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A study of morphological alterations of *Meyerozyma guilliermondii* (JN128648) cells during mannan synthesis

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ABSTRACT

A study was made of the morphology of yeast cells grown in a continuous culture at a high concentration of carbon source and a low concentration of ammonium sulphate. The morphological changes of the yeast cells during their mannan biosynthesis under continuous culture were investigated by using Scanning Electron Microscopy (SEM). We examined the morphological changes of yeast cells under optimal conditions at 0, 24, 48, 72, 96, 120 and 144 hrs of incubation in a continuous culture. The morphology of the yeast cells during the biosynthesis of mannan was markedly different from that of control. The SEM analysis showed that at 0 hrs of incubation the cells are normal, regular and ovoid. The cells started to divide and release mannan into the culture medium after 24 hrs of incubation. After 48 hrs of incubation the budding cells were more prominent and production of mannan increased logarithmically. The matured cells lead to lysis after 72 hrs of incubation. After 96 hrs of incubation the regenerated cells became more prominent and perfectly spherical. After 120 hrs of incubation there was no further increase in the total biomass.

Key words: *Meyerozyma guilliermondii*, polysaccharides, mannan, biosynthesis

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INTRODUCTION

The cell wall of yeast is composed of a thick layer of polysaccharides (80-90%) mainly glucan and mannan and serves as the interface between cell and the neighbouring environment. It provides osmotic and physical protection and determines the shape of the cell [1]. Several papers have reported that *Saccharomyces cerevisiae* releases cell wall polysaccharides, particularly mannoproteins, into the extracellular medium during yeast growth [2-5]. This release may be a consequence of cell wall-controlled hydrolysis of the mother cell to allow the emergence of the bud [6, 7]. Several environmental factors such as temperature, carbon source or initial and final pH of the medium have been shown to influence the amount of cell wall polysaccharides secreted into the fermenting medium [8, 9]. Previous studies reported that the release of exocellular polysaccharides occurs during the growth of yeast cells [4, 10, 8].

Microbial exopolysaccharides have found application in the food, pharmaceutical and other industries [11]. The polymer types reported for yeast producers include mannans, glucans, glucomannas, galactomannans and phosphomannans [12-16].

The objective of this study was to investigate the morphological alterations of yeast cells during mannan synthesis, effect of growth rate, growth conditions including temperature, sugar concentration, supplementation of ammonium salts to the culture medium and the polysaccharide release. The results of our study may be relevant for more general biotechnological purposes, as there is an increasing interest in the production of glucan and mannan for agrofood, pharmaceutical, industrial and cosmetic purposes.

MATERIALS AND METHODS

Source of organism

The potential polysaccharide producing marine yeast *Meyerozyma guilliermondii* (JN128648) was isolated and identified in Microbial Biotechnology, Mahatma Gandhi University, Kerala, India. The yeast strain was maintained on Yeast Malt (YM) agar containing yeast extract 0.3%; malt extract 0.3%; peptone 0.5%; glucose 1%; agar 2%; pH 6.0) and was stored at 4 °C.

Starter cultures were prepared by transferring cells from YM agar slants to 50 mL of sterilized medium followed by incubation at 22°C for 72 hrs.

Shake culture experiments

Shake culture experiments were done to determine the growth characteristics and morphology of cells of *Meyerozyma guilliermondii* in a continuous culture. Freshly prepared inoculum was used to inoculate the production medium. For the preparation of inoculum, a loop full of yeast cells was transferred in 50 ml of inoculum medium (Sea water). The flask was loaded on a rotary shaker incubator at a speed of 220 rpm at 22°C for 72 hours. 1% inoculum

was inoculated into the fermentation medium containing 0.25% ammonium sulfate and 5% sucrose for the production of mannan. The flask was loaded on a rotary shaker incubator at a speed of 220 rpm at 22°C for 144 hours. The initial pH was adjusted to 5.6.

Scanning Electron Microscopy

The morphological changes of the yeast cells during their mannan biosynthesis under continuous culture were investigated by using Scanning Electron Microscopy (SEM) experiments. An aliquot of 1.5 ml of yeast culture at 0, 24, 48, 72, 96, 120 and 144 hrs was centrifuged at 8000 rpm in a refrigerated centrifuge for 15 minutes. The pellet was washed with sterile saline of 50/100 and fixed in 0.5 ml of 2.5 % glutaraldehyde prepared in sterile saline at 4°C overnight. The pellet was washed repeatedly with saline and dehydration was done through an acetone series of 70-100 % and kept overnight in a dessicator. The particle was spread on SEM stubs, dried in critical point dried apparatus, platinum coated and observed under Scanning Electron Microscope.

Analytical methods

Dry weight of the yeast biomass was determined at 105°C. The total amount of carbohydrates in the crude polysaccharides was determined using the phenol-sulphuric acid method [17].

RESULTS AND DISCUSSION

Marine yeast constantly holds a special significance in the research area for the past few years and is known to produce compounds with diverse biological properties. The discovery of bioactive compounds is a never ending process for novel drugs and other biomolecules with antimicrobial and other therapeutic properties.

To study the cell morphology alterations during mannan biosynthesis in a continuous culture, the cells at 0, 24, 48, 72, 96, 120 and 144 hrs of incubation were observed under Scanning Electron Microscopy. The morphology of the yeast cells during mannan biosynthesis was markedly different from that of the control (Fig. 1a). The SEM analysis showed that the cells at 0 hr of incubation were normal, regular and ovoid in shape (Fig. 1b). After 24 hrs of incubation the cells became enlarged, started rapidly growing and dividing. The cells started to produce mannan in the liquid medium at this time (Fig. 1c). After 48 hrs of incubation the cells became enlarged and aggregated. Budding cells were more prominent and production of mannan increased logarithmically. This result indicated that a high production of polysaccharides helps to mediate cell to cell aggregation (Fig. 1d). The biomass of yeast cells increased after 72 hrs of incubation, the matured cells became more aggregated, swollen and started lysis (Fig. 1e). After 96 hrs of incubation the matured cells were more irregular and regenerated cells became more prominent and perfectly spherical. During growth of the daughter cell, the buds gradually became ovoid (Fig. 1f). After 120 (Fig. 1g) and 144 hrs (Fig. 1h) of incubation the cells had an irregular, uneven and perforated shape and an extensive lysis of

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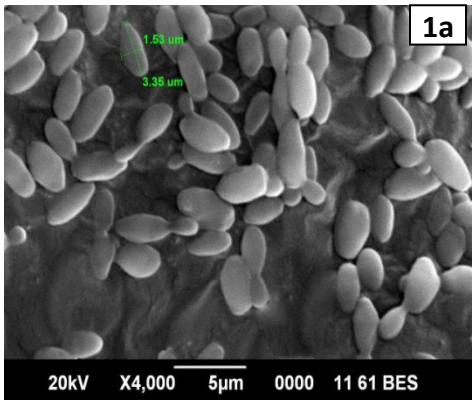
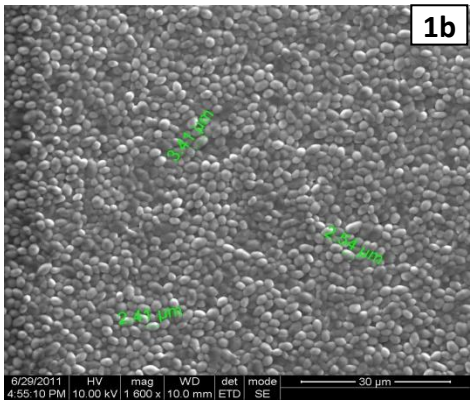
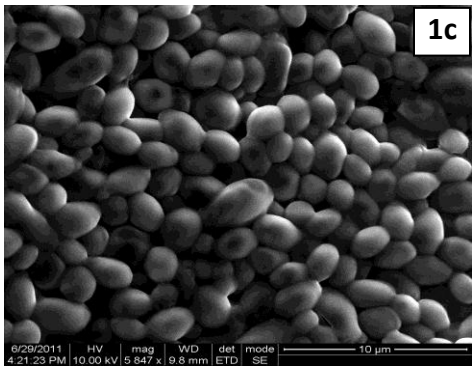
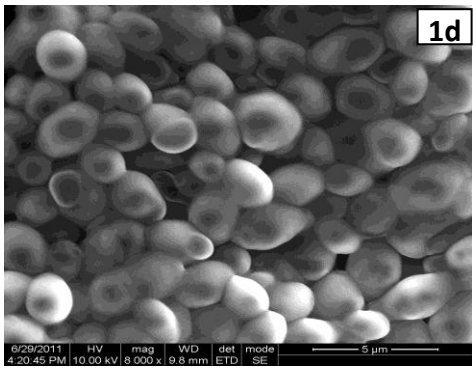
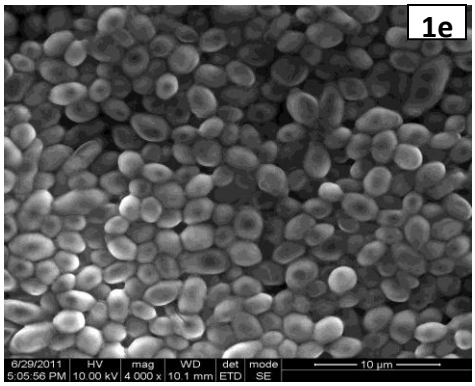
cells occurred. At this stage the polysaccharide production reached its maximum. This suggests that the release of mannan into the culture medium may also be by the autolysis of cells in addition to the extracellular release.

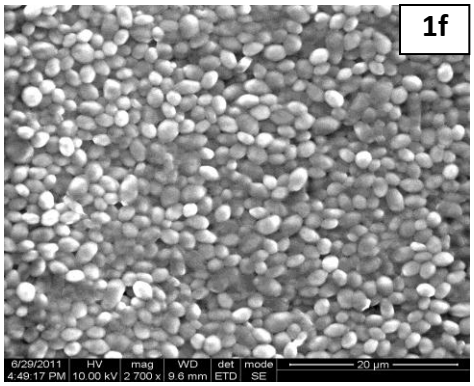












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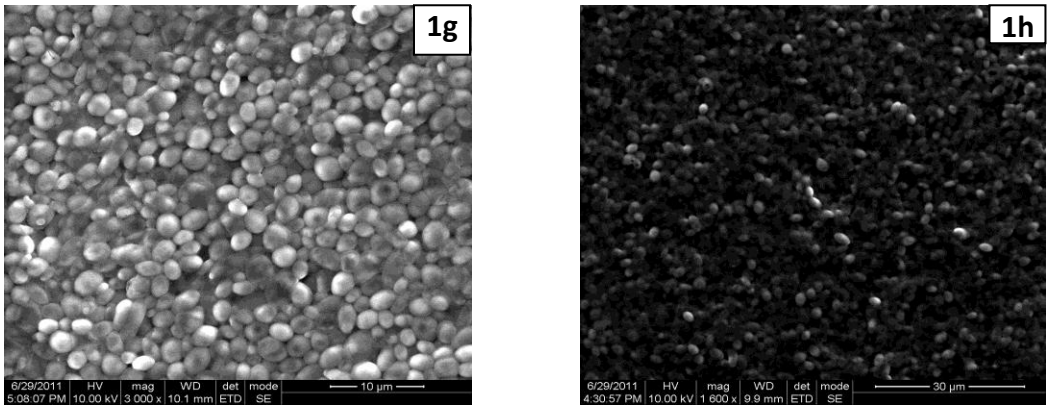

Fig. 1 - SEM images of *Meyerozyma guilliermondii* (JN128648) cells (1a - control, 1b - 0 hr, 1c - 24 hrs, 1d - 48 hrs, 1e - 72 hrs, 1f - 96 hrs, 1g - 120 hrs, 1h - 144 hrs)

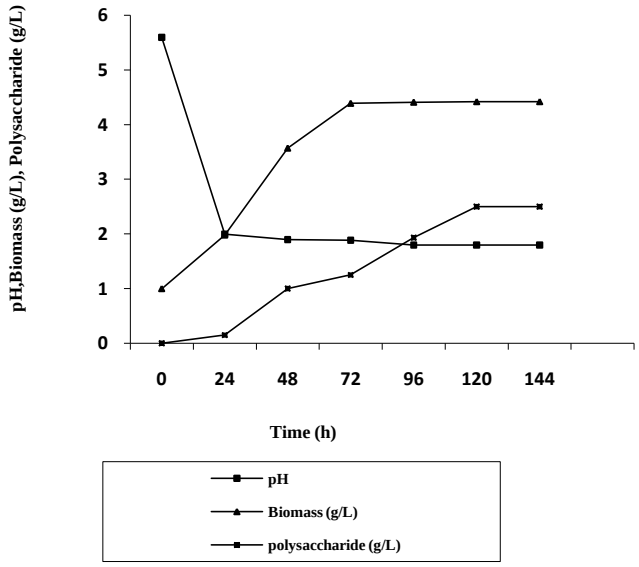

Fig. 2 - The effect of pH, Biomass production and polysaccharide synthesis at 0 - 144 hrs of incubation.

It has been noted that the important characteristic of most microorganisms is their strong dependence on the extracellular pH for cell growth and polysaccharide production. The pH plays a key role in the production and growth of marine yeast. The exomannan synthesis was activated at the acidic range of pH from 5.6 to 2.0 and increased when the pH of the culture broth decreased naturally to 2.0 during the fermentation (Fig. 2). The result clearly indicated that the marine yeast can survive at low acidic pH. This acidic pH enhances both growth and polysaccharide production. The release of the mannan from the walls of the yeast also depended on the components in the culture media. The presence of a greater proportion of mannan in the walls appeared to be associated with yeast grown in cultures containing a high concentration of carbon source (sucrose) and a low concentration of nitrogen source (ammonium sulphate) in the media. This suggests that the synthesis of mannan and biomass production may be promoted by sucrose (Data not shown).

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CONCLUSION

In the present study we found that the marine yeast *Meyerozyma guilliermondii* had variable response to different environmental stress in a continuous culture. The production of the polysaccharide mannan started at the early logarithmic phase and reached maximum at late stationary phase (120 hrs of incubation). SEM analysis revealed the morphological changes during the period. Regeneration of new cells and budding was prominent till 96 hrs of incubation which covers the logarithmic phase and early stationary phase. Autolysis of the yeast cells started at 72 hrs of incubation reaching maximum at the late stationary phase. The above results lead to the conclusion that the strain of *Meyerozyma guilliermondii* releases polysaccharide during ageing on account of autolysis. Interestingly the pH of culture medium decreased from 5.6 to 2.0 at the early logarithmic phase and remained same till late stationary phase suggesting its important role in mannan production. Further studies are in progress to determine whether the release of mannan into culture media is an integral part of the adaptation response of the strain to the environmental conditions.

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REFERENCES

[1] Klis FM, Mol P, Hellingwerf K, Brul S. FEMS Microbiology Reviews 2002; 26: 239–256.
[2] Boivin S, Feuillat M, Alexandre H, Charpentier C. AJEV 1998; 49 (3): 325–332.
[3] Dupin IVS, McKinnon BM, Corey R, Boulay M, Markides AJ, Jones GP, Williams PJ, Waters EJ. JAFC 2000; 48: 3098–3105.
[4] Llauberes RM, Dubourdieu D, Villettaz JC. JSFA 1987; 41: 277–286.
[5] Rosi I, Gheri A, Domizio P, Fia G. Revue des OEnolologues 2000; 94,18–20.
[6] Charpentier C, N'guyen Van Long T, Bonaly RC, Feuillat M. Appl Microbiol Biotechno 1986; 24: 405–413.
[7] Fleet GH. Cell wall. In: Rose AH, Harrison JS (Eds.), The Yeasts; Yeast Organelles, vol. 4. Academic Press, London, 1991; pp. 199–277.
[8] Guilloux-Benatier M, Guerreau J, Feuillat M. AJEV 1995; 46: 486–492.
[9] Rosi I, Giovani G. Effect of some winemaking variables on the production of exopolysaccharides by Saccharomyces cerevisiae. In: Lonvaud-Funel, A., et al. (Ed.), 7th International Symposium of OEnology, pp. 2003; 324–328.
[10] Feuillat M. Revue des OEnologues 1994; 73: 15–19.
[11] Sandford PA, Cotterel LW Pettitt DJ. Pure and Appli chemi 1984; 56: 879-895.
[12] Elinov NP, Ananyeva EP, Vitovskaya GA. Microbiologia 1992; 4: 615-621.
[13] Chiura H, Iizuka M, Yamamoto T. Agricultural and Biological Chemistry 1982; 46: 1723-1733.
[14] Chiura H, Iizuka M, Yamamoto T. Agricultural and Biological Chemistry 1982b; 46, 1733-1742.

[15] Head PJ, Kristiansen B. Biotechnology and Bioengineering 1985; 27; 1516-1519.
[16] Peterson GR, Nelson GA, Cathey CA, Fuller GG. Applied Biochemistry and Biotechnology 1989; 20/21: 845-867.
[17] Dubois M, Gilles K, Hamilton Y, Robers P, Smith F. Anal Chem 1956; 28: 350-356.