ISOLATION OF POLYGALACTURONASE-PRODUCING STRAINS OF *SACCHAROMYCES CEREVISIAE* FROM DIFFERENT SOURCES IN SAUDI ARABIA

Basheer A. Al-Sum, Ali H. Al-Bahkali, Shafik A. Filflan, S. Hadi and Mohammad A. Moslem*

Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT

Six yeast strains belonging to different species of *Candida*, *Trichosporon*, and *Cryptoccocus* were isolated from apple, apricot, mandarin, kiwi-fruit, and oranges procured from markets in Riyadh. Eleven strains belonging to species of *Candida, Rodotorula, Sacharomyces, Kluyveromyces* and *Kloeckera* were isolated from dairy products and sorghum grains, at the College of Agriculture, KSU. These seventeen strains along with two reference strains of *Saccharomyces cerevisiae* were tested for polygalcturonase (PG) activity with plate assay technique, in this study. PG activity was scored on the basis of color intensity on a scale of 1-5 in ascending order. All the isolates showed PG activity of varying order. Six PG-producing strains isolated from fruits are being reported for the first time from Saudi Arabia. One of the isolates, *Saccharomyces cerevisiae*-MB1 showed a remarkably high score for PG activity as compared to the reference strains.

KEYWORDS:
Yeasts, polygalacturonase (PG), *Saccharomyces cerevisiae*.

1. INTRODUCTION

Yeasts are unicellular fungi, round to oval in shape, which multiply by budding; but some of them may also form pseudo-hyphae comprising series of rectangular cells like *Saccharomyces ceriviciae* being one of the most important species of yeasts [1]. Members of this species grow wherever fluid sugar is present, for example in food materials, fruits, plant secretions etc. [2, 3]. They may also be present as saprophytes in soil and other media [4], and live as commensals or parasites in association with humans or livestock [5]. They have the ability to ferment carbohydrates with the help of enzymes capable of converting simple sugars into alcohol and carbon dioxide [6].

Besides its role in fermentation, *S. cerevisiae* has frequent use in experiments on genetics and cloning due to its rapid growth and easy manoeuvrability in culture [7].

Pectins are carbohydrate polymers consisting mainly of galacturonic acid units connected by glycosidic linkages which can be digested by enzyme polygalacturonase (PG) produced by many fungi and bacteria [8]. This enzyme is used in the food industry to solublize cell wall material, especially during the extraction and filtration of fruit juices [9].

Yeasts are known as PG source [10]. In this study, an attempt was made to isolate PG-producing fungi from local sources, and evaluate their enzyme production efficiency in comparison with the standard strains.

2. MATERIALS AND METHODS

Yeast samples were isolated from fruits like apple, apricot, mandarin, kiwi, and orange, available in Riyadh. Some isolates were obtained from sorghum grains and dairy products, at the College of Agriculture, KSU. *S. cerevisiae* strains, 1389 (wild type) and IM1-8b, used as references for the purpose of comparison, were obtained from Professor Villa, University of Santiago.

Isolation of yeasts was carried out using dilution method [11], and the isolates were purified and cultured on potato dextrose agar, Sabouraud dextrose agar, and corn meal agar media. Subsequently, pure colonies were maintained on YPD (yeast, peptone, dextrose) medium preferred for yeasts [12-14]. Cultures were incubated at 25-30 °C for 3 days. Yeast cultures were identified and distinguished, on the basis of morphology, stain reaction, and germ tube test by examination under the microscope.

PG activity was detected by plate assay method [15] using ruthenium red dye on yeast nitrogen base medium (YNB) after 3-5 day incubation at 30 °C. Enzyme activity was scored on the basis of color intensity on a 1-5 scale in ascending order, brightest being 5 and dimmest being 1. Cultures without color development were scored as '0' and excluded from the observations. Five plates were screened for each strain and means were tabulated with standard deviation of the sample.

3. RESULTS AND DISCUSSION

Microscopic examination confirmed exenic nature of individual yeast cultures (Fig, 1). Hence, these colonies were considered to be fit for determining PG activity in cultures.

Yeast cultures isolated from different sources have been listed in Table 1. All the fruits tested in this study proved to be sources of yeasts. Two species of *Candida,* and one species each of *Trichosporon* and *Cryptococcus,* were isolated from the five fruit species. Except for apricot which yielded two fungi, *Trichosporon mucoides* and *Candida guilliermondii*, all other fruits harbored only one fungus each. *Candida* was found on four out of five fruits. Among the yeasts isolated earlier at the College of Agriculture also, *Candida* was the dominant species. Six strains isolated from different fruits were found to show PG activity, of which 4 belonged to *Candida*, and one each to *Trichsporon* and *Cryptococcus*. In a similar study, Trindade et al. [16] found *Candida, Cryptococcus, Kloeckera, Kluyveromyces, Rhodotorula, Trichosporon* and *Saccharomyces species,* besides many other yeasts to be associated with some Brazilian fruits and their pulp. Spencer et al. [17] isolated 3 species of *Candida* including *guilliermondii*, and many other types of yeast from decaying oranges, limes, mandarins, and grapefruits. Strains isolated previously also showed PG activity of varying order. Yeast strains isolated during the present study have not been reported previously from Saudi Arabia.

Yeasts may cause rot in fruits and fruit pulps leading to quality deterioration and associated commercial losses. However, PG, the same enzyme playing a major role in this damage may be utilized to the benefit of food and beverage industry for its ability to solubilize the pectin component of cell wall [18]. Besides yeasts, some related fungi may also produce this enzyme. Guessous et al. [9] reported PG production by *Geotricum candidium.* In the present study, six strains of yeasts belonging to species of *Candida*, *Trichsporon* and *Cryptococcus,* were isolated from different fruits and were found to show PG activity (Table 1). To the best of our knowledge, this is the first report of PG production by these strains from Saudi Arabia. Of the two reference strains used in the present study, *S. cerevisiae* 1389 – wild type is known to show high pectolytic activity, while activity of strain IM1-8b is highly selective vis-à-vis carbon source, but is much lower as compared to the wild type strain [19]. One of the strains isolated during the present study (*S. cerevisiae*-MB1) showed remarkably higher capability of PG production with a color score of 4.6±0.08 (Table 1) as compared to score 3.4±0.20 for the reference strain. Other strains showed activities ranging between scores 1.4±0.08 and 3.4±0.08. Next to *S. cerevisiae* selection and the wild type, *Candida* strains showed higher PG activity with regard to other strains. However, not all *S. cerevisiae* strains showed a high activity. McKay [20] tested 33 strains of *S. cerevisiae* for PG activity, and found only 9 strains to be positive, and highlighted the importance of fermentation activity on plant products by these strains.

PG production ability of different species and strains of yeasts varies greatly as demonstrated by daSilva et al. [21]. They tested 300 isolates from different tropical fruits and reported that only 21 isolates belonging to 6 genera of yeasts produce PG. *Kluyveromyces wickerhamii* isolates secreted the highest amount of PG, followed by *K. marxianus* and *Stephanoascus smithiae*. Other yeast species showed lower PG activity. Gainvors et al. [22] studied 33 yeast species and found that only one strain of *S. cerevisiae* was able to secrete the enzyme. The present study has yielded a promising *S. cerevisiae* strain with high PG activity.

TABLE 1 - PG activity of some yeast strains isolated from different sources.

Yeast Strain	Source	Strain	PG score
Candida pelliculosa*	**Dairy products**	**MB3**	**2.4±0.08**
Rhodotorula minuta*	**Dairy products**	**MB4**	**1.8±0.18**
Candida colliculosa*	**Dairy products**	**MB5**	**2.8±0.18**
Saccharomyces cerevisiae*	**Dairy products**	**MB1**	**4.6±0.08**
Saccharomyces fragilis *	**Dairy products**	**MB2**	**3.2±0.18**
Kluyveromyces marixianus *	**Dairy products**	**MB6**	**2.8±0.05**
Saccharomyces lipopolitica*	**Dairy products**	**MB7**	**3.2±0.05**
Candida famata*	**Yellow apple**	**MB13** **MB14**	**2.2±0.04** **2.6±0.07**
Trichosporon mucoides*	**Apricot**	**MB15**	**2.2±0.05**
Candida guilliermondii*	**Apricot**	**MB16**	**3.0±0.10**
Cryptococcus albidus*	**Mandarin**	**MB17**	**2.0±0.08**
Candida guilliermondii*	**Kiwi**	**MB18**	**3.2±0.05**
Candida guilliermondii*	**Orange**	**MB19**	**3.4±0.08**
Kloeckera japonica*	**Sorghum**	**MB8**	**1.6±0.06**
Candida tropicalis*	**Sorghum**	**MB9**	**2.6±0.08**
Candida ciferii*	**Sorghum**	**MB10**	**3.4±0.06**
Candida guilliermondii*	**Sorghum**	**MB12**	**3.0±0.12**
S. cerevisiae ***	**-**	**IM1-8b**	**1.4±0.08**
S. cerevisiae ***	**-**	**1389-wild type**	**3.4±0.20**

* Strains obtained from College of Agriculture, KSU; **Strains isolated during this study; ***Reference strains

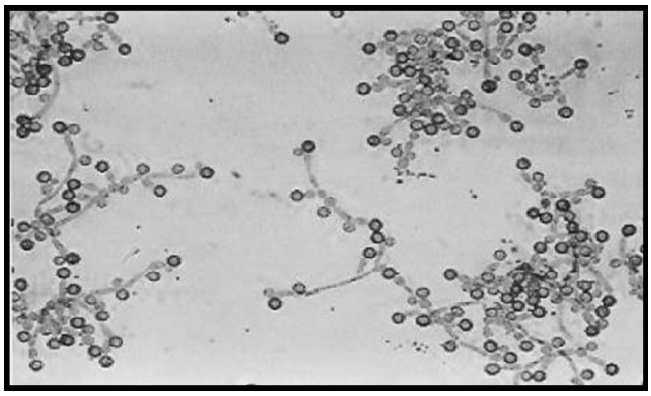

FIGURE 1 – Exenic yeast colonies under the microscope (x400).

ACKNOWLEDGEMENTS

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

REFERENCES

[1] Sleigh JD and Timbury MC (1994). Medical Bacteriology: fungal infection, 4th edn. Longman group, UK.

[2] Nyanga LK, Nout MJR, Gadaga TH, Theelen B, Boekhout T, and Zwietering MH (2007). Yeasts and lactic acid bacteria microbiota from masau (*Ziziphus mauritiana*) fruits and their fermented fruit pulp in Zimbabwe. Int. J. Food Microbiol. 120: 159-166.

[3] Loureiro V (2000). Spoilage yeasts in foods and beverages: characterisation and ecology for improved diagnosis and control. Food Res. Int. 33: 247-256.

[4] Botha A (2011). The importance and ecology of yeasts in soil. Soil Biol. Biochem. 1: 1-8.

[5] Kam AP and Xu J (2002). Diversity of commensal yeasts within and among healthy hosts. Diagnostic Microbiol. Infec. Disease 43: 19-28.

[6] Madigan M and Parker B (2000). Biology of Microorganisms, Ninth edition, Prentice-Hall, New Jersey. Pp. 407-413.

[7] Carlson M (2000). The awesome power of yeast biochemical genomics. Trends Genet. 16: 49-51.

[8] Blanco P, Diaz A, and Villa TG (1994). Production and partial characterization of an endopolygalacturonase from *Saccharomyces cerevisiae*. Canad. J. Microbiol. 40: 974-977.

[9] Guessous Z, Lebbar S, Ouhssine M, Mokhtari A, and El-Yachioui M (2001). Endo and exopolygalacturonase from *Geotricum candium*: partial purification and characterization. Sci. Lett. 3: 1-13.

[10] Murad HA and Foda MS (1992). Production of yeast polygalacturonase on dairy wastes. Bioresource Technol. 41: 247-250

[11] Nina G-G, Zalar P, deHoog S, and Plemenitas A (2000). Hypersaline waters in salterns natural ecological niches for halophilic black yeasts. FEMS Microbiol. Ecol. 32: 235-240.

[12] Ellis MB (1976). More dematiaceous hyphomycetes. Common Wealth Mycological Institute, Kew, Surrey, England.

[13] Ellis MB (1971). Dematiaceous hyphomycetes. Common Wealth Mycological Institute, Kew, Surrey, England.

[14] Barnett HLC (1960). Illustrated genera of imperfect fungi. Burgess, Publ. Co. Minneapolits. Pp. 241.

[15] Mckay AM (1988). A plate assay method for the detection of fungal polygalacturonase secretion. FEMS Microbiol. Lett. 56: 355-358.

[16] Trindade RC, Resende MA, Silva CM, and Rosa CA (2002). Yeasts associated with fresh and frozen pulps of Brazilian tropical fruits. Syst. Appl. Microbiol. 25: 294-300.

[17] Spencer DM, Spencer JFT, DeFigueroa L, and Heluane H (1992). Yeasts associated with rotting citrus fruits in Tucumán, Argentina. Mycol. Res. 96: 891-892.

[18] Blanco P, Sieiro C, and Villa TG (1999). Production of pectic enzymes in yeasts. FEMS Microbiol. Lett. 175: 1-9.

[19] Blanco P, Serio C, Diaz A, and Villa TG (1997). Short communication: Differences between pectic enzymes produced by laboratory and wild-type strains of *Saccharomyces cerevisiae*. World J. Microbiol. Biotechnol. 13: 711-712.

[20] Mckay AM (1990). Degradation of polygalacturonic acid by *Saccharomyces cerevisiae.* Lett. Appl. Microbiol. 11: 41-44.

[21] daSilva EG, Borges MdF, Medina C, Piccoli RH, and Schwan RF (2005). Pectinolytic enzymes secreted by yeasts from tropical fruits. FEMS Yeast Res. 5: 59-865.

[22] Gainvors V, Lemaresqutes FH, and Blarb A (1994). Detection of polygalacturonase, pectin lyase and pectin esterase activities in *Saccharomyces cerevisiea.* Yeast 10: 1311-1319.

Received: November 09, 2011
Accepted: February 08, 2012

CORRESPONDING AUTHOR

Mohammad A. Moslem
Department of Botany and Microbiology
College of Science
King Saud University
P.O. Box 2455
Riyadh 11451
SAUDI ARABIA

Phone: +966-1-4675832
E-mail: mbmoslem@ksu.edu.sa