Taguchi Design of the Experimental approach to Increase the Biomass and Cell Wall Chitosan Contents of Zygomycetous Dimorphic Fungus *Benjaminiella Poitrasii*

S.R. MANE[1, 2], E. K. PATHAN[3], G. PATIL[4], S. G. TUPE[5], V. GHORMADE[4], B. P. CHAUDHARI[1,2] AND M.V. DESHPANDE[5*]

[1]*Biochemical Sciences Division, CSIR-National Chemical Laboratory, Pune-411008, India.*

[2]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad-201002, India.*

[3]*Department of Biosciences and Bioengineering, Indian Institute of Technology-Bombay, Powai, Mumbai-400076, India.*

[4]*Nanobioscience, Agharkar Research Institute, Pune-411004, India.*

[5]*Greenvention Biotech Pvt. Ltd., Pune-412202, India.*

ABSTRACT

A dimorphic fungus Benjaminiella poitrasii contains high chitin/chitosan (35% of the cell wall) in the mycelial (M) form than its yeast (Y) form (20% of the cell wall). However, the relative proportion of chitosan is more in yeast form cells (chitosan: chitin ratio, 6:1) than mycelial cells (chitosan: chitin ratio, 3:1). Using the Taguchi design of experimental (DOE) approach, interactions among eight different parameters showed that carbon source (starch, 10 g/L), incubation time (48 h), inoculum (M and Y mixed 10%), yeast extract (6 g/L) and peptone (10 g/L), were optimum for maximum biomass production. Under these conditions, the chitosan yield from the mycelia was 60.89±2.30 mg/g of dry biomass, while from the yeast cells was 28.29±2.7 mg/g. The molecular weights of chitosan isolated from M and Y cells were 41.28 kDa and 21.72 kDa, respectively as measured by gel permeation chromatography. The degree of deacetylation of chitosans as 88.17-90.72% DDA measured by ^{1}H-NMR. Furthermore, chitosans from M and Y cells inhibited the growth of plant pathogenic Fusarium oxysporum (MIC_{90}0.1 and 0.4 mg/mL) and Ustilago maydis (MIC_{90}0.4 and 0.8 mg/mL) at lower concentrations as compared to chitosan isolated from marine sources (MIC_{90} 0.8 and >1.6 mg/mL).

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Correspondence author e-mail: mvdeshpande1952@gmail.com
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KEYWORDS: *Antifungal activity, Degree of deacetylation, Fungal chitosan, Molecular weight, Taguchi design of experiment.*

1. INTRODUCTION

Chitosan is a natural β-(1→4)-linked glucosamine polymer produced by deacetylation of chitin, a β-(1→4)-linked *N*-acetylglucosamine polymer. The main commercial source of chitin/chitosan is landings of marine organisms such as, crabs, lobsters, and shrimps. However, the supply of marine waste is seasonal and limited, resulting in variability in the source material. The fungi containing chitin/chitosan in their cell walls can be an alternative commercially viable source. Ghormade *et al.,* (2017) extensively reviewed the possible fungal sources for chitin and chitosan production[1]. The fermentation of zygomycetous fungi *viz., Absidia coerulea, Benjaminiella poitrasii*, *Cunninghamella elegans*, *Gongronella butleri*, *Mucor rouxii* and *Rhizopus oryzae*, was preferred for chitosan production due to a higher percentage of chitosan in their cell wall [1]. The attempts were also made to optimize the fermentation conditions to increase biomass production, ultimately increasing the chitosan yield. Previously, we have optimized the mycelial biomass production in *B. poitrasii* by using an one variable at time (OVAT) approach [2]. Xu *et al.*, 2003 also used an OVAT and orthogonal matrix method to optimize submerged conditions for *Paecilomyces tenuipes* C240 strain[3]. However, the optimization of fermentation parameters by OVAT approach becomes tedious and time-consuming. Alternatively, statistical methods can be used to study the interaction of variables in generating the process response. Taguchi design of experiments (DOE) developed by Genichi Taguchi is one such tool. It involves studying the system by a set of independent variables (factors) over a specific region of interest (levels). Taguchi's parameter design concept is related to finding the appropriate design factor levels to make the system insensitive to variations in noise (uncontrollable factors). Kim *et al.*, 2005 used this statistical approach to optimize submerged culture conditions to produce mycelial biomass and exopolysaccharides (EPS) by *Agrocybe cylindracea*[4]. Under the optimal culture condition, the maximum EPS concentration achieved bout three times higher than the basal medium[4].

In the present study, fermentation conditions were optimized to increase the biomass and extractable cell wall chitosan contents of *B. poitrasii* using the Taguchi DOE approach. The effect of interactions between different fermentation parameters on growth and chitosan contents of *B. poitrasii* were also studied. Further, biophysical properties of chitosan isolated from *B. poitrasii* yeast and mycelia grown under optimized fermentation conditions were studied. Furthermore, the antifungal potential of *B. poitrasii* yeast and hyphal chitosan was also evaluated against plant pathogenic fungi.

2. MATERIALS AND METHOD

2.1 Fungal strains and growth conditions

The parent strain of *B. poitrasii* (NCIM 1240) isolated

at CSIR-National Chemical Laboratory, Pune, was maintained on YPG agar (g/L: yeast extract, 3; mycological peptone, 5; glucose, 10; agar, 20, pH adjusted to 6.5) slant. The plant pathogens *Fusarium oxysporum* CMI113138 and *Ustilago maydis* PRL 1549 were maintained on 2% potato dextrose agar (PDA). All the slants were stored at 4°C and routinely sub-cultured every week.

2.2 Optimization of fungal biomass using taguchi design of the experimental approach

The optimization of media components and other incubation conditions *B. poitrasii* biomass was done using the Taguchi design of experimental approach (DOE). Eight different control factors were identified to evaluate their role in the objective function, *i.e.,* optimizing medium for maximum biomass production. The factors selected were starch, yeast extract (YE), peptone, pH, temperature, the ratio $MgSO_4$: KH_2PO_4, inoculum size, and incubation time. The temperature factor has been assigned with only two levels, namely 25°C and 28°C, whereas three were selected for the remaining seven factors. Based on the number of factors and levels, L18 ($2^1X\ 3^7$) orthogonal array was used for

TABLE 1. Design of experiment to optimize fermentation conditions to increase *B. poitrasii* biomass and chitosan contents using L18 orthogonal array.

Control factors								
Expt. No.	**Temp (°C)**	**Starch (%)**	**pH**	**YE (%)**	**Peptone (%)**	**Inoculum Size (%)**	**Incubation Time (h)**	**$MgSO_4$: KH_2PO_4**
1	25	0.5	5.5	0.3	0.5	8	24	1:2
2	25	0.5	6.5	0.6	1	10	36	1:2.5
3	25	0.5	7.5	0.9	1.5	12	48	1:3
4	25	1	5.5	0.3	1	10	48	1:3
5	25	1	6.5	0.6	1.5	12	24	1:2
6	25	1	7.5	0.9	0.5	8	36	1:2.5
7	25	1.5	5.5	0.6	0.5	12	36	1:3
8	25	1.5	6.5	0.9	1	8	48	1:2
9	25	1.5	7.5	0.3	1.5	10	24	1:2.5
10	28	0.5	5.5	0.9	1.5	10	36	1:2
11	28	0.5	6.5	0.3	0.5	12	48	1:2.5
12	28	0.5	7.5	0.6	1	8	24	1:3
13	28	1	5.5	0.6	1.5	8	48	1:2.5
14	28	1	6.5	0.9	0.5	10	24	1:3
15	28	1	7.5	0.3	1	12	36	1:2
16	28	1.5	5.5	0.9	1	12	24	1:2.5
17	28	1.5	6.5	0.3	1.5	8	36	1:3
18	28	1.5	7.5	0.6	0.5	10	48	1:2

the design. In the present study, all 8 columns were assigned with different factors (Table 1 depicts the details).

The growth experiments were carried out in a 1000 mL Erlenmeyer flask containing 200 mL of optimized medium. The different inoculums were prepared in the respective medium using sporangiospores (1.6 x 10^7/100 mL), incubated for 24h under shaking conditions and used for inoculations. All flasks were incubated at 28°C for 48h on a rotary shaker (180 rpm). The mycelial biomass was harvested by filtration through Whatman filter paper No. 1 and washed with distilled water (3-4 times). The above filtrate solution was centrifuged at 7100rpm () for 20 min to harvest the yeast cells and washed with distilled water several times to remove the starch. The removal of starch was confirmed with the iodine test. For instance, 1g of wet biomass (yeast or mycelia) was taken into a test tube, and a few drops of iodine solution were added. No change in color indicated the absence of starch in biomass. The dried mycelial and yeast biomass was further used for the isolation of chitosan.

2.3 Effect of different inoculum types on the growth of fungal biomass

B. poitrasii, *viz.,* sporangiospores, mycelia, yeast cells, alone and in combination, were used as an inoculum to obtain biomass. *B. poitrasii* growth from slant was gently scraped to obtain sporangiospores, free from yeast, mycelium, and zygospores, as determined by light microscopy. The yeast inoculum was obtained by inoculating 1.6 x 10^7 sporangiospores in 100 mL YPG medium under shaking conditions (180 rpm) at 37°C for 24h. To prepare mycelial inoculum, sporangiospores were inoculated in 100 mL YP medium and incubated at 28°C, 180 rpm for 24h. The mix inoculum was prepared by growing the sporangiospores in the optimized medium at 28°C, 180 rpm for 24h. All the experiments were carried out in a 1000 mL Erlenmeyer flask containing 200 mL of optimized medium, inoculated with different inoculum types and incubated at 28°C, 180 rpm for 48h. The biomass was harvested and dried until constant weight.

2.4 Extraction of chitosan from *B. poitrasii* biomass

The biomass was washed and suspended in cold distilled water and broken by mechanical shaking with glass beads (0.45-0.5 mm) in a Braun homogenizer. The absence of cytoplasmic material was confirmed by light microscopy as described earlier by Khale and Deshpande 1992[5]. The purified cell wall samples were lyophilized and stored at -20°C. Chitosan was extracted as described by Mane *et al.,* 2017[2]. The alkali-insoluble fraction (AIF) was separated by centrifugation at 7100 rpm for 15 min and neutralized by washing with cold distilled water. The pellet obtained was treated with 2% acetic acid at 95°C for 24h, followed by centrifugation at 7100 rpm for 30min and the supernatant was collected. Chitosan was precipitated from the supernatant using 1N NaOH. A white precipitate of chitosan obtained at pH 8.5-10 was washed with acetone and dried at 50°C to a constant weight.

2.5 Biophysical characterization of chitosan

2.5.1 Fourier Transform Infrared (FTIR) spectroscopy

The samples were analyzed using FTIR spectrophotometer (IRAfinity-1S, Shimadzu, Japan). The FT-IR spectrum of chitosan was recorded in the 400-4000 cm^{-1} region. The sample dried at 105°C for 1h was powdered and then mixed with KBr with appropriate control. The degree of deacetylation was determined using the absorbance ratio A_{1320}/A_{1420} [6].

2.5.2 Gel permeation chromatography

The gel permeation chromatography to determine the molecular weight of chitosan samples was carried out using OHpak SB-800 HQ series columns (Agilent Technologies, USA). For instance, chitosan samples were dissolved in acetic acid (2%) solution to get the final concentration of 2 mg/mL and filtered to remove the debris, if any. The 0.2 M acetic acid/0.1 M sodium acetate was used as mobile phase. The 50 ìL of chitosan sample was run through the column at 0.8 ml/min flow rate at 40°C. The column was calibrated using Pullulan 20201 Shodex standards and molecular weight of chitosan samples were determined[7].

2.5.3 ^{1}H-Nuclear Magnetic Resonance

Chitosan sample (10 mg) was dissolved in a solution containing 1.96 mL of D_2O and 0.04 mL of deuteriated HCl. The samples were kept on magnetic stirrer for half

an hour to confirm complete dissolution of the chitosan. Further the samples were lyophilized and dissolved in 1 mL of D_2O, 600 μL of the sample was used to record the NMR spectra. All the NMR spectra were taken on Bruker AV400 NMR spectrophotometer. The Degree of Deacetylation (DDA) was calculated using formula as mentioned in Lavertu *et al.,*[8]

2.6 Antifungal assay

Chitosan extracted from *B. poitrasii* was dissolved in 2% acetic acid solution to make 10 mg/mL stock. The solution was kept for stirring overnight for dissolution of chitosan. The final pH of the solution was adjusted to 5 using 1 N NaOH. The antifungal activity was tested against two plant pathogenic fungi, *F. oxysporum* CMI 113138 and *U. maydis* PRL 1549 by Clinical Laboratory Standards Institute's (CLSI) broth micro-dilution assay (CLSI document M27-A3 and CLSI M38-A2) as described earlier by Pulya *et al.,* 2016[9]. The stock was diluted in RPMI 1640 medium and added to the first row of a 96-well microtitre plate. The chitosan samples were serially diluted using RPMI 1640 medium in successive wells to get the final concentration in the range of 0.0125-1.6 mg/mL. *F. oxysporum* and *U. maydis* spores ($2x10^4$/mL) were suspended in the medium and inoculated (100 μL) in the microtitre plate wells. Acetic acid (2%) served as a control. All plates were incubated for 48h. After incubation, the growth was assessed visibly and recorded. The minimum inhibitory concentration (MIC) was defined as the concentration required to inhibit>90% of the growth compared to the control.

3. RESULTS

3.1 Multiple variable approaches for optimization of biomass production by *B. poitrasii*

The experimental data were processed using excel sheets prepared by Dr. P. R. Apte, TIFR, Mumbai, India (http://www.tifr.res.in/~apte) for DOE using the Taguchi approach. The individual influence of the factors at the assigned levels, severity indices for different interactions between factors and ANOVA were calculated. The biomass (dry weight, unless otherwise mentioned) after 48h of incubation was in the range of 2-6.6 g/L and 0.1-2.83 g/L for mycelium and yeast, respectively (Table 2).

The maximum biomass of *B. poitrasii* hyphae (6.50-7.00 g/L) was obtained in experiment no. 8

TABLE 2. Effect of interactions between different fermentation parameters on growth and chitosan contents of *B. poitrasii* studied using L18 orthogonal array

Expt. No.	Final pH	Mycelia		Yeast cells	
		Biomass (g/L)	Chitosan* (mg/g)	Biomass) (g/L)	Chitosan* (mg/g)
1	7.07	2.16±0.33	29.33±0.48	0.63±0.30	8.88±0.32
2	8.25	4.93±0.43	30.28±0.40	0.9±0.10	10.48±0.36
3	8.31	4.76±0.15	31.46±0.45	0.26±0.05	9.15±0.29
4	7.37	5.94±0.48	33.98±0.37	1.7±0.10	12.79±0.21
5	7.90	3.80±0.55	30.71±0.51	1.46±0.15	11.43±0.34
6	7.90	5.32±0.44	33.71±0.53	0.97±0.15	8.28±0.21
7	7.46	4.81±0.21	30.44±0.39	2.53±0.87	14.48±0.36
8	8.20	6.62±0.44	38.49±0.35	0.96±0.15	8.42±0.10

9	8.21	4.11 ±0.21	28.95 ± 0.44	1.3±0.20	12.44±0.25
10	7.77	4.74±0.39	30.16±0.38	1.56±0.25	13.87±0.83
11	7.03	2.58±0.50	28.88±0.31	2.83±0.12	15.33±0.26
12	7.56	2.50±0.52	31.18±0.38	0.14±0.04	6.41±0.19
13	7.39	5.57±0.15	35.30±0.42	0.5±0.17	7.56±0.37
14	6.81	3.44±0.37	32.65±0.47	0.7±0.26	9.36±0.12
15	7.47	3.86±0.27	33.21±0.25	0.16±0.05	4.49±0.32
16	6.67	3.03±0.94	35.85±0.32	0.26±0.25	6.58±0.23
17	7.90	4.23±0.15	30.34±0.27	0.11±0.01	5.52±0.13
18	7.09	5.08±0.27	29.83±0.28	0.12±0.005	5.79±0.18

*The mycelia and yeast cells were homogenized in liquid nitrogen and chitosan was isolated as mentioned under Materials and Methods.

(Table 2). The factors *viz.,* incubation temperature of 25°C, starch (1.5%), yeast extract (0.9%), peptone (1%), $MgSO_4$ and KH_2PO_4 at 1:2 proportion in the growth medium of pH 8, and incubation time of 48h, were found to be optimum for production of maximum mycelial biomass (6.62 ± 0.44 g/L) and chitosan (38.49 ± 0.35 mg/g of dry biomass) (Table 2). The optimum conditions for yeast biomass production were found to be as of experiment no. 7 (Table 2).

3.2 Effect of different types of inoculums on the growth of *B. poitrasii* biomass

The yeast inoculum produced higher proportions of yeast cells (3.15±0.05 g/L) and fewer mycelia ((1.37±0.21 g/L). The least biomass was obtained with mycelial inoculum (2.60±0.22 g/L of M and 1.88±0.10 g/L of Y) (Table 3).

The mixed inoculum (Y+M) produced the highest biomass (4.71±0.19 g/L of M and

TABLE 3. Effect of different types of inoculums on the growth and chitosan contents of *B. poitrasii*

Inoculum Type	Biomass (g/L)		Chitosan dry wt. (mg/g of dry biomass)	
	M	Y	M	Y
Spores	4.27±0.16	1.80±0.18	57.20±1.1	26.23±2.7
Mycelia	2.60±0.22	1.88±0.10	38.10±0.5	27.41±2.4
Yeast	1.37±0.21	3.15±0.05	36.30±0.8	25.63±1.6
Mix (M+Y)	4.71±0.19	2.70±0.10	58.72±1.0	23.64±1.2

M, mycelia; Y, yeast cells.

2.70±0.10 g/L of Y) followed by spore inoculum (4.27±0.16 g/L of M and 1.80±0.18 g/L of Y) in the optimized media. Therefore, the mixed inoculum containing both the yeast and mycelium cells was used in further experiments.

3.3 Validation of optimized conditions to produce *B. poitrasii* biomass and cell wall chitosan

The software automatically calculated optimized process conditions on pooled ANOVA: (g/L): yeast extract 6; peptone 15; KH_2PO_4, 4; $MgSO_4$ 2; starch, 10; and 1 ml trace metal solution containing [(mg/mL): $FeSO_4.7H_2O$, 5; $MnSO_4$, 1.5; $ZnSO_4$, 3.34; $CoCl_2$, 2.0], pH 6.5, inoculum 10%, incubation temperature, 28°C, incubation time, 48h and agitation 180 rpm. The validation experiment was carried out under the above-optimized process conditions (Table 4).

TABLE 4. Yield of *B. poitrasii* chitosan from One Variable at Time (OVAT) and Validation experiment using Taguchi Design of experimental approach (DOE).

Method	Biomass Dry wt. (g/L)		Chitosan (mg/g dry wt.)		% DDA (FTIR)		Molecular weight (kDa)	
	M	Y	M	Y	M	Y	M	Y
OVAT Method	10.00±0.50	ND	51.00±0.52	ND	92.89	ND	42.82	ND
Taguchi Method	9.17±0.32	1.78±0.28	60.89±2.30	28.29±2.7	88.51	87.30	41.68	19.49

M, mycelia; Y, yeast cells; ND, not determined. From 1 L optimized medium, 10.95±0.3 g biomass and 608±2.5 mg of chitosan was obtained using Taguchi DOE approach.

It produced seven times more biomass (9.17±0.32 g/L of mycelial biomass and 1.78±0.28 g/L yeast biomass) than the biomass obtained in basal medium (1.28±g/L)[2]. The Taguchi optimized produced substantial amount of both mycelial and yeast biomass which increased the final yield of chitosan as compared to the OVAT approach (Table 4).

3.4 Degree of deacetylation of chitosan isolated from *B. poitrasii* (M and Y)

The degree of deacetylation (DDA) is one of the most important factors responsible for chitosan's physical and chemical properties. The solubility of chitosan is mainly dependent on the DDA. The fungal chitosan showed characteristic peaks for O-H stretching at 3450 cm^{-1}, for amide II at 1650 cm^{-1}, at 1420 cm^{-1} for C-H deformations, for amide III, C-N stretching at 1320 cm^{-1} and at 1035 cm^{-1} for O bridge stretching in FTIR spectroscopy. These peaks are comparable with the FTIR spectra obtained for commercial chitosan from marine source (Fig. 1).

In validation experiment, % DDA of chitosan isolated from mycelia and yeast cells were 88.51% and 87.30%, respectively. The chitosan from marine source showed 79.26% of DDA, lower as compared to the fungal chitosan.

Further, chitosan samples from validation experiments were also analyzed by ^{1}H-NMR spectroscopy. The characteristic peak of H1

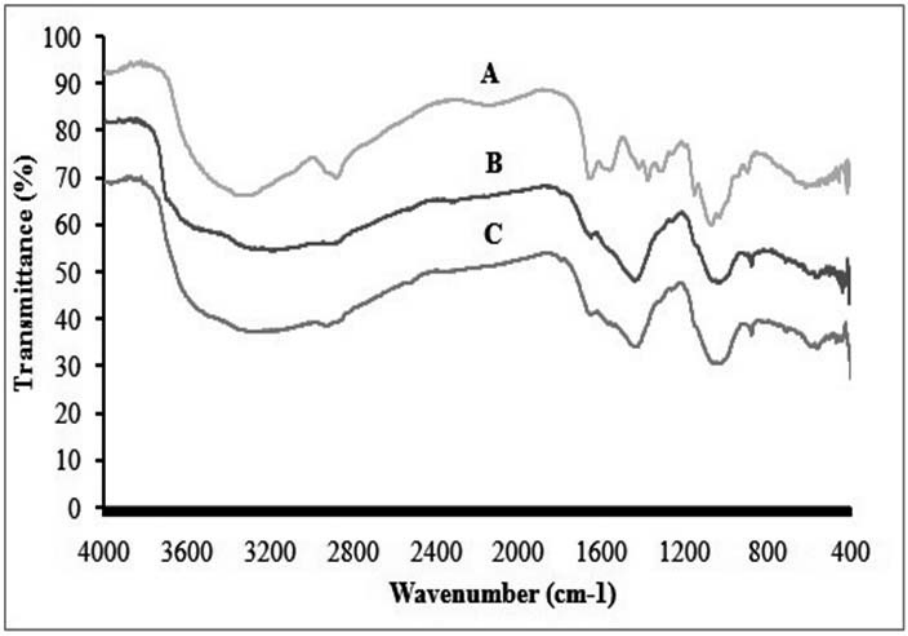

Figure 1. FT-IR profiles of *Benjaminiella poitrasii* mycelium (B) and yeast (C) chitosans comparedwith chitosan obtained from marine source (A).

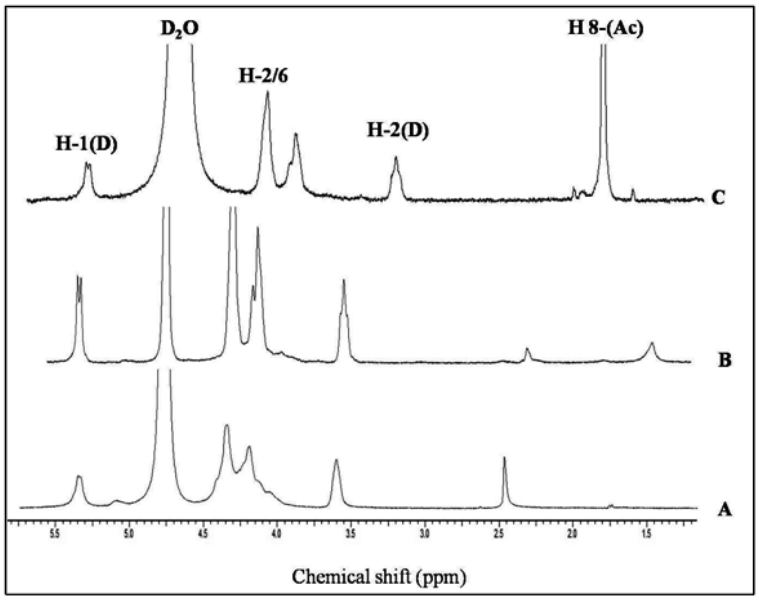

Figure 2. ^{1}H-NMR profiles of chitosan from marine source (A), *B. poitrasii mycelium* (B) and B. *poitrasii* yeast (C).

proton (a deacetylated monomer, H1D) and peak of three protons of acetyl group (H-Ac) for chitosan were observed. These peaks observed in fungal chitosan samples were comparable with the commercial chitosan from marine source (Fig. 2). The % DDA as calculated from ^{1}H-NMR of marine chitosan was 86.51 whereas for *B. poitrasii* hyphal and yeast chitosan were 90.72 and 88.17, respectively.

3.5 Molecular weight of chitosan isolated from *B. poitrasii* mycelia and yeast cells by gel permeation chromatography

The molecular weight of chitosan samples from validation experiment was determined by gel permeation chromatography (GPC). The molecular weight of mycelial and yeast chitosan samples by GPC were 41.28 kDa and 21.72 kDa, respectively (Fig. 3). These

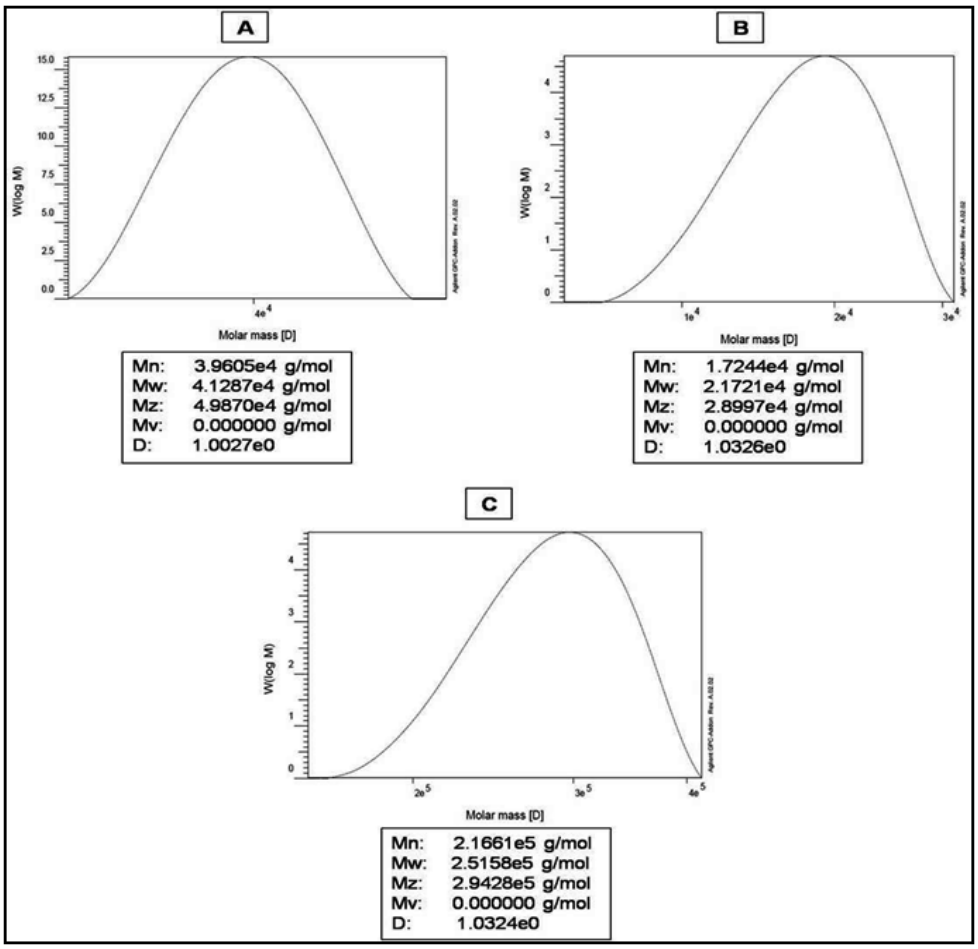

Figure 3. Gel permeation chromatography to determine the molecular weight of chitosan samples isolated from (A) *B. poitrasii* mycelia, (B) *B. poitrasii* yeast and (C) Marine sources.

values were found to be comparable with the values obtained with viscometry method. The molecular weight of commercial chitosan from marine sources as determined by GPC was 251 kDa (Fig. 3).

3.6 Antifungal activity of *B. poitrasii* chitosan against plant pathogens

The chitosan extracted from *B. poitrasii* yeast and mycelia were tested against plant pathogenic fungi *viz., F. oxysporum* and *U. maydis*. The mycelial chitosan inhibited the growth of *F. oxysporum* (MIC_{90} 0.1 mg/mL) and *U. maydis* (MIC_{90} 0.4 mg/mL) more effectively than the yeast chitosan (MIC_{90} 0.4 and 0.8 mg/mL, respectively). The marine chitosan showed least effectiveness under studied conditions (MIC_{90} 0.8and >1.6 mg/mL) (Table 5).

4. DISCUSSION

Media optimization is a crucial step in increasing the production of fungal biomass that ultimately increases the yield of extractable chitosan. The Conventional optimization procedures involve altering one parameter at a time, keeping the rest of the parameters constant, enables understanding the impact of only those parameters on the process. Further, optimization by the conventional approach becomes erroneous. On the contrary, statistical optimization methods consider the interaction of variables in generating the process response. Chenthamarakshan *et al.,*[10] described the optimization of extracellular production of laccase from *Marasmiellus palmivorus* LA1 by Taguchi method using L8 orthogonal array. The optimization improved the laccase yield by 17.6-fold. The process parameters for the growth of *Aspergillus sp.* to remove the copper and nickel were also optimized using the Taguchi method[11]. In the present study, optimized conditions gave 7-fold higher biomass than the biomass obtained in the basalmedium[2]. Previously, it was reported that peptone's source might affect the % germ tube formation in *B. poitrasii*[12]. A similar effect was observed during the two optimization methods. Earlier, the concentration of peptone was less (10 g/L), resulting in more mycelial biomass (10 g/L). However, in the case of Taguchi media optimization, the higher peptone concentration (15 g/L) gave at-par mycelial biomass (9.17 g/L) with a substantial amount of yeast cells

TABLE 5. Antifungal activity of chitosan polymers against plant pathogenic fungi.

Chitosan source	MIC_{90} (mg/mL)	
	Fusarium oxysporum	*Ustilago maydis*
B. poitrasii (M)	0.1	0.4
B. poitrasii (Y)	0.4	0.8
Marine chitosan	0.8	>1.6
Control	ND	ND

ND, not detected; 2% acetic acid (used to dissolve chitosan polymer) was served as control; The antifungal assay was carried out using CLSI micro-broth dilution method; All the assays were carried out in triplicates and average values are presented.

(1.78 g/L). So, ultimately the total yield of biomass was increased (10.95 g/L). The DDA is a crucial biophysical characteristic in deciding the quality and solubility of chitosan polymer[1]. The chitosan extracted from yeast and mycelia of *B. poitrasii* showed ~90% DDA. The chitosan isolated from another zygomycetous fungus *Mucor rouxii*, grown in three different media, showed variation (82.2-89.8%) in % DDA [13]. The chitosan's molecular weight varies depending on the source of isolation. Chitosans from crabs and shrimps have high molecular weights as compared to fungal chitosans[1]. The molecular weight of chitosan isolated from *Cunninghamella elegans* was 27.2 kDa[14]. Munoz *et al.,* reported thechitosan extraction from *A. niger* (molecular weight 190 kDa) to synthesize hydrogels for biomedical applications[15]*.* The chitosan also has antiviral, antibacterial, and antifungal properties that can be used for different applications. Previously, Mane *et al.,* showed that *B. poitrasii* mycelial chitosan (molecular weight 42.82 kDa) inhibited the growth of *A. niger*, *Candida albicans*, *Candida glabrata*, and *Cryptococcus neoformans* (MIC_{90} 0.025-0.8 mg/mL)[2]. In the present study, *B. poitrasii* mycelial and yeast chitosan (molecular weights 41.68 and 19.49 kDa, respectively) were compared for their antifungal potential against dreadful plant pathogenic fungi *F. oxysporum* and *U. maydis*. The high molecular weight chitosan from the marine source was the least effective among tested chitosans against plant pathogens. It suggested that the molecular weight of chitosan along with % DDAare important factors in defining its antifungal potential.

5. CONCLUSIONS

Taguchi DOE approach used in the present study for media optimization considers the interaction between multiple factors affecting the growth and cell wall chitosan content of zygomycetous fungus *B. poitrasii*. The biomass (yeast and mycelia) obtained under optimized growth conditions gave maximum extractable chitosan, which has low molecular weight and high % DDA, the properties that define its antifungal potential.

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CONFLICT OF INTEREST

No conflict of interest was declared.

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