40	0.093
B	Peptone (g/L)	0.25	0.500	0.75	0.231
C	Arginine (g/L)	0.75	1.000	1.25	0.231
D	Soluble starch (g/L)	3.00	4.000	5.00	0.926
E	Glycerol (g/L)	2.00	3.000	4.00	0.926
F	Dextrose (g/L)	2.00	3.000	4.00	0.926
G	$CaCO_3$ (g/L)	2.00	3.000	4.00	0.926
H	K_2HPO_4 (g/L)	0.75	1.000	1.25	0.231
J	$MgSO_4$ (g/L)	0.75	1.000	1.25	0.231
K	$(NH_4)_2SO_4$ (g/L)	1.00	2.000	3.00	0.926
L	Inoculum size (%)	5.00	10.000	15.00	4.629

TABLE 2. Plackett-Burman experimental design for factors affecting antifungal and antioxidant activity of *Streptomyces acrimycini* strain MBS-HRS-EG (MYI1)

Run no.	Factor A	Factor B	Factor C	Factor D	Factor E	Factor F	Factor G	Factor H	Factor J	Factor K	Factor L
1	1	-1	1	1	-1	1	1	1	-1	-1	-1
2	-1	-1	1	-1	1	1	-1	1	1	1	-1
3	0	0	0	0	0	0	0	0	0	0	0
4	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
5	-1	1	1	-1	1	1	1	-1	-1	-1	1
6	1	-1	-1	-1	1	-1	1	1	-1	1	1
7	1	1	-1	-1	-1	1	-1	1	1	-1	1
8	-1	1	-1	1	1	-1	1	1	1	-1	-1
9	1	-1	1	1	1	-1	-1	-1	1	-1	1
10	1	1	-1	1	1	1	-1	-1	-1	1	-1
11	-1	1	1	1	-1	-1	-1	1	-1	1	1
12	-1	-1	-1	1	-1	1	1	-1	1	1	1
13	1	1	1	-1	-1	-1	1	-1	1	1	-1
14	0	0	0	0	0	0	0	0	0	0	0

TABLE 3. Screening media for fermentation bioactivity metabolites production by strain MBS-HRS-EG (MYI1)

	Antifungal activity against different molds and yeasts*					
	C. albicans **ATCC.10231**	***C. tropicalis*** **ATCC.18807**	***A. niger*** **NRRL-3**	***F. oxisporium*** **ATCC.76616**	***F. solanii*** **ATCC.52628**	***R. solanii*** **ATCC.10186**
M1	15	13	14	11	13	11
M2	17	14	15	13	11	12
M3	13	12	Nil	Nil	14	12
M4	18	16	17	16	14	13
M5	18	18	20	16	16	15

* Determined by agar diffusion method where diameter of inhibition zone in mm was measured

TABLE 4. Antifungal and antioxidant activity of *Streptomyces acrimycini* strain MBS-HRS-EG (MYI1) obtained in Plackett-Burman design

Run No.	Antifungal activity against different molds and yeasts*						Scavenging activity % (DPPH)
	C.albicans	***C.tropicalis***	***A.niger***	***F.oxisporium***	***F.solani***	***R.solani***	
1	18	20.5	17.5	20.5	17.5	16	99.5
2	11	11	13	16	11	11.5	97.8
3	26	28	27	29	27.5	27	99.9
4	10.5	10.5	11.5	13	9	9	97.5
5	20	23	24.5	24.5	19	18	99.2
6	23.5	24.5	23	25	24.5	24.5	99.4
7	16.5	14	11	16.5	13.5	17.5	98.4
8	11	10	12	13	13	13	98.1
9	20.5	23	20	23.5	19	18.5	99.01
10	29.5	26	24.5	28.5	27.5	27.5	99.7
11	28	26	27	28	25	26	99.5
12	13.5	11	10.5	13.5	14	15.5	98.1
13	18	17	16	18.5	24	17	98.8
14	29.5	27.5	27	30	28	26	99.9

* Determined by agar diffusion method where diameter of inhibition zone in mm was measured

TABLE 5. Broad features (Principal data) of Plackett-Burman models obtained for different biological activities of strain *Streptomyces acrimycini* strain MBS-HRS-EG (MYI1)

Model specification	Antifungal activity against *C.albicans*	Antifungal activity against *C.fusarium*	Antifungal activity against *A.niger*	Antifungal activity against *F.oxisporium*	Antifungal activity against *F.solani*	Antifungal activity against *R.solani*	Scavenging activity %
p-value	0.0181	0.0195	0.0125	0.0094	0.0261	0.0070	0.0020
Significance and validity of model	Significant. There is only a 1.81% chance that a "Model F-Value" could occur due to noise	Significant. There is only a 1.95% chance that a "Model F-Value" could occur due to noise	Significant. There is only a 1.25% chance that a "Model F-Value" could occur due to noise	Significant. There is only a 0.94% chance that a "Model F-Value" could occur due to noise	Significant. There is only a 2.61% chance that a "Model F-Value" could occur due to noise	Significant. There is only a 0.7% chance that a "Model F-Value" could occur due to noise	Significant. There is only a 0.2% chance that a "Model F-Value" could occur due to noise
*F.P							
R^2	0.9819	0.9961	0.9975	0.9981	0.9768	0.9986	0.9959
Adj. R^2	0.9277	0.9765	0.9849	0.9887	0.9071	0.9916	0.9835
Signal/noise ratio	13.300	19.998	25.828	30.628	11.914	39.965	28.657
**Factors of significant effect	A, B, D, J, K, L	A, B, C, D, E, J, K, L	A, B, C, D, E, J, K, L	A, B, C, D, E, G, J, K, L	A, B, J, K	A, B, D, E, J, K, L	A, B, C, D, E, G, J, K, L
Factors of no effect	H, F	H	H	F	H, G	-	H, F
Most affecting factors	J, A, K	J, A, L	J, C, E	J, A, C, L	A, K, J	K, A, J	A, J, D
% Contribution of most affecting factors	44.9	50.86	48.87	48.32	47.18	42.37	49.71
Model equation	Activity (mm) = +0.083333 +26.66667 * Sodium pyruvate +8.66667 * Peptone +3.66667 * Arginine +1.75 * Soluble starch +0.91667 * Glycerol - 1.0 * CaCO3 -13.0 * MgSO4 +2.25 * Ammonium sulphate +0.4 * Inoculum size	Activity (mm) = –0.70833 + 27.917*Sodium pyruvate + 5.17*Peptone+ 8.167*Arginine +1.375 * Soluble starch +1.54167 * Glycerol - 0.45833 * Dextrose - 0.375 * CaCO3 - 14.83333 * MgSO4 +1.20833 * Ammonium sulphate +0.44167 * Inoculum size	Activity (mm) = +4.04167 +11.25 * Sodium pyruvate +6.5 * Peptone +8.5 * Arginine + 1.04167*Soluble starch +1.95833 * Glycerol -0.70833 * Dextrose -0.29167 * CaCO3 -15.16667 * MgSO4 +1.45833 * Ammonium sulphate +0.35833 * Inoculum size	Activity (mm) = +3.83333 + 20.417*Sodium pyruvate + 5.8333*Peptone +7.16667 * Arginine +1.125 * Soluble starch +1.70833 * Glycerol - 0.875 *CaCO3 -0.83333 * K_2HPO_4 -12.83333 * $MgSO_4$ +1.54167 * Ammonium sulphate +0.35833 * Inoculum size	Activity (mm) = –3.25 +29.16667 * Sodium pyruvate +9.0 * Peptone +4.66667 * Arginine +1.25 * Soluble starch +0.91667 * Glycerol -1.0 * Dextrose - 9.33333 * MgSO4 +2.91667 * Ammonium sulphate +0.21667 * Inoculum size	Activity (mm) = –1.5 +23.33333 * Sodium pyruvate +8.0 * Peptone +1.58333 * Soluble starch +1.0 * Glycerol -0.16667 * Dextrose -0.5 * CaCO3 +1.0 * K2HPO4 -9.33333 * MgSO4 +2.5 * Ammonium sulphate +0.43333 * Inoculum size	Activity (%) = +95.64 + 3.84167 * Sodium pyruvate + 0.79667 * Peptone + 0.87 * Arginine + 0.23417 * Soluble starch + 0.1175 * Glycerol + 0.099167 * CaCO3 - 1.53 * MgSO4 + 0.13250 * Ammonium sulphate + 0.036833 * Inoculum size

*F.P =Fitness parameters; ** **Factors of no effect =** (not included in the model)

TABLE 6. Pearson's correlation coefficient between different biological activities of strain MBS-HRS-EG (MYI1)

Activity against	Activity against *C.albicans*	Activity against *C.fusarium*	Activity against *A.niger*	Activity against *F.oxisporium*	Activity against *F.solani*	Activity against *R.solani*	Scavenging activity % (DPPH)
C.albicans	1.00	0.95	0.92	0.97	0.94	0.97	0.93
C.fusarium	0.95	1.00	0.97	0.99	0.91	0.90	0.96
A.niger	0.92	0.97	1.00	0.98	0.88	0.87	0.90
F.oxisporium	0.97	0.99	0.98	1.00	0.91	0.92	0.93
F.solani	0.94	0.91	0.88	0.91	1.00	0.93	0.92
R.solani	0.97	0.90	0.87	0.92	0.93	1.00	0.90
Scavenging activity % (DPPH)	0.93	0.96	0.90	0.93	0.92	0.90	1.00

TABLE 7. Cytotoxicity assessment of strain MBS-HRS-EG (MYI1) fermentation broth against mammalian cell lines

Cell lines	Inhibition of proliferation (%)				IC_{50} µg/mL
	Concentrations				
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	
HCT116	1.40	14.03	31.93	47.80	91.5±0.103
MCF-7	12.10	26.83	40.00	60.73	75.8±0.090
A431	0.37	2.20	14.50	37.67	108.5±0.105
A549	1.53	12.70	26.67	57.80	85.2±0.121

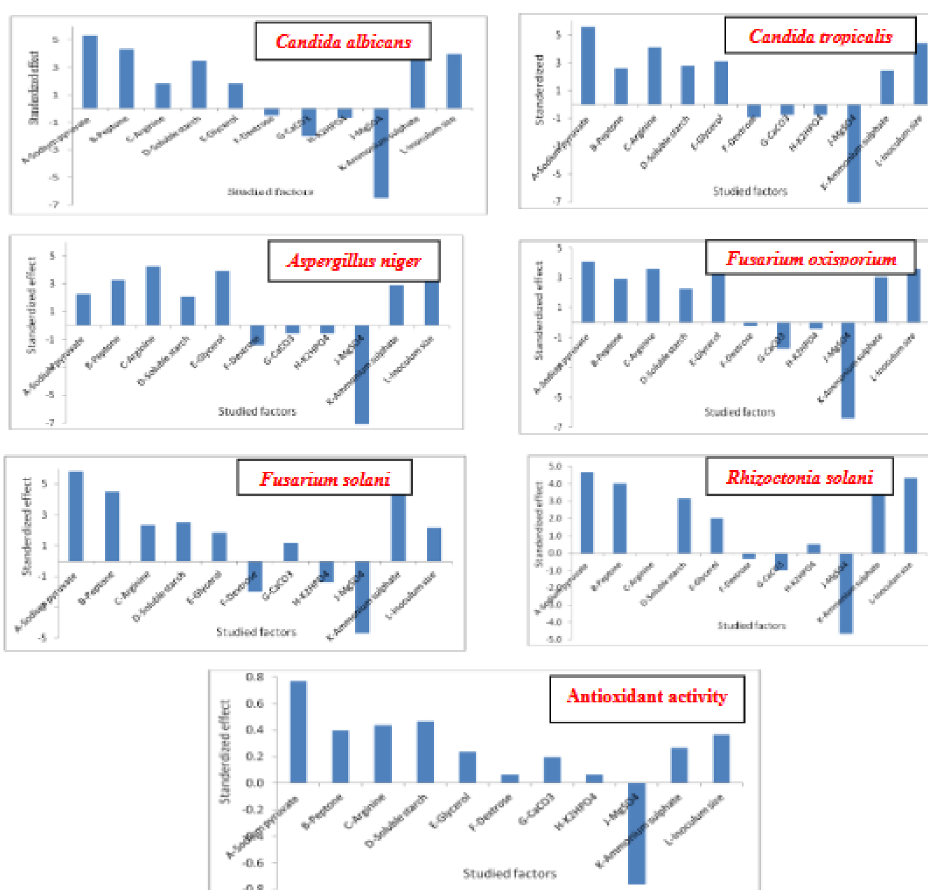


Fig. 1. Effect of different factors on antifungal and antioxidant activity of strain MBS-HRS-EG (MYI1) against different pathogenic strains as inferred from Plackett-Burman model.

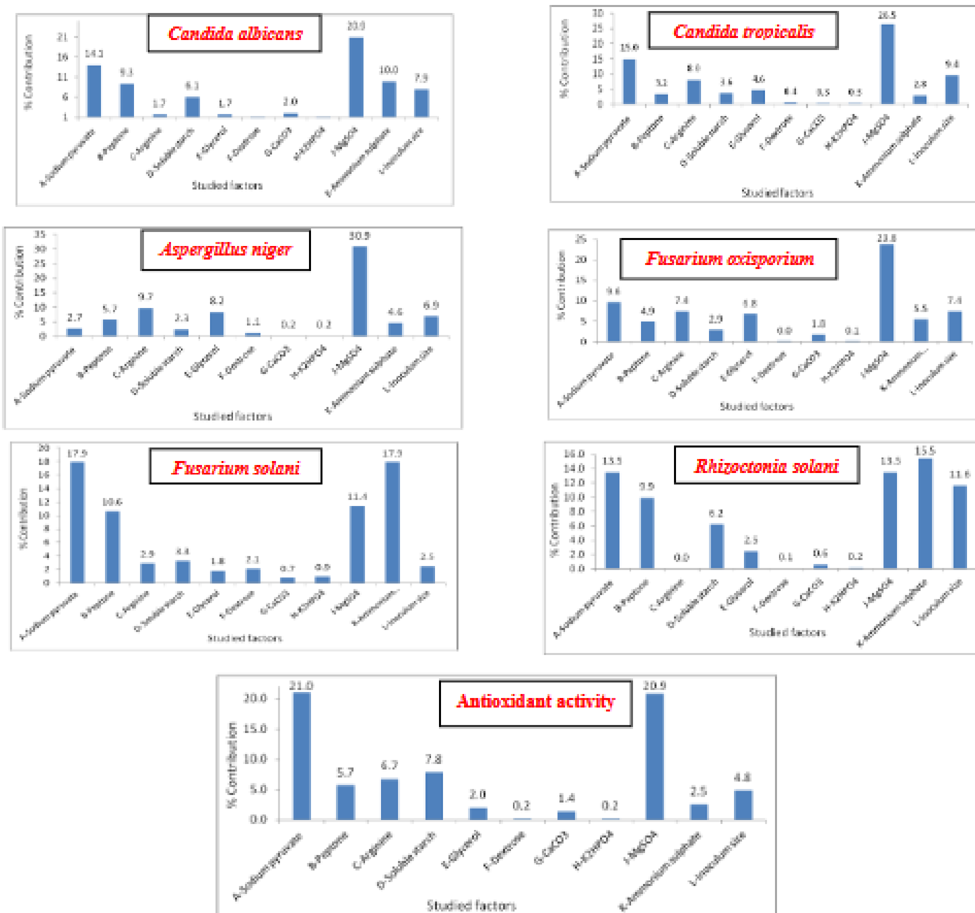


Fig. 2. Percent contributions of different tested factors on antifungal and antioxidant activity of strain MBS-HRS-EG (MYI1) against different pathogenic strains as inferred from Plackett-Burman model

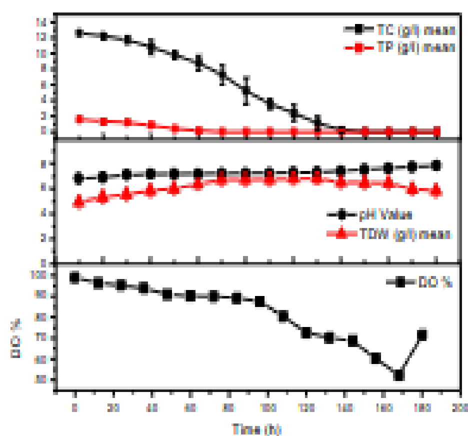


Fig. 3. Effect of agitation speed 200 rpm on total carbohydrates, total protein, pH value, total dry weight and DO percentage during the fermentation of strain MBS-HRS-EG (MYI1) in 7.5 L bioreactor

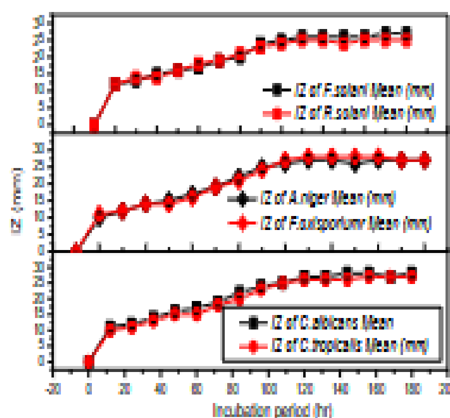
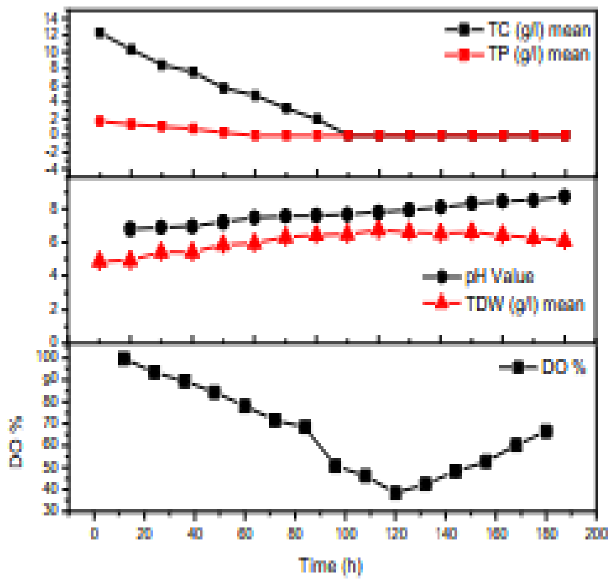


Fig. 4. Effect of agitation speed 200 rpm on antifungal metabolites production by strain MBS-HRS-EG (MYI1) in 7.5 L bioreactor.



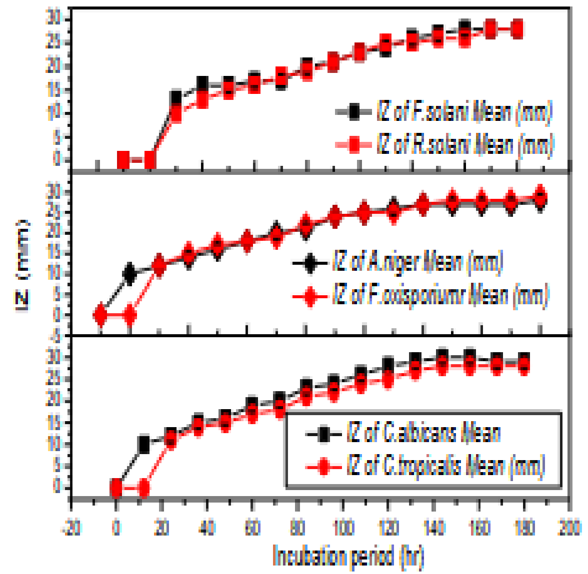
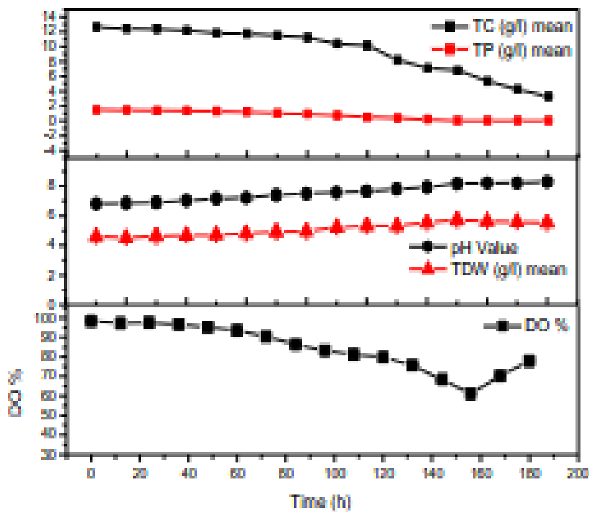


Fig. 5. Effect of agitation speed 400 rpm on total carbohydrate, total protein, pH value, total dry weight and DO percentage during the fermentation of strain MBS-HRS-EG (MYI1) in 7.5 L bioreactor.

Fig. 6. Effect of agitation speed 400 rpm on antifungal metabolites production by strain MBS-HRS-EG (MYI1) in 7.5 L bioreactor



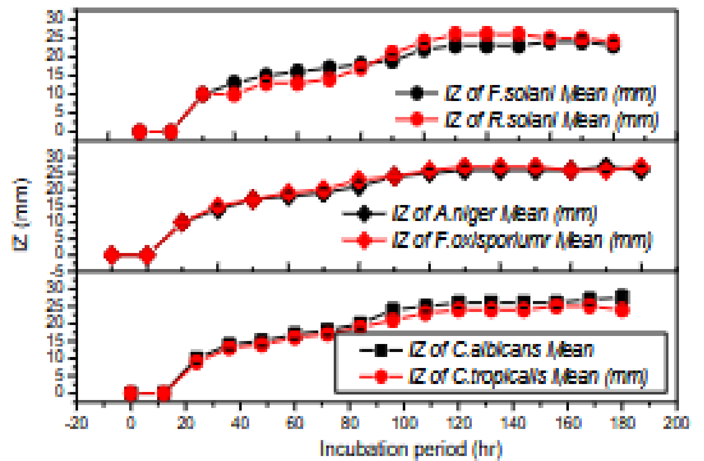


Fig. 7. Effect of agitation speed 600 rpm on total carbohydrates, total protein, pH value, total dry weight and DO percentage during the fermentation of strain MBS-HRS-EG (MYI1) in 7.5 L bioreactor

Fig. 8. Effect of agitation speed 600 rpm on antifungal metabolites production by strain MBS-HRS-EG (MYI1) in 7.5 L bioreactor.

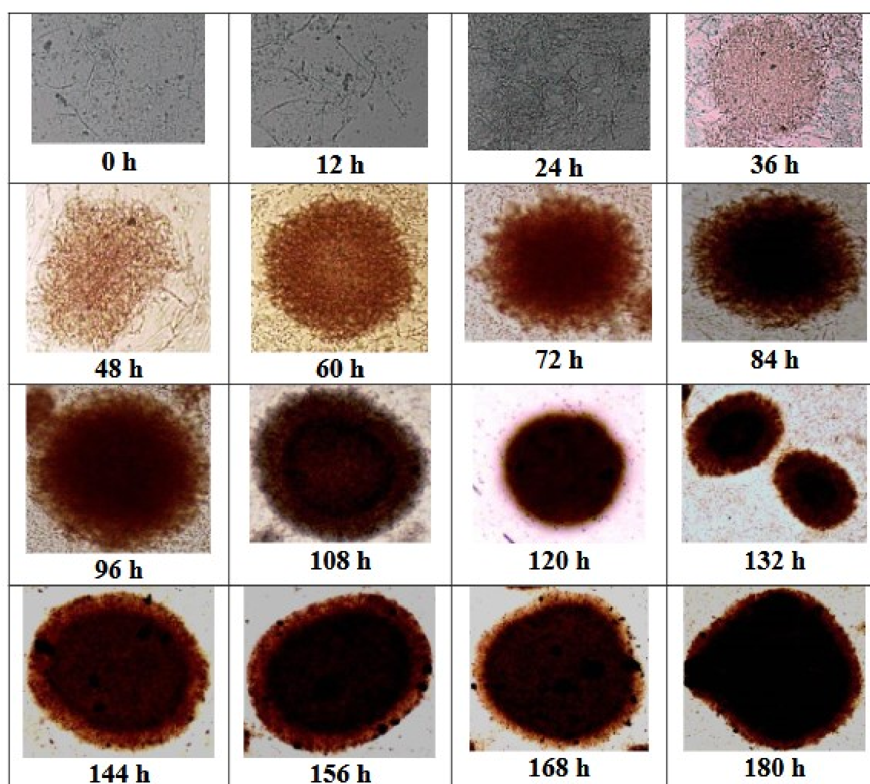


Fig. 9. Light microscopic photomicrograph of characteristic pellets growth of strain MBS-HRS-EG (MYI1) at different cultivation periods on stirred tank bioreactor 600 rpm.

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تحسين إنتاجية نواتج الأيض الثانوية والفعالة بيولوجيا من ستربتوميسيس أكريميسيناي (MBS-HRS-EG (MYI1 بإستخدام تصميم بلاكيت بيرمان وزيادة الإنتاجية بإستخدام المخمر الحيوي 7.5 لتر مع عمل التقيمات الحيوية كمضادات للفطريات، مضادات أكسدة ومضادات للسرطان

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الملخص

تُعرف الأكتينوميسيتات البحرية، وخاصة جنس الإستربتوميسيس، بإنتاجها لمركبات نشطة بيولوجيًا متنوعة تعمل كمضادات للفطريات ومضادات للأكسدة وكذلك كمضادة للسرطان. تهدف هذه الدراسة إلى تحسين إنتاجية نواتج الأيض الثانوية و النشطة بيولوجيًا بواسطة سلالة *Streptomyces* MBS-HRS-EG (MYI1) ID: PP477812.1 *acrimycini* البحرية من خلال التخمرات المغمورة. تضمنت هذة الدراسة فحص الأنشطة المضادة للفطريات ومضادات الأكسدة باستخدام طريقة الإنشارمن خلال الآجار وطريقة DPPH. تم عمل دراسة مسحيه على البيئات المنتجه للنواتج الثانويه واختيار افضلها انتاجاً، و تم تحسين انتاجيه افضل البيئات المختارة باستخدام النموذج الاحصائى والذى يسمى تصميم بلاكيت-بيرمان (PBD) مع أحد عشر عاملاً وأربعة عشر دوره. أظهر التحليل الإحصائي نماذج مهمة ذات عدم توافق قدره 0.212 وR2 أعلى من 0.9768 لجميع الاستجابات. و كانت كبريتات المغنسيوم، بيروفات الصوديوم، وكبريتات الأمونيوم من متغيرات النشاط الحيوي الرئيسية والأكثر تأثيرا فى زيادة الانتاج. تم اختبار الظروف المثلى الناتجه من دراسة التحليل الاحصائى وتطبيقها على مستوى المفاعل الحيوي سعة 7.5 لتر بسرعات دوران مختلفة من 200 الى 600 لفة فى الدقيقه، حيث أظهرت الدراسة إنتاجية أعلى للمركبات النشطة بيولوجيًا عند 200 و400 دورة في الدقيقة. كشف تقييم السمية الخلوية عن فعالية المركبات النشطة بيولوجيًا ضد الخلايا السرطانية المختلفة، حيث تتراوح قيم IC_{50} من 75.8 إلى 108.5 ميكروجرام/مل.

الكلمات الدالة: الإستربتوميسيس البحرية – التحسين الإحصائي من خلال بلاكيت بيرمان – مضادات فطريات، مضادات أكسدة ومضادات للسرطان – زيادة الإنتاجية على مستوى المفاعل الحيوي سعة 7.5.