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ORIGINAL PAPER

Induction of metabolic variability of the endophytic fungus *Xylaria* sp. by OSMAC approach and experimental design

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Abstract

This work has as the main focus, to analyze the behavior of physic-chemical variations from the fungus *Xylaria* sp., through the OSMAC (One Strain, Many Compounds) approach as an efficient way of obtaining new compounds. To perform such inductions and to explore the variability of the metabolic network of this microorganism, a factorial design was designed to induce variability (or enhancement) of metabolites. In view of chemometric insights, the planned inductions were imposed on the microorganism and variations in the chemical profile were observed in the crude extracts. Through mass spectrometry (HR-ESI–MS) and nuclear magnetic resonance-based profiles, combined with multivariate analysis through Principal Component Analysis (PCA), it was observed a marked variability of signals, confirming the efficacy in the metabolic alteration, defining the culture medium and agitation as the most important variables in the metabolic variability of the fungus. However, the extract mass is more significant for the agitation variable, and there is no relationship between the mass of crude extract and the amount of molecular signals of the complex matrices studied.

Keywords Fungal metabolites · Endophytic fungi · Dereplication · Factorial design · NMR · ESI–MS

Introduction

Endophytic fungi are a group of microorganisms that inhabit the interior of the tissue plant, at least during part of the life cycle of its host (Clay 1988) and consist of a promising source of bioactive natural products because they are able to biosynthesize a large variety of secondary metabolites. The fungus of the genus *Xylaria* has demonstrated a rich source of secondary metabolites with high chemical and biological variability (Zhang et al. 2015). Several secondary metabolites produced by fungi of the genus *Xylaria* have been identified at literature, as follows: terpenoids, diterpenes, triterpenes, steroids, cytochalasins and cyclopeptide (Becker and Stadler 2021).

Those metabolites have bioactivity such as antifungal, anticholinesterase, antimalarial and cytotoxic activities, showing the potential of these fungi genera in the production of bioactive substances. Many of the main drugs currently come from microorganisms like Xylariales, and some of their genera constitute one of the most prolific sources of secondary metabolites in the fungal kingdom (Helaly et al. 2018), opening up the possibility of rational exploitation of these species.

Thus, only a minority of genes are expressed under standard laboratory conditions and many valuable compounds are neglected. To overcome this drawback and provide a better exploration of the metabolic potential of microorganisms, many regulatory strategies are performed to activate dormant pathways to facilitate discovery of new natural products by modifying the culture conditions; stress; co-culture and genomic approaches (Pan et al. 2019).

OSMAC (One Strain, Many Compounds) is a useful method that works on the concept that a microorganism can

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produce several secondary metabolites using particular techniques, for example, change of physico-chemical parameters such as pH, substrates (culture media), temperature, agitation and light during fungal culture (Hewage et al. 2014). The OSMAC approach allows the induction of silent biogenetic cluster pathways, allowing the accumulation of new compounds during the fermentation process (Hemphill et al. 2017), which are activated in specific conditions (Selegato et al. 2019).

Many secondary metabolites of great economic importance are produced in low yields or even are not produced by some fungal species due to the use of unfavorable cultivation conditions (Bills et al. 2008). However, production of bioactive metabolites as a result of manipulation of growing conditions has been shown to be important in the discovery of novel major bioactive metabolite (Liu et al. 2016), which isolated three new polyketides. Wortmannilactones polyketides were purified from the same organism using the OSMAC approach, varying only the cultivation conditions. It is reported that change in the composition of the culture medium produced new compounds of pharmacological interest (Wijesekera et al. 2017).

There are reports that such variations can completely change the metabolic profile of many microorganisms, inducing metabolic pathways not previously activated. Thus, a wide variability of metabolites may be obtained or boosted using this approach (Wang et al. 2013). So, OSMAC presents itself as a tool with great potential to exploit the chemical diversity of the secondary metabolites of several genera of microorganisms. The choice of the parameter to be changed is usually random; therefore, such variations are known as random inductions. In general, it is carried out a lot of experiments making numerous changes in order to verify the increase of metabolic variability in a particular microorganism. The handling of such conditions has been shown to have substantial impact on the quantity and diversity of production of secondary metabolites (VanderMolen et al. 2013).

Aimed to express and optimize the experimental conditions held inductions not random, but rather planned inductions, supported by chemometric tools to create an experimental design based on factorial design to maximize the achievement of results because make inductions planned instead of random inductions imply: process time reduction; operational cost reduction related to culture media and organic solvents used in the extraction of metabolites; improved process yield; choice of targeted form of variables.

Materials and methods

Fungal strain

One strain of the genus *Xylaria* was obtained from the culture collection of the microorganisms from Laboratory of the Department of Organic Chemistry—Institute of Chemistry (UNESP). This endophytic fungus was isolated from *Casearia sylvestris* plant. The fungus was maintained in sterile water at a storage temperature of approximately 4 °C.

Activation and production of inoculum

The strain was activated and propagated on nutrient agar, consisting of: beef extract (1 g L^{-1}); yeast extract (2 g L^{-1}); peptone (5 g L^{-1}); NaCl (5 g L^{-1}); and agar (15 g L^{-1}). The pH value of the media was 6.8. The strain activated was propagated at 25 ± 1 °C for 10 days. After the incubation period, agar plugs containing fungal mycelium were used as inoculum for the induction of metabolic variability of *Xylaria* sp. by submerged fermentation.

Induction of metabolic variability of Xylaria sp.: One Strain, Many Compounds (OSMAC)-based experimental design strategy

Submerged fermentation was employed for induction of metabolic variability of *Xylaria* sp. Tests occurred in 500 mL Erlenmeyer flasks with 300 mL of reaction medium. Agar plugs containing fungal mycelium were transferred aseptically to the media and incubated at 25 ± 1 °C until the fungus reached the stationary phase (28 days). Composition of the medium for induction of metabolic variability and physical parameters of the incubation varied according to experimental design.

Three significant factors (independent variables) were studied by 2^3 full factorial design (FFD): growth media, agitation, and luminosity. Independent variables established in the research were qualitative (type of growth media, absence or continuous presence of luminosity and agitated or static culture) and based on studies previously conducted with the genus *Xylaria* (Table 1). The response variable was crude extract (mass in mg) in all tests. Statistical analysis of the data was associated with a scan of the metabolic profile of crude extracts by HPLC–DAD-MS and ^{1}H-NMR. The independent variables were tested at two different levels (+ 1 and − 1) resulting in eight assays. Statistical analysis of each variable and their interactions were evaluated at 5% significance (95% confidence interval) with Minitab® 19 software

Table 1 Actual and coded values of the independent variables 2^3 FDD

Independent variable	Levels	
	(−1)	(+1)
Growth media	Czapek	Nutrient
Agitation	Static	100 rpm
Luminosity	Absent	24 h

(Minitab Inc., State College, Pennsylvania, USA). The same software generated the figures and tables presented in the results. Table 1 lists the independent variables involved in the process and their respective levels. OSMAC experiments were performed in duplicate under homogeneous conditions and the experimental sequence was randomized to minimize the effects of uncontrolled factors.

Extract preparation

After the incubation period, the biomass produced in the different tests was removed by vacuum filtration using a Whatman #4 filter. The filtrates were subjected to three successive liquid–liquid extractions with ethyl acetate—AcOEt (150 mL). Organic phase was combined and the solvent was removed under vacuum on a rotary evaporator.

High-performance liquid chromatography with diode array detection (HPLC–DAD-MS) analysis

Crude extracts obtained in 2^3 FFD were analyzed by HPLC–DAD-MS. Samples were prepared by weighing 4 mg of each extract and solubilizing them in 1 mL acetronitrile. The chromatograph used was a Shimadzu LC coupled to pumps model-6 AD Shimadzu SCL-10VP control system, Shimadzu, UV diode array detector (VIS-Shimadzu 1520 PC—Diode Array). The separations were accomplished using a reversed-phase Phenomenex Kinetex column (150 × 4.6 mm, 5 µm). Elution was done with binary system of H_2O-ACN containing 0.1% of formic acid in each phase. An exploratory gradient was performed according to the following ratio: H_2O: ACN (1:19, $v.v^{-1}$) to 0:1 ($v.v^{-1}$) at 40 min.

Metabolic profiles of extracts *Xylaria* sp. culture were obtained by coupling HPLC (Shimadzu Prominence Model High-pressure pumps, LC-20AD model, flow range: 0.0001–10.0000 mL min^{-1} spectrophotometric detector "Photodiode Array" model SPD-M20A, Shimadzu. Degasser, DGU-20A-5 Model, Shimadzu) to the High-Resolution Mass Spectrometer Bruker, micrOTOF II using electrospray ionization. Parameters used for ionization: capillary voltage was 3500 V and source temperature was 200 °C. The nebulizer gas was nitrogen, at a pressure of 5.5 bar. The gas flow rate was set at 10 L min^{-1}. The same samples, with same concentration (1.0 mg mL^{-1}), were prepared to obtain the chromatographic profile of the crude extracts. The data were processed using Bruker software Data Analysis 4.1.

Nuclear magnetic resonance spectroscopy (NMR) analysis

The acquisition ^{1}H-NMR data were at Bruker Avance 600 with cryoprobe core 3 mm. The samples were prepared in 3 mm tubes, solubilizing 1 mg of the crude extract in 240 µL of deuterated methanol (CD_3OD) from Cambridge Laboratories Isotope with 99.8% purity. ^{1}H-NMR analyses were performed for each sample and the data processed with Mestrenova software version 6.0.2 and Tospin 4.0 (Bruker Corporation).

Results and discussion

Design experimental and statistical analysis

Tests predicted by the 2^3 FFD attest to the versatility of *Xylaria* sp. to produce biomass in chemically defined culture medium (Czapek) or in complex medium (nutrient broth), regardless of the previously established physical parameters (absence or continuous presence of luminosity and shaking or static culture). Therefore, production of metabolites with different culture media is distinct, such as been observed comparing a chemically defined medium where the only carbon source is sucrose and complex medium where the carbon sources are associated with organic nitrogen sources (beef extract, yeast extract, and peptone).

Amounts (mg) of the crude extracts from the submerged fermentation of *Xylaria* sp. ranged between 20.9 and 101.4 mg (Table 2). The largest mass of crude extract (101.4 mg) occurred in run 3, with Czapek media, absence of luminosity and under agitation (100 rpm). Yet, a lower mass of crude extract (20.9 mg) was detected in run 6 with nutrient broth, presence of luminosity and under static conditions.

Therefore, effects involved in the mass crude extractions were statistically evaluated by the normal plot of the standardized effects, Pareto charts, and plots of the main effects and interactions.

Table 2 Matrix with coded values for the variables and response mass crude extract

Test*	Growth media	Agitation	Luminosity	Crude extract (mg)
1	− 1	− 1	− 1	61.1
2	+ 1	− 1	− 1	31.1
3	− 1	+ 1	− 1	101.4
4	+ 1	+ 1	− 1	54.6
5	− 1	− 1	+ 1	23.5
6	+ 1	− 1	+ 1	20.9
7	− 1	+ 1	+ 1	95.9
8	+ 1	+ 1	+ 1	21.4

*All experiments were performed in duplicate and the determinations of mass crude extract

In the normal plot of the standardized effects, the effects and interactions with negative coefficients (negative signs) are located in the region less than 50%, whereas the factors and interactions that have positive coefficients (positive signs) are in the region above 50%. All non-significant factors and interactions in the mass of the crude extract approach a straight line (center line crossed the zero value on the abscissa at 50% probability), while those considered significant in the response are distanced from the straight line (Fig. 1).

Main effects A (growth media), B (agitation) and C (luminosity) e the interaction terms AB (growth media × agitation) and ABC (growth media × agitation × luminosity) were significant in the variable response mass of crude extract. The negative signs of the main effects (A e C) and interaction terms (AB e ABC) indicate a reduction in the mass crude extract when their levels were changed. The positive sign of the main factor B causes an in the mass crude extract.

Standardized effects, in absolute values, may also be represented on Pareto charts (Fig. 2). Effects higher than 2.31 ($P = 0.05$), located to the right of the dividing line, are significant. The absolute standardized values of the effects of each factor and of the interactions correspond to the Student's *t*-test calculated for each independent variable.

The main effect plots of crude extract production are shown in Fig. 3. In this context, the change from Czapek medium level (low level) to the nutrient medium level (high level) in independent variable growth media would cause a decrease in the crude extract mass around 38.5 mg. A similar effect is observed in independent variable luminosity, since conducting the experiments continuously in the presence of light (high level) would cause a mean reduction of 21.63 mg in the mass of the crude extract. In contrast, a different impact of the independent variable agitation was observed in the response variable. When the *Xylaria* sp. strain is incubated under agitation (100 rpm, high level), an increase of 34.13 mg in the mass of the crude extract is suggested.

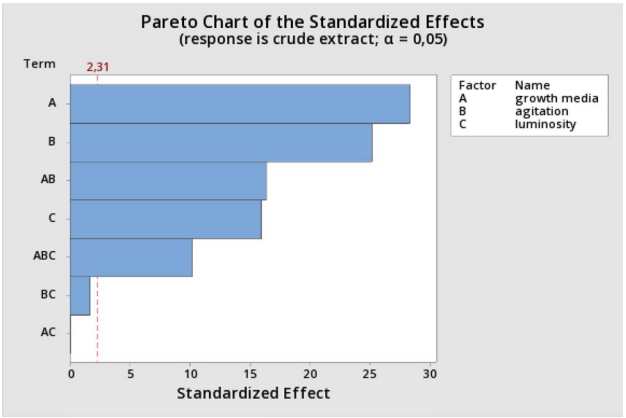

Fig. 2 Pareto chart of standardizes effects for response mass crude extract (Minitab® 19 software Minitab Inc., State College, Pennsylvania, USA)

The analysis of Fig. 4 shows the effects of the interactions for response mass crude extract. All interactions were pointed and considered, including those that were not significant in the response mass crude extract. The interaction between growth media and agitation had the greatest impact on the mass of crude extract. When the fungus is cultivated in Czapek (high level) and under agitation (high level), the largest mass determination of the crude extract (101.4 mg) was recorded. However, when this same combination of factors is evaluated, there is a reduction in the mass of the crude extract if *Xylaria* sp. is grown in nutrient broth (low level), regardless of the incubation conditions (static or shaking condition). In addition, the combination growth media and luminosity provided an increase in the mass of the crude extract when tests were performed with Czapek medium (low level) in the absence of light (low level). In the same conditions of incubation (absence

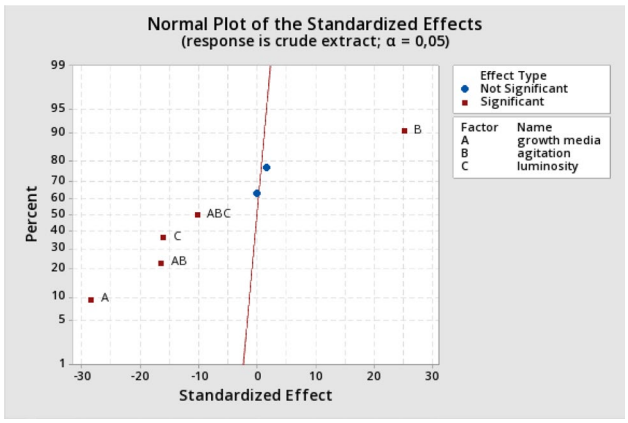

Fig. 1 Normal probability plot of standardized effects for response mass crude extract (Minitab® 19 software Minitab Inc., State College, Pennsylvania, USA)

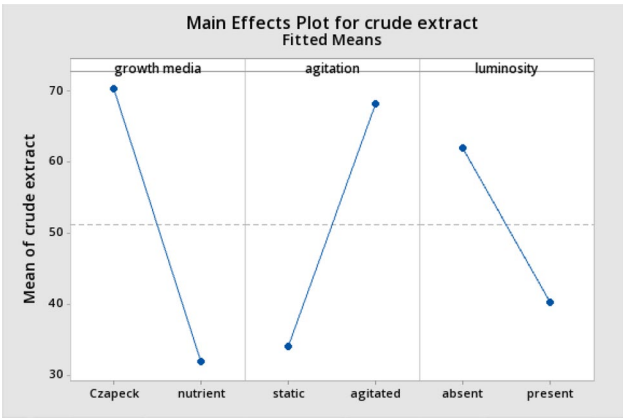

Fig. 3 Main effects plot for response mass crude extract (Minitab® 19 software Minitab Inc., State College, Pennsylvania, USA

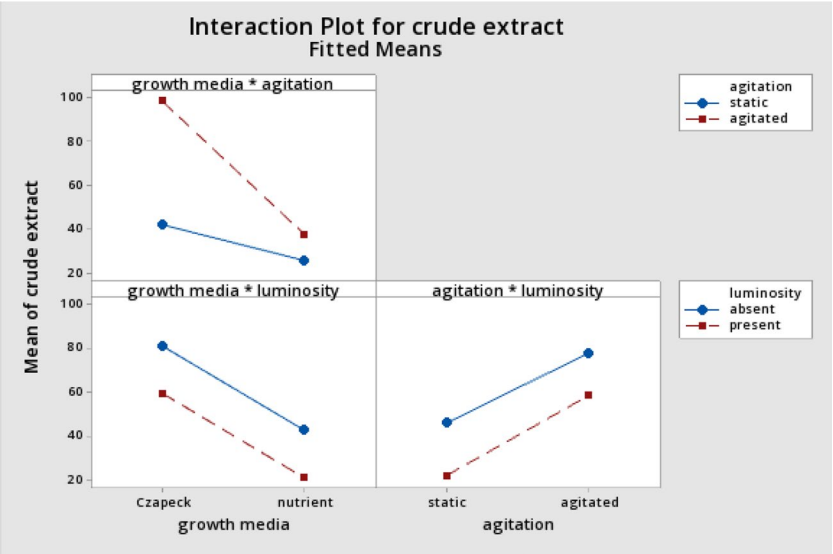

Fig. 4 Interaction effects plot for response mass crude extract (Minitab® 19 software Minitab Inc., State College, Pennsylvania, USA)

of light), the mass of the crude extract decreases if the fungus is grown in nutrient medium (high level). In addition, cultures conducted in nutrient medium (high level) in the presence of light (high level) showed the lowest mass determinations of the crude extract (20.9 mg). In the agitation and luminosity combination, the highest mass values of the crude extracts were verified in cultures conducted in the absence of light (low level) and under agitation (high level).

Considering all the factors involved in the yield of the crude extract, the model is predicted. Table 3 shows the estimated effects and the regression coefficients, accompanied by the corresponding values of the Student's *t*-test and the probability (*P*). Significance of the regression coefficients was verified by these tests.

value with the tabulated *F* value (Table 4). In the analysis of variance only the significant parameters in which the *P* value was under 0.05 were considered and the non-significant factors added to Lack of fit. Tests suggested that the model behaved linearly for the response variable (mass crude extract).

ANOVA applied to experimental design with *Xylaria* sp. showed R^2 at 99.48%. Under the conditions investigated, the regression F test (382.14) was higher than the tabulated value (3.33) and no significant lack of fit (1.41) reaffirmed that the data fit the linear model.

Then, after discarding the interactions that were not significant in the model, the experimental design data were checked for the normal distribution. In the normal distri-

$$\begin{aligned}\text{crude extract (mg)}: \; & 51.213 - 19.238\ \text{growth media} + 17.063\ \text{agitation} \\ & - 10.813\ \text{luminosity} - 11.088\ \text{growth media} \times \text{agitation} - 0.038\ \text{growth media} \\ & \times \text{luminosity} + 1.137\ \text{agitation} \times \text{luminosity} - 6.888\ \text{growth media} \times \text{agitation} \times \text{luminosity}\end{aligned}$$

In the sequence, the model was validated by analysis of variance (ANOVA) with *F* test, comparing the models' *F* bution of data, the residuals tend to approach a straight line. For the evaluated response (mass crude extract), the

Table 3 Estimated effects and coefficients for response mass crude extract

Factors	Effect	Coef. Regression	*t*-Student	*P*
Constant	79.49	51.213	75.62	0.000
Growth media	− 38.47	− 19.238	− 28.41	0.000
Agitation	34.12	17.063	25.20	0.000
Luminosity	− 21.62	− 10.813	− 15.97	0.000
Growth media × agitation	− 22.17	− 11.088	− 16.37	0.000
Growth media × luminosity	− 0.07	− 0.038	− 0.06	0.957
Agitation × luminosity	2.27	1.137	1.68	0.132
Growth media × agitation × luminosity	− 13.77	− 6.888	− 10.17	0.000

Table 4 ANOVA of the regression model for response mass crude extract

Source of variation	Sum of squares	Degrees of freedom	Mean of squares	*F* test
Regression	15175.9	5	3035.17	382.14
Residual	79.4	10	7.94	
Lack of Fit	20.7	2	10.36	1.41
Pure error	58.7	8	7.34	
Total	15255.3	15		

Regression: $F_{0.05;5,10}$ (*F* tabulated) = 3.33 R^2 = 0.9948; R^2 adj = 0.9922 Residual: $F_{0.05;2,8}$ (*F* tabulated) = 4.46

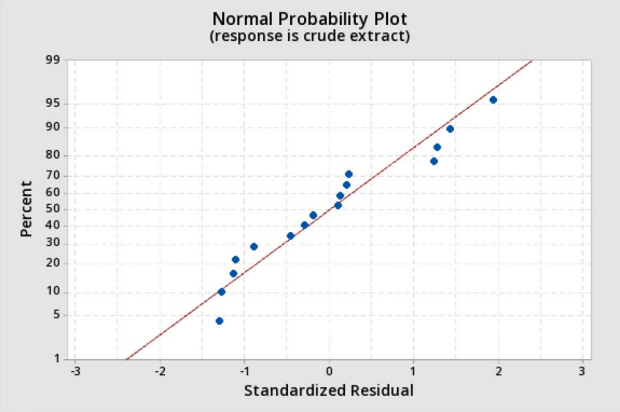

Fig. 5 Normal probability plot of the residual for mass crude extract (Minitab® 19 software Minitab Inc., State College, Pennsylvania, USA)

residues followed a normal distribution. In addition, standardized residues ranging from − 3 to + 3 suggested the absence of outliers and the regression hypotheses were satisfied (Fig. 5).

Therefore, to produce crude extract reduced model at the range set by the study was: crude extract (mg) : 51.213 − 19.238 growth media + 17.063 agitation − 10.813 luminosity −11.088 growth media × agitation − 6.888 growth media× agitation × luminosity

Research discussion so far has focused on evaluating, through experimental design, the impact of different factors on the production of crude extracts from submerged cultivation of *Xylaria* sp. Construction of a statistical model, which estimated the amount of crude extract generated by pre-established nutritional conditions and physical parameters for fermentation with *Xylaria* sp., was pertinent and revealed that the variable culture medium was the most impacting factor in the generation of these crude extracts. In addition, the interference independent of the physical parameters of the fermentation in the exploration of metabolic variability of *Xylaria* sp. in Czapek and nutrient media is discussed. The, the variable culture medium is fixed and the metabolic variability discussed as a function of changes in the fermentation operational conditions (agitation and luminosity). Such as, the data were grouped into two large blocks. At this stage, comparisons of tests obtained from cultivation in Czapek are presented, and subsequently the ones obtained from the cultivation nutrient. The discussion of chromatographic profiles and NMR spectra was, thus, organized.

Chromatographic profile by HPLC–DAD-MS analysis

Figures S6 and S7 show the chromatographic profiles of conditions 1 and 3 performed on Czapek medium, respectively. Test 1 was performed with low levels (agitation and luminosity), so it was an experiment performed under static conditions and without light. And test 3 with agitation (high level) and absence of light (low level). Results demonstrated that conducting the experiments under agitation (test 3) increased the crude extract mass by about 40 mg. However, it was observed that including this operational variable (agitation) in the tests under absence of light caused a decrease in the number of signals when comparing the chromatographic profiles since tests 1 and 3 recorded 44 and 41 signals (chromatographic bands or peaks) automatically detected, respectively.

In the analysis of the profiles of the TIC (Total Ion Chromatogram) of HPLC–DAD-MS is possible to verify that the planned inductions induce the fungus to change its metabolism. When the regions of the chromatograms are observed, it is evident that there are specific signs present in one TIC, and which are absent in another. Test 1 is depicted in blue and test 3, red.

Most of the metabolites produced are probably the same based on common retention time values but different signals are shown in the comparison of the two assays in different culture conditions. Some regions are similar, but there are signs, such as 13, 24, 26, 29, that do not appear in test 3 (red). Agitation and light are factors which can change the metabolic profile of a microorganism, and the difference in quantity of signs can prove it, making clear that such inductions can arouse or inhibit certain metabolic pathways of the fungus.

This time, the comparison is between the test 5, static (low level) and exposed to light (high level), with test 7, cultivation under agitation (high level) and incidence of light

(high level). Experiments conducted statically and exposed to light show an increase in the mass of crude extract by about 308%. However, as previously pointed out in tests 1 and 3, the increase in the mass of the crude extract did not reflect an increase in the number of signals. Tests 5 and 7 recorded, respectively, 53 and 46 signals in the chromatograms (Figs. S8 and S9).

The regions of the chromatograms (from 10 to 25 min and 20 to 29 min) demonstrated that tests 5 and 7 exhibit similar bands, but there are signals in test 5 not appear in the other one (12, 20, 22).

The highest metabolic variability was associated with *Xylaria* sp. in nutrient broth. The chromatograms for the tests under these experimental conditions exhibited the highest signal numbers. Interestingly, although the results suggest greater metabolic variability when *Xylaria* sp. was cultivated in nutrient broth, highest crude extract mass values were recorded when the fungus grew in Czapek medium. Figures S10 and S11 comprise the chromatograms of the nutrient medium extracts to explore the metabolic changes induced by the variation of the culture conditions. Test 2 was performed with under static conditions and without light (low levels), and test 4 with agitation (high level) and no light (low level). As demonstrated in Czapek assays, the inclusion of agitation (test 4) increased the crude extract mass by about 23.5 mg. However, the inclusion of agitation, under absence of light, promoted an increase in the number of signals when comparing the chromatographic profiles. Tests 2 and 4 recorded 63 and 74 signals, respectively.

The regions of the chromatograms demonstrated (from 6 to 33 min and 20 to 31 min) that tests 2 and 4 exhibit similar peaks but there are signs in test 2 that do not appear on test 4, such as 19, 20, 30, 36, 37, 39, 40, 42, 48 e 49. In test 2 despite having only 63 signals, these are more intense compared to test 4.

Figures S12 and S13 comprise the tests 6 (in orange) and 8 (in gray). Experiment 6 was conducted statically (low level) and exposed to light (high level), while test 8 was performed under agitation (high level) and exposed to light (high level). Tests 6 and 8 showed practically the same values of crude extract mass. Unlike previous notes, the inclusion of agitation in test 8 during incubation of *Xylaria* sp. did not trigger an increase in crude extract mass. The highest number of chromatographic signals was observed in test 8, in this type of culture condition 63 signals were observed. Test 6 showed 55 signs.

To explore the two chromatograms more closely, the regions from 5 to 15 min and 18 to 31 min exhibited considerable similarity; however, signals 35, 14 and 18 showed in test 8 are not present in test 6. In contrast, the chromatographic signals 12, 35, 45 and 48 showed in test 6 were not identified in test 8.

Metabolites profile of crude extracts by ^{1}H-NMR analysis

The ^{1}H-NMR spectra of all extracts from the factorial design are compared (Fig. S14) in attempt to further understand the behavior of the fungus *Xylaria* sp. under influence of selected variables, and provide another results to assert that OSMAC was reached, or simply prove that metabolites production was boosted. ^{1}H-NMR technique, which possesses a universal detection capability, allows comparison among the variation of the metabolic profile produced by the fungus. The ^{1}H-NMR spectra are shown for test 1, 3, 5 and 7, which correspond to cultivations in Czapek medium.

^{1}H-NMR spectra comparison showed hydrogen aromatic/olefinic signals that feature oxidation of aromatic systems with chemical displacement between 6.5 and 9.0 ppm with broad signals, characteristics of amine group hydrogen.

It is important to verify that tests 1 and 3 were conducted both trials were grown in the dark (absence of light), however, test 3 was run under agitation.

Figure S14 shows that there is indeed a variation in the profile of complex matrices obtained from the same type of culture medium. The tests 5 and 7, both were cultivated in the presence of light, but 7 was stirred throughout the process.

The ^{1}H-NMR spectra of the assays using nutrient medium, which correspond to the tests pairs 2, 4, 6 and 8 (Fig. S15).

The spectra 2 and 4 are very similar. However, the highlighted region shows that there are signs that do not appear in the test 4, but which are evident in the test 2. The only difference in the production of extracts from these two tests is that the test 4 was grown under agitation during the whole period.

The differences between the ^{1}H-NMR spectra of the samples 6 and 8 (region highlighted in blue) between δ_H 5 and 8.5 are very similar for both trials. However, test 8 highlighted a singlet in δ_H 9.7 (characteristic of labile hydrogen) that did not appear in any other test with nutrient medium. Both experiments were performed under light, although the last test was maintained with stirring during the entire growth period.

Like this, the comparative study envolving the OSMAC approach with *Aspergillus* fungus cultured in different culture media gave rise to different compounds such as eight new cyclopentenone and cyclohexenone, with potencial for inhibitory activity against acetylcholinesterase, toxicity towards brine shrimp, and antibacterial (Yao et al. 2020).

Furthermore, some types of bioactive compound fusarielin were obtained by supplementing the culture medium with $CaBr_2$. In addition, we were able to induce the production of the known fusarielins A and B, and of two new fusarielins K and L, along with the 80-fold enhancement of the new fusarielin J (Hemphill et al. 2017).

In recent works, the OSMAC-NMR approach showed that it is a clever way to identify molecular variations in complex matrices (Liu et al. 2017).

To analyze the metabolic variability in the different extracts of the fermentation of the fungus *Xylaria* sp., the multivariate analysis technique called PCA (Principal Component Analysis) was used, which is a statistical technique that linearly transforms a original set of variables, initially correlated with each other, in a substantially smaller set of uncorrelated variables that contain most of the information from the original set (Abdelhafez et al. 2020). This technique allowed to group a set of data according to the variation of its characteristics. Figure S16 shows the Scores graph obtained through multivariate analysis of the form's nuclear magnetic resonance data, using the Principal Component Analysis (PCA) tool.

Analyzing the profile of the PCA scores graph, it is noted that the tests 1, 7 and 3 are in the same quadrant, having more similar chemical characteristics; likewise, tests 2 and 8 show similarity; as well as extracts from tests 4 and 6. Experiment 5 stands out, which presents a greater diversity of chemical profile when compared to the others.

Comparing carefully ^{1}H-NMR spectra, all the chromatograms and PCA, it is observed that there was a change in the metabolism of the microorganism, which is a strong evidence that there was variation in the metabolic profile of the fungus *Xylaria* sp., and we can say that OSMAC was obtained through an experimental design.

Conclusions

According to the results presented, it is concluded that a planned induction of the fungus *Xylaria* sp. secondary metabolism was obtained through an experimental design.

Chromatographic profiles and ^{1}H-NMR spectra are clear metabolic evidence that chemical variability has occurred, which means that OSMAC approach successfully worked. Both the culture media and light and agitation were proven to be parameters that influence the biosynthesis of secondary metabolites by *Xylaria* sp.. Finally, factorial designs allow to predict the number of tests to be performed, thereby reducing the use of solvents and the growth medium, contributing to the principles of rational use of resources and green chemistry, as well as exploitation of microbial biosynthetic capability.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02283-w.

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