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ORIGINAL ARTICLE

Statistical optimization of culture conditions for biomass production of probiotic gut-borne *Saccharomyces cerevisiae* strain able to reduce fumonisin B_1

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biomass production, cane molasses, fumonisin B_1, *Saccharomyces cerevisiae*, statistical optimization.

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Abstract

Aim: To evaluate the ability of probiotic *Saccharomyces cerevisiae* RC016 strain to reduce fumonisin B_1 (FB_1) *in vitro* and to optimize the culture conditions for the growth of the yeast employing surface response methodology.
Methods and Results: Using Plackett–Burman screening designs (PBSD) and central composite designs (CCD), an optimized culture medium containing (g l^{-1}) fermentable sugars provided by sugar cane molasses (CMs), yeast extract (YE) and $(NH_4)_2HPO_4$ (DAP) was formulated. The *S. cerevisiae* RC016 strain showed the greatest binding at all assayed FB_1 concentration. The CMs, YE, DAP concentrations and incubation time influenced significantly the biomass of *S. cerevisiae* RC016.
Conclusion: A combination of CMs 17%; YE 4·61 g l^{-1} and incubation time 60 h was optimum for maximum biomass of *S. cerevisiae* RC016.
Significance and Impact of the Study: The importance of this work lies in the search for live strains with both probiotic and fumonisin B_1 decontamination properties that could be sustainably produced in a medium just containing cheap carbon, nitrogen and phosphorus sources and would be included in a novel product to animal feed.

Introduction

Fumonisins (FBs), a structurally related mycotoxin group produced by *Fusarium verticillioides* and *Fus. proliferatum*, are common contaminants of corn and corn-based products worldwide (Shephard *et al.* 1996). Although several FBs have been identified, fumonisin B_1 (FB_1) and B_2 (FB_2) are the most important and constitute up to 70% of the FBs found in naturally contaminated food and feed. Fumonisin B_1 is the di-ester of propane-1,2,3-tricarboxylic acid (tricarballylic acid, TCA) and 2-amino-12,16- dimethyl-3,5,10,14,15-pentahydroxyeicosane, in which the C14 and C15 hydroxyl groups are esterified with the terminal carboxyl group of TCA (Fig. 1). Fumonisin B_1 is phytotoxic to corn (Lamprecht *et al.* 1994), cytotoxic to various mammalian cell lines (Abbas *et al.* 1993) and is a carcinogen in rat liver and kidney (IARC 2002). Moreover, it is considered possible carcinogen to humans being classified as class 2B (IARC 2002). The occurrence of FB_1 in home-grown corn has been associated with an increased risk of oesophageal cancer in humans (Shephard *et al.* 1996).

Many strategies, including biological control, have been investigated to manage, reduce and ultimately eliminate FB_1 contamination of crops (Khan *et al.* 2001; Dal Bello *et al.* 2002; Dawson *et al.* 2002; Schisler *et al.* 2002; Diamond and Cooke 2003; Luz *et al.* 2003; Varga and Toth 2005).

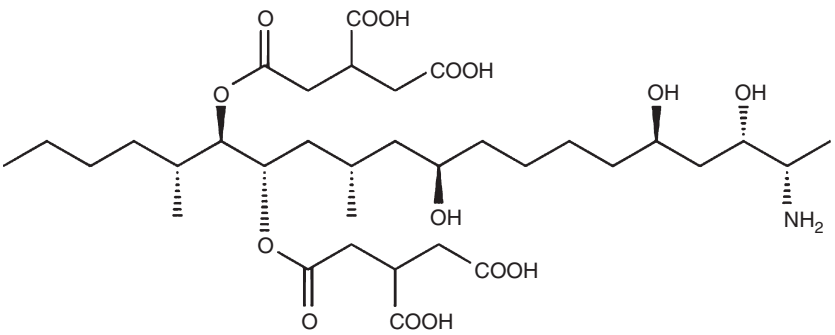

Figure 1 Fumonisin B_1.

Previous studies have demonstrated that *Saccharomyces cerevisiae* RC016, isolated from animal environment, were able to reduce aflatoxin B_1 and to retain mycotoxin-binding ability under gastrointestinal conditions (Armando *et al.* 2011). Furthermore, this strain demonstrated to have probiotic properties such as coaggregation and antimicrobial activity against pathogenic enterobacteria and was also able to improve ruminal fermentation (Armando *et al.* 2011; Dogi *et al.* 2011). These authors produced *S. cerevisiae* RC016 biomass using yeast peptone dextrose (YPD) as culture medium. However, the use of this medium is currently limited due to its high production costs. Thus, optimization of the fermentation medium is included in the strategic analysis of the viability of the biotechnological process. Optimization of any bioprocess can be conducted either by changing one factor at a time or by varying several factors at the same time and examining their effects and interactions using statistical analysis.

The statistical design of experiments is an organized approach that yields more reliable information per experiment than unplanned approaches. Statistical data analysis enables visualization of the interactions among several experimental variables, leading to the prediction of data in areas not directly covered by experimentation (Kalil *et al.* 2000; Montgomery 2001).

Molasses is a by-product of sugar refining plants from beet or cane sugar. The main sugar present in molasses is sucrose (50–65%), which is easily assimilated by *S. cerevisiae*. It has been shown that the molasses, despite its low content of nitrogen and phosphorus, is a good nutritive medium for fungi and bacteria (Zumbado-Rivera *et al.* 2006), it contains compounds that promote the development of biomass as high contents of carbohydrates (sucrose, glucose and fructose), proteins, fats, calcium, phosphorus, amino acids and vitamins, among others (Téllez 2004; Yépez 2005).

Economic evaluation of the yeast production process has suggested that the major contributor to the overall cost is the cost of carbon substrate. Thus, it is desirable to produce biomass yeast from cheap carbon sources or even from a waste product, such as sugar cane molasses (CMs).

The aim of this study was to examine *S. cerevisiae* RC016 for its ability to reduce FB_1 and maximize biomass production by defining the major components of the yeast production medium and their optimal concentrations. A systematic and statistical optimization was carried out.

Materials and methods

Microorganisms, growth medium and cultural conditions

Saccharomyces cerevisiae strain RC016 isolated from animal ecosystem (pig gut) was obtained from the collection centre at the National University of Rio Cuarto, Argentina. Stock cultures were maintained at −80°C in 30% (v/v) glycerol. Working cultures were prepared from frozen stocks by two transfers in yeast extract-peptone-dextrose (YPD) broth (5 g yeast extract, 5 g peptone, 40 g dextrose, 1000 ml water) and incubation at 37°C for 24 h.

Saccharomyces cerevisiae strain inoculum preparation

Inoculum of *S. cerevisiae* was prepared from a 25°C overnight culture in YPD and harvested by centrifugation. Then, cells were resuspended in peptone water (0·1% w/v), and serial decimal dilutions were done to obtain 10^7 cells ml^{-1}. The cell suspension concentration was determined using a haemocytometer. Viability was confirmed by standard plate count method using YPD agar.

Mycotoxin-binding assay

The FB_1 binding assay was performed according to Bueno *et al.* (2007) with modifications. Stock solution of

solid FB_1 (Sigma, St. Louis, MO, USA) was suspended in methanol to obtain FB_1 concentration of 2 mg ml^{-1}. Solutions of FB_1 (1, 5, 20 and 50 μg ml^{-1}) were prepared in phosphate-buffered saline (PBS) (pH 7·3). Yeasts (10^7 cells ml^{-1}) were washed twice with PBS and incubated at 37°C for 1 h in a shaking bath with 1 ml of PBS at each FB_1 concentration, separately. Then, cells were pelleted by centrifugation at 5000 ***g*** at room temperature for 15 min, and the supernatant containing unbound mycotoxin was collected and stored at −20°C for high-performance liquid chromatography (HPLC) analysis. Positive (PBS + mycotoxin) and negative (PBS plus yeast cells) controls were included for all experiments. The experiment was conducted in triplicate.

Detection and quantification of fumonisin B_1

Fumonisin B_1 was detected and quantified according to Shepard *et al.* (1990) and modified by Doko *et al.* (1995). The HPLC with fluorescence detection (λexc 335 nm; λem 440 nm) consisted of a C18 column (Supelcosil LC-ABZ, Supelco; 150 × 4·6 mm, 5 μm particle size) connected to a precolumn (Supelguard LC-ABZ, Supelco; 20 × 4·6 mm, 5 μm particle size). Methanol/Sodium dihydrogen phosphate (75 : 25) solution adjusted to pH 3·35 with orthophosphoric acid was used as mobile phase, at a flow rate of 1·5 ml min^{-1}. An aliquot (50 μl) of this solution was derivatized with 200 μl of o-phthaldialdehyde (OPA) solution obtained by adding 5 ml of 0·1 mol l^{-1} sodium tetraborate and 50 μl of 2-mercaptoethanol to 1 ml of methanol containing 40 mg of OPA. The injection volume was 50 μl, and the retention time was around 7 min. The detection limit of the technique was 20 μg l^{-1}. Fumonisin B_1 quantification was performed by peak height measurements and comparing with a reference standard solution.

Statistical optimization for biomass production assay

Chemicals, microorganism, and culture conditions

Saccharomyces cerevisiae RC016 was maintained at 4°C on slants of YPD agar. For Erlenmeyer flasks inoculation, a loopful of the slants cultures was transferred into 20 ml YPD broth contained in Erlenmeyer flasks and incubated for 18–24 h with agitation (200 rpm) provided by an orbital shaker, keeping the ratio 'volume flask/volume medium' in 5 : 1 (pre-inoculum). Before inoculation, pre-inoculum cells were washed three times with sterilized distilled water by centrifugation (9000 ***g*** for 5 min) and suspension. Filter-sterilized (0·22 μm-pore membranes of cellulose acetate) yeast extract (YE) solutions were aseptically added to the other media components previously sterilized at 121°C for 15 min. Unless indicated, temperature for all experimental cultures was maintained at 28 ± 1°C, and pH values between 4·5 and 5·5.

For bioreactor assays, batch fermentations were carried out in a mechanically stirred 5-l New Brunswick FS300 fermentor (New Brunswick Scientific Co., Edison, NJ, USA) equipped with pH, temperature and dissolved oxygen concentration sensors. Agitation and aeration were varied to maintain dissolved oxygen concentration above 40% saturation. The pH was maintained between 4·5 and 5·5 units by addition of H_2SO_4 (18 N) or Na_2CO_3 (20% w/v) solutions. Foam production was controlled by the addition of antifoam (silicone Antifoam 289; Sigma). The fermentation media in the bioreactor (3-l working volume) contained different concentrations of diammonium phosphate (DAP), YE and CMs (50·75% w/w, fermentable sugars). Aliquots of 20 ml culture were withdrawn for biomass determinations.

Experimental designs and statistical analysis

As a first step in the optimization of yeast biomass production in shaken flasks, a Plackett–Burman screening design (PBSD) (Plackett and Burman, 1946) was used to analyse the main medium constituents influencing *S. cerevisiae* RC016 production.

In the Plackett–Burman design, each variable was represented at two levels, high (+1) and low (−1); thus, low and high factors settings were coded as −1 and +1, while the mid-point was coded as 0 and was run to evaluate the linear effects of CMs concentration (X1), which varied from 5 to 20% (w/v); DAP concentration (X2), from 0 to 10 g l^{-1}; YE (X3), from 0 to 3 g l^{-1}; inocula concentration (X4), from 0·5 to 2% (v/v) and incubation time (X5), from 20 to 48 h. The results were fitted with a first-order model.

A Box–Wilson central composite design (CCD) (Box and Wilson 1951) with five settings for each of the three factors levels (molasses, yeast extract concentration and incubation time) was used to evaluate the quadratic effects and two-way interactions among these variables. In yeast biomass production, the variable levels X_i were coded as x_i according to

$$x_i = \frac{X_i - X_o}{\Delta X_i}, i = 1, 2, 3 \ldots k$$

where x_i and X_i are the dimensionless (codified) value and the actual value of an independent variable, respectively; X_0 is the real value of an independent variable at centre point, and ΔX_i is the step change.

In CCD, CMs concentration (X_1) had a lower limit of 10% and an upper limit of 30% (w/v); yeast extract concentration (X_2) had a lower limit of 1 and an upper limit of 5 g l^{-1}, and incubation time (X_3) was varied between 24 and 60 h. Response surface methodology was used to analyse this experimental design. The second-degree model used to fit the response to the independent variables was

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{j=2}^{k} \sum_{i=1}^{j-1} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2$$

where Y is the predicted response, $x_i x_j$ are the input variables that influence the response variable Y, β_0 is the intercept, β_i is the i the lineal coefficient, β_{ii} is the ith quadratic coefficient and βij is the ij the interaction coefficient.

All experimental designs were done at least three times. Statistical and numerical analyses were carried out by means of the analysis of variance (ANOVA) and multiple regressions, using the softwares Essential Regression v. 2·205, Mathematica v. 3·0 and Origin v. 6·0.

Analytical determinations

For routine assays, biomass production was monitored by the optical density at 640 nm ($OD_{640\ nm}$), after washing the cells three times with PBS 0·05 mol l^{-1} (pH 6·5) by centrifugation (5000 ***g***, 5 min) and suspension. Growth was also evaluated as cell dry weight (CDW) (g l^{-1}) after cells were washed with distilled water as described previously.

The equivalences obtained among these two parameters were as follows: CDW (g l^{-1}) = $OD_{640\ nm}$ × 0·59 g l^{-1}

Results

Fumonisin B_1 binding

The ability of *S. cerevisiae* RC016 strain to reduce FB_1 is summarized in Table 1. Fumonisin B_1 reduction percentages varied according the used mycotoxin concentration. Reduction percentages were 27% at 1 μg ml^{-1}; 43% at 5 μg ml^{-1}; 68·5% at 20 μg ml^{-1} and 78·66% at 50 μg ml^{-1} FB_1 concentrations.

Screening of essential culture conditions for biomass production

The equation obtained for cell growth using PBSD was as follows:

$$\text{Response measured} = \text{OD}_{640\text{nm}} = -12.34 + \mathbf{0.841_{x1}} + \mathbf{1.368_{x2}} + \mathbf{3.439_{x3}} + 0.119_{x4} + \mathbf{0.564_{x5}}$$

[*The P-values for regression coefficients in bold characters were significant at $P < 0{\cdot}05$.] The lack of fit of the regression model was not significant, and the P value of the F test ($F_{\text{significative}} = 0{\cdot}00344 < 0{\cdot}05$) demonstrated a high significance for the regression. The F value (8·329) was higher than the critical value obtained from tables for a 5% significance level with 5 and 9 degrees of freedom.

Fifteen trials were carried out to analyse the effect of five variables on yeast biomass production, and the results are shown in Table 2. The goodness-of-fit of the model was checked by the determination of the coefficient $R^2 = 0{\cdot}822$. Table 3 shows that CMs (%), YE (g l^{-1}), DAP (g l^{-1}) and incubation time (h) are the most significant factors ($P < 0{\cdot}05$). And then, CMs, YE concentrations and incubation time were selected for a further optimization to obtain a maximum response.

Optimization of selected culture conditions for biomass production

Table 4 presents the actual and coded values of the three variables in the experimental design, as well as the responses corresponding to yeast biomass production. Data obtained were analysed by multiple regressions. The experimental results of the CCD were fitted and explained with a second-order polynomial function (Eqn. 2).

Table 1 Fumonisin B_1 binding levels (μg ml^{-1} and percentage%) by *Saccharomyces cerevisiae* RC016 at different toxin concentrations

	FB_1 (μg ml^{-1})*							
	1		5		20		50	
Saccharomyces cerevisiae RC016	Reduction level							
	μg ml^{-1}	%	μg ml^{-1}	%	μg ml^{-1}	%	μg ml^{-1}	%
	0·27a	27	2·15b	43	13·9c	69·5	39·33d	78·66

*Letters in common are not significantly different according to Fisher's protected LSD test ($P < 0{\cdot}001$). Binding level at each concentration was statistically analysed separately.

Table 2 Plackett–Burman design for screening of significant factors affecting biomass (OD 640 ηm) of *Saccharomyces cerevisiae* RC016

	Factor										
Assay	CMs (%)		DAP (g l^{-1})		YE (g l^{-1})		Inoculum (%)		Incubation time (h)		Response* (OD 640 ηm)
1	+1†	20	−1	0	+1	3	+1	2	−1	20	27·4
2	−1	5	−1	0	+1	3	+1	2	+1	48	40·6
3	0	12·5	0	5	0	1·5	0	1·25	0	34	36·5
4	−1	5	−1	0	−1	0	−1	0·5	−1	20	5·67
5	0	12·5	0	5	0	1·5	0	1·25	0	34	35·5
6	+1	20	−1	0	−1	0	−1	0·5	+1	48	29·2
7	+1	20	+1	10	+1	3	−1	0·5	+1	48	54·8
8	−1	5	+1	10	+1	3	−1	0·5	+1	48	36·9
9	+1	20	+1	10	−1	0	+1	2	−1	20	26·5
10	0	12·5	0	5	0	1·5	0	1·25	0	34	35·1
11	+1	20	+1	10	−1	0	+1	2	+1	48	53·7
12	−1	5	+1	10	+1	3	+1	2	−1	20	25·0
13	−1	5	−1	0	−1	0	+1	2	+1	48	9·8
14	+1	20	−1	0	+1	3	−1	0·5	−1	20	23·9
15	−1	5	+1	10	−1	0	−1	0·5	−1	20	21·8

CMs, sugar cane molasses; DAP, $(NH_4)_2HPO_4$, YE, yeast extract.
All assays were performed at 28°C and 250 rpm.
*Data are the average ± SD of three independent experiments.
†Numbers in the first column are the coded values of the variables.

Table 3 ANOVA of the model applied for *Saccharomyces cerevisiae* RC016 biomass production

Source of variation	DF	Sum of squares	Mean square	*F* value	*P* value
Model*	5	82	423·08	8·329	0·00344
Residual	9	18	50·80		
Lack of fit	7	18	65·16	125·3091	0·00794
Pure error	2	0	0·520		
Total	14	100			

Source of variation	Sum of squares	*P* value	Standard Error
CMs (%)	0·841	0·01344	0·274
DAP (g l^{-1})	1·368	0·00886	0·411
YE (g l^{-1})	3·439	0·03347	1·372
Inoculum (%)	0·119	0·675	0·274
Incubation time (h)	0·564	0·00399	0·147

DF, Degrees of freedom.
*r^2 = 0·822, adjusted r^2 = 0·724, coefficient of variation = 23·12.
Statistically significant at 95% of confidence level.

Table 5 shows the results of ANOVA. The lack of fit of the regression model was not significant, and Fischer's *F* test demonstrated a high significance ($P < 0·05$) for the regression.

Employing response surface methodology (RSM), the combination of factors that maximized biomass production of the strain RC016 was as follows: (a) CMs, 17%; YE, 4·61 (g l^{-1}) and incubation time, 60 h; for which the model predicts an $OD_{640\ nm}$ = 68·41.

Saccharomyces cerevisiae RC016 biomass production for different concentrations of the components were predicted from these plots (Fig. 2). The maximum predicted production of biomass is indicated by the surface confined in the response surface diagram.

Table 4 Central composite design for *Saccharomyces cerevisiae* RC016 biomass production optimization

Trial	Factor concentration						Response* (DO 640 ηm)
	CMs (%)		YE (g l^{-1})		Incubation time (h)		
1	0†	20	0	3	0	42	51·1
2	+1	30	+1	5	+1	60	55·9
3	−1	10	−1	1	+1	60	51·0
4	+1	30	−1	1	−1	24	26·0
5	0	20	0	3	+1	60	66·8
6	−1	10	+1	5	+1	60	66·7
7	0	20	0	3	0	42	52
8	−1	10	+1	5	−1	24	29·6
9	−1	10	−1	1	−1	24	31·8
10	+1	30	−1	1	+1	60	51·1
11	0	20	−1	1	0	42	41·1
12	+1	30	+1	5	−1	24	25·5
13	−1	10	0	3	0	42	44·7
14	0	20	+1	5	0	42	48·7
15	0	20	0	3	−1	24	32·4
16	0	20	0	3	0	42	53·3
17	+1	30	0	3	0	42	40·6

CMs, sugar cane molasses; YE, yeast extract.
*Data are the average ± SD of three independent experiments.
†Numbers in the first column are the coded values of the variables.
All assays were performed with inoculum (1%), $(NH_4)_2HPO_4$ (DAP) (10 g l^{-1}), at 28°C and 250 rpm.

Table 5 ANOVA of the model applied for *Saccharomyces cerevisiae* RC016 biomass production

Source of variation	DF	Sum of squares	Mean square	*F* value	*P* value
Model*	9	97	287·06	27·28	0·000122
Residual	7	3	10·52		
Lack of fit	5	3	14·39	16·6658	0·05757
Pure error	2	0	0·863		
Total	16	100			

DF, Degrees of freedom.
*r^2 = 0·972, adjusted r^2 = 0·937, coefficient of variation = 7·174.

Validation of the model in shaken flasks and bioreactors

Different combinations of the cultural involved variables were used, and the experimental and predicted responses were compared by the mathematical model both in shaken flasks and bioreactors. The results of validation experiments after 24- and 60-h incubation at 28°C at the used CMs and YE concentrations are shown in Table 6. As can be seen, the results obtained widely validated the mathematical model. On the other hand, the absolute values of the standardized residues were within the acceptable limits (data not shown).

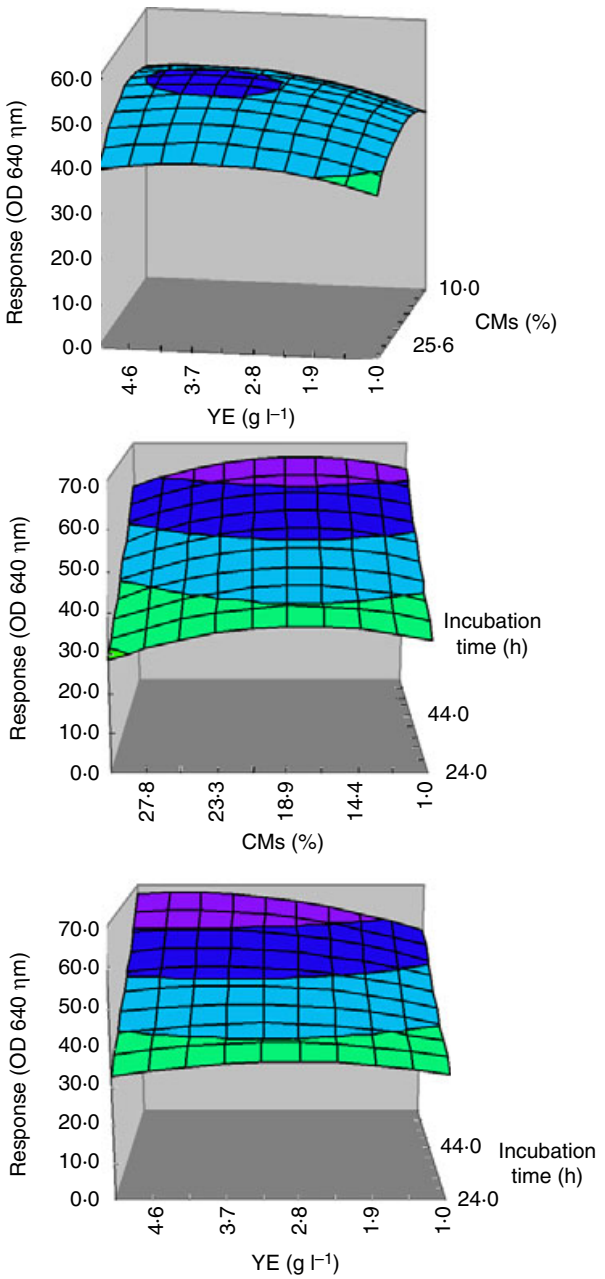

Figure 2 Response surface plots showing the effect on *Saccharomyces cerevisiae* RC016 biomass production ($OD_{640\ \eta m}$) of: (a) CMs (%) and YE (g l^{-1}), (b) incubation time (h) and CMs (%), (c) incubation time (h) and YE (g l^{-1}). CMs, sugar cane molasses; and YE, yeast extract concentration. (■) 60·0–70·0; (■) 50·0–60·0; (■) 40·0–50·0; (■) 30·0–40·0; (■) 20·0–30·0; (■) 10·0–20·0; (■) 0·0–10·0.

Discussion

The present work shows the *in vitro* FB_1 decontamination ability of *S. cerevisiae* RC016 strain. Probiotics are a great promise for reducing the bioavailability of consumed

Table 6 Validation results in a bioreactor for predicted and obtained biomass concentrations, for different combination of CMs (%) and yeast extract concentrations (w/v) and incubation time (h)

Validation assay	Obtained biomass (g CDW* l^{-1})	Predicted biomass (g CDW l^{-1})	Absolute standardized residual	Yield (g CDW g^{-1} FS**)	Productivity (g CDW l^{-1} h^{-1})
Optimized medium (CMs†, 17%; YE‡, 0·46 g l^{-1}); incubation time, 60 h)	39·59	40·36	−0·77	0·468	0·673
Optimized medium (CMs, 17%; YE, 0·46 g l^{-1}); incubation time, 24 h)	15·63	15·52	0·11		

CDW, cell dry weight; CMs, sugar cane molasses; YE, yeast extract; FS, fermentable sugars.

mycotoxins. The union of binder probiotics with toxins reduces their availability and, consequently, the absorption of the toxin in the gastrointestinal tract. Reduction in other major mycotoxins, aflatoxin B_1, zearalenone and ochratoxin A, by the probiotic *S. cerevisiae* RC016 strain has also been shown *in vitro* (Armando *et al.* 2011, 2012). In the absence of a simple detoxification method for foods and feeds contaminated by FB_1, the use of yeast selected strains appears as a promising approach to reduce their toxicological effects.

The *S. cerevisiae* RC016 strain showed an efficient reduction percentage at the four tested FB_1 levels, significantly increasing the removal capacity with increasing mycotoxin initial concentration. Similar results were informed by Niderkorn *et al.* (2006) who reported that lactic acid bacteria were able to bind FBs. It was also reported that glucomannans of the cell wall of *S. cerevisiae* were able to bind fumonisins (Bakutis *et al.* 2005). Their great binding capacity resulted from the large area available for exchange. Thus, 500 g of glucomanns from yeast cell wall have the same adsorption capacity as 8 kg of clay (Yiannikouris and Jouany 2002).

This study reports the FB_1 decontamination capability, although not determining mycotoxin decontamination mechanism involved. Studies to examine whether this yeast strain is able to adsorb and/or degrade the mycotoxin are in progress.

As there are no reports on statistical optimization of cultural conditions for biomass production of *S. cerevisiae* with probiotic and mycotoxin decontamination properties, the main variables involved with strain RC016 growth were screened by means of PBSDs. The Plackett–Burman design is a powerful method for screening significant factors. The obtained results demonstrated that inoculum size did not significantly affected cell growth at the selected ranges, but CMs, YE and DAP concentrations and incubation time significantly affected ($P < 0·05$) the growth of the studied strain.

In a second step, the optimal levels of the three significant variables identified in the initial screening experiments were defined to obtain maximal biomass production. Therefore, CCD was carried out to optimize the levels of YE, CMs concentration and incubation time in the fermentation broth.

The goodness-of-fit of the model was checked by the determination coefficient (R^2). In this case, the value of the coefficient ($R^2 = 0·972$) indicated that only 2·8% of the total variations are not explained by the model. The value of the adjusted determination coefficient (adjusted $R^2 = 0·937$) indicated a high significance of the model (Haaland 1987). At the same time, a relatively lower value of the coefficient of variation (CV = 7·17) indicated an improved precision and reliability of the experiments (Montgomery 2001). The significance of each coefficient was determined by student's *t*-test and *P* values, as well as regression coefficients and standard errors. Judging by the regression coefficients and *t* values (Haaland 1987), it can be concluded that yeast biomass production was primarily determined by the linear and quadratic terms of the model and that significant interaction existed between YE concentration and incubation time.

Response surface methodology (RSM) showed to be a convenient statistical methodology to optimize the main fermentation condition to produce high cell concentration of this *S. cerevisiae* strain able to reduce FB_1 intended to be used at an industrial scale, because it allowed fine-tuning of the economically most important production variables. The final composition of the optimized culture conditions resulted in an overall fivefold increase compared with that using the nonoptimized medium (data not shown) for yeast biomass production.

Saccharomyces cerevisiae RC016 with beneficial properties and mycotoxins reduction abilities is a good candidate for cell biomass production. High yield and productivity values could be obtained; 0·468 g CDW g^{-1} fermentable sugars in cane molasses and 0·673 g CDW l^{-1} h^{-1}, respectively, could be obtained in a batch fermentation using a cheap and simple medium. The addition of yeast extracts slightly (<10%) increased those values.

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Conflict of Interests

The authors do not have any conflict of interests.

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