An Acad Bras Cienc (2020) 92(3): e20191140 DOI 10.1590/0001-3765202020191140
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

AGRARIAN SCIENCES

Isolation and identification of *Saccharomyces cerevisiae* for fermentation of rice polishing in Livestock feeding

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Abstract: Biomass of *Saccharomyces cerevisiae* wasenhancedin rice polishing by fermentation to increase protein contents of feedfor its use in livestock. Broth culture of *Saccharomyces cerevisiae* (2.6×10^8CFU/mL) was prepared from culture obtained by continuous streaking. The isolated culture was identified morphologically by Gram staining and confirmed by biochemical characteristics. Rice polishing was sieved to remove larger particles. Then it was distributed to 4 treatments in triplicates. Treatments were represented as rice polishing (RP), rice polishing plus *Saccharomyces* culture (RPSC), rice polishing plus ammonium sulphate (RPAS), rice polishing plus *Saccharomyces* culture plus ammonium sulphate (RPSCAS).Fermentation was provided for 144 hours at 32^0C,while samples were collected after every 24 hours. Samples were dried, ground and subjected to proximate analysis. It was observed that protein content was increased from 11% to 21.51% and maximum increment was obtained after 144 hours of incubation in RPSC treatment. Ether extract and ash were increased from 14% and 10% to 16.96% and 11.11% in RPSCAS respectively. A significant reduction in neutral detergent fiber was observed after fermentation. It is concluded that *Saccharomyces cerevisiae* has potential to improve mineral and protein contents of rice polishing by fermentation process with or without addition of nitrogen source.

Key words: *Saccharomyces cerevisiae*,rice polishing, protein, livestock, fermentation.

INTRODUCTION

Nutrition is the most important aspect in livestock production as feed costs usually 70% of the total production cost (Imran et al. 2016). Nutritional programs for livestock mainly focus on providing a precise level of various nutrients to explore optimum production performance and ultimately healthy profitability (Gaafar et al. 2010). Among different nutrients, protein possesses the major importance regarding its role in animal production and limited resource availability (Van Zanten et al. 2016). These protein requirements are fulfilled usually by fodders and concentrates which are conventional protein sources. However, these sources adversely affect the profit margin due to its high price. Moreover, their availability is not sufficient to keep pace with increased production of modern day breeds due to improved genetic potential (Deb et al. 2016). Therefore, there should also be inclusion of non-conventional protein sources in livestock feed that can compensate the shortage of conventional sources.

Saccharomyces cerevisiae is an unicellular fungus distributed widely in nature and rich in protein, vitamins and minerals (Rodríguez et al. 2011). Moreover, it has a good balance of amino acids and also reported with probiotic properties (Adedayo et al. 2011, Amata 2013). Chemical

composition for live yeast culture consists of dry matter (DM) 93%, crude protein (CP) 44.50%, ether extract (EE) 1.10%, ash 3.50%, crude fiber (CF) 2.75%, metabolizable energy (ME) 1990 Kcal/Kg (Küçükersan et al. 2009). Several studies has been reported with positive influence of *Saccharomyces cerevisiae* on DM intake (Titi et al. 2008), nutrient digestibility (Obeidat et al. 2018, Ullah et al. 2017), live weight gain (Yalcin et al. 2011), feed conversion ratio (Lesmeister et al. 2004), ruminal parameters (rumen pH, ruminal ammonia concentration), milk production and milk composition (Ayad et al. 2013). Therefore, it can be a better replacement of conventional protein sources (Sharif et al. 2018). Moreover, it also has the ability to produce single cell protein (SCP) by growing on large number of solid substrates such as citrus, pulp, pineapple waste, potato waste etc (Adedayo et al. 2011). Therefore, it is comparatively a better choice for SCP production due to its rapid multiplication and eco-friendly nature.

Rice polishing is also being used in livestock ration due to its rich energy value and economical price. Its nutritive value has a close resemblance with maize in supply of total digestible nutrients (Shih 2003). Moreover, it contains high amounts of copper, phosphorous, iron, potassium and zinc (Hossain et al. 2012). While comparing with other cereal grains like wheat and corn, it is reported with better amino acid assortment and profile (Khalique et al. 2004). It also contains Y-oryzanol and some antioxidants which are of great medical importance (Iqbal et al. 2005). Its low protein can also be enhanced by fermentation using *Saccharomyces cerevisiae.*

It was hypothesized that protein content of rice polishing can be increased along with other nutrients by fermentation using *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Research trial was conducted jointly at the labs of Institute of Microbiology and Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad, Pakistan. The study was approved by the scrutiny committee of the institute and ethical considerations were observed while cannulation in animal (Reference No. dgs/257-60).

Sampling and culturing of *Saccharomyces cerevisiae*

Isolation of *Saccharomyces cerevisaie* was done from rumen liquor. Sample from cannulated buffalo bull was collected 7 hours post feeding. It was spread on petri plate using swabbing technique. The isolated culture was identified morphologically by Gram staining and confirmed by biochemical characteristics. Broth culture of *Saccharomyces cerevisaie* was prepared in the lab having concentration of 2.6×10^{8}CFU/ml.

Incubation for fermentation

One kg of rice polishing was obtained from local market. It was sieved to remove the larger foreign particles. Then it was packaged in polythene bags for further use. Packaged rice polishing was distributed to 4 treatments in triplicates (Figure 1). Treatments were represented as RP (only rice polishing), RPSC (rice polishing along with *Saccharomyces* culture), RPAS (rice polishing with ammonium sulphate) and RPSCAS (rice polishing with *Saccharomyces* culture and ammonium sulphate). Moisture was adjusted to 65% and was autoclaved. Then, all flasks were incubated at 32°C with continuous shaking for 144 hours. Samples were collected in sterile environment after every 24 hours. Collected samples were dried using hot air oven at 45°C to avoid any nutrient damage. These dried samples were ground and packaged for further analysis.

Chemical analysis

Air tight packaged samples were undergone proximate analysis. Dry matter was determined by oven drying at 105°C for 24 hours and ash content by burning samples at 600°C for 3 hours(AOAC 2000). Nitrogen content was determined by Kjeldhal's method and CP was calculated as N% × 6.25(AOAC 2000). Neutral detergent fiber and acid detergent fiber were determined by using sodium sulphite (Van Soestet al. 1991).

Statistical analysis

Data on dry matter, crude protein, ash, ether extract, neutral detergent fiber and acid detergent fiber were subjected to Analysis of Variance (ANOVA) using statistical package for social sciences (SPSS 2009). Means were compared using Tukey's test. Level of 0.05 was chosen as probability level for analysis.

RESULTS

Isolated yeastwas identified as it appeared Gram positive and oval shaped after staining procedure. Both single cell and budding stage were seen under microscope (Figure 2). Presence of *Saccharomyces cerevisiae* was further confirmed by yeast plus (Figure 3). Highest values for CP were observed in RPAS and RPSCAS followed by RPSC and RP respectively. After 48 hours of incubation, CP values had also shown significant differences (p<0.05) across the treatments. Highest values for CP were observed in RPSC, RPAS and RPSCAS followed by RP. After 72 hours of incubation, highest statistical values were observed in treatment containing *Saccharomyces* culture and rice polishing while lowest values were recorded in treatment having only rice polishing. After 96 hours of incubation, highest statistical values for CP were observed in RPSC treatment followed by RPAS and RPSCAS. After 120 hours of incubation, highest statistical values for CP were observed in RPSC while lowest values were recorded in RP and RPSCAS. At the end of complete incubation period, highest CP was observed in treatment containing *Saccharomyces* culture with rice polishing while lowest values were recorded in rice polishing alone and RPSCAS. Best results for CP were found in treatment containing *Saccharomyces* culture after 144 hours of incubation (Table I).

Ether extract values for rice polishing were given as percentage on DM basis. Highest values for ether extract were recorded in treatment containing rice polishing, *Saccharomyces* culture and ammonium sulphate. While lowest values

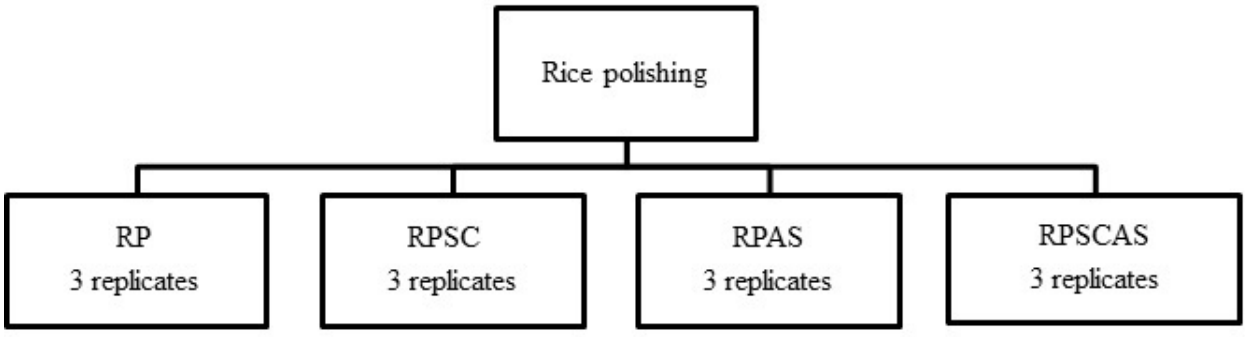

Figure 1. Flow diagram of research experiment. RP= Rice polishing,RPSC=Rice polishing plus *Saccharomyces* culture,RPAS=Rice polishing plus ammonium sulphate, RPSCAS=Rice polishing plus ammonium sulphate and *Saccharomyces* culture.

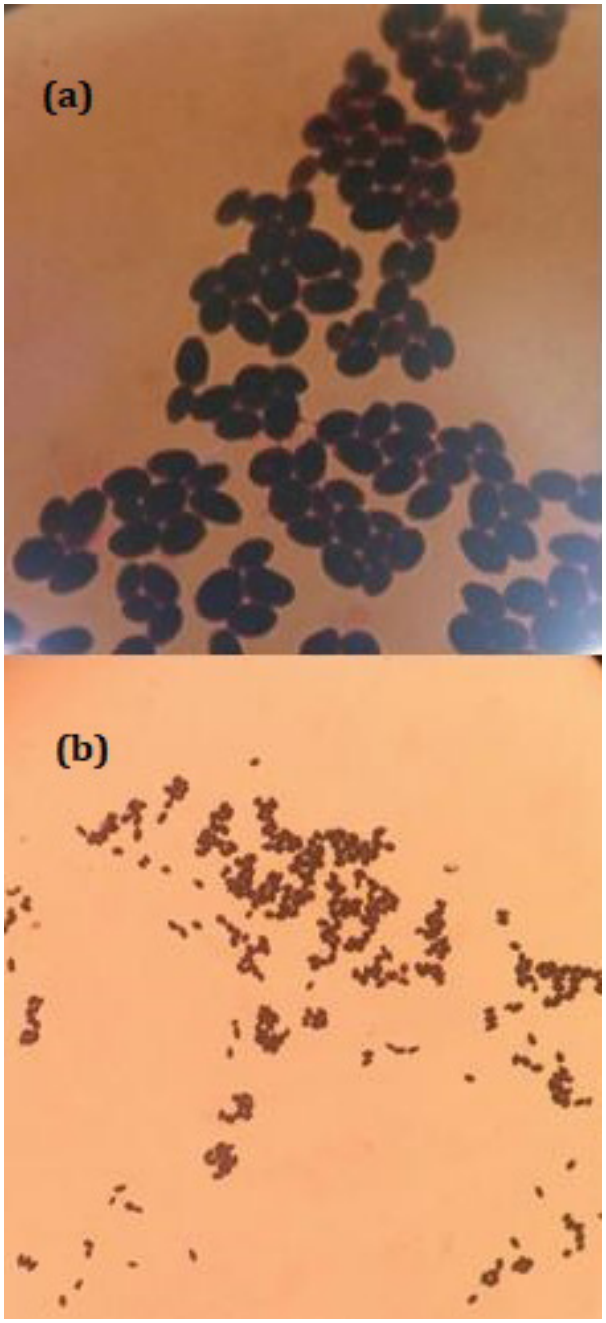

Figure 2. (a, b): Single cell and budding stages of *Saccharomyces cerevisiae* after gram staining.

were recorded in treatment having ammonium sulphate. Ash values for rice polishing were given as percentage on DM basis. Highest values for ash were observed in RPSCAS, RPSC and RP followed by RPAS. However, there was no significant difference (p>0.05) between RP and RPAS. The NDF had highest values in RP and RPSC while results were non-significant among all the treatments for ADF (Table II).

DISCUSSION

Single cell protein is the biomass produced by different types of microorganisms like yeast fungi, bacteria and algae (Saeed et al. 2016). Different substrates and microorganisms have been used for this purpose while in this experiment, *Saccharomyces cerevisiae* in rice polishing was used as active microorganism and substrate to investigate change in various nutrients after fermentation. It was observed that highest values of CP were noticed in RPSC after 144 hours of incubation. Results were in accordance with Oboh & Akindahunsi(2003) who observed increase in crude and true protein content in cassava byproducts. These results were also supported by Correia et al.(2007) who reported an increase in CP of pineapple waste after 120 hours of incubation. Increase in CP content can be attributed to the secretion of extracellular enzymes like amylase by *Saccharomyces cerevisiae* which helps in catalysis of complex polysaccharides in the substrate and metabolized into its constituent elements (Correia et al. 2007). Moreover, protein increment was due to the biomass production after proliferation of *Saccharomyces cerevisiae* on given substrate (Antai & Mbongo 1994). However, different results were observed by Aruna et al. (2017) who observed more increase in protein when inorganic nitrogen supplementation was done while rice polishing has some negative relationship of protein increment with inorganic nitrogen supplementation. More increase after nitrogen supplementation can be due to the nutritive nature of nitrogen. This nitrogen increases the microbial growth by exerting

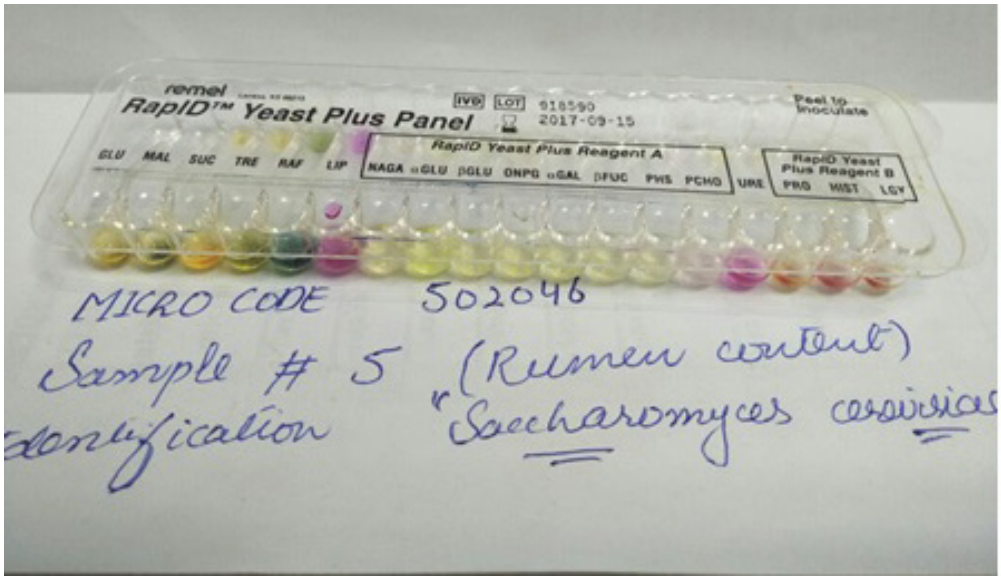

Figure 3. **Generation of code with yeast plus remel kit.**

positive effect on pH of the medium (Oboh & Akindahunsi 2003).

Ether extract content of rice polishing was also increased after fermentation with highest values observed with inorganic nitrogen supplementation and inclusion of *Saccharomyces* culture (RPSCAS). Results are in line with the findings of Aruna et al. (2017) who recorded increase in fat content of yam peels after fermentation with supplementation of inorganic nitrogen. Increase in fat content may be as a result of possible bio-conversion carbohydrate to fat and also certain fungal species are capable of building up fat during the fermentation process (Akindumila & Glatz 1998). The increase in the fat content could also be attributed to the fact that the fungi could secrete microbial oil (Araoye 2004). Ash content was highest in the presence of *Saccharomyces cerevisiae* with or without addition of nitrogen. These finding were supported by those of Aruna et al. (2017) who observed an increase in ash content of yam peels from 3.98% to 7.01% after fermentation. Increase in ash content indicates that *Saccharomyces* has some particular roles to increase inorganic minerals in the substrate through various biosynthetic and hydrolytic mechanisms (Adeyemi et al. 2012). The NDF content of rice polishing was decreased addition of after inorganic nitrogen along with *Saccharomyces* culture which indicates the fiber degradation and improved palatability while ADF content remained non-significant across the treatments. Results regarding decrease in NDF content were in line with findings of Nasehi et al.(2017) who also observed a significant decrease (p<0.05) in NDF content of different substrates i.e. rice straw, wheat straw, soyabean straw, canola straw etc. This decrease in NDF content of wastes treated with various fungal species may be due to the degradation of substrates cell wall components by extracellular enzymes secreted by active fungus Akinfemi et al. (2010). It has two different enzyme systems one of which is of hydrolytic nature responsible for degradation of polysaccharides while other is oxidative and lignolyticwhich causes opening of phenyl rings by degradation of lignin (Sánchez 2009).

Table I. Crude protein content of rice polishing at different periods of fermentation.

Hours	RP	RPSC	RPAS	RPSCAS	SE*	*p*-value
24	11.13^{c}	16.4^{b}	19.66^{a}	19.29^{a}	1.06	0.005
48	11.3^{b}	18.22^{a}	17.86^{a}	17.86^{a}	0.89	0.013
72	10.93^{c}	19.29^{a}	17.13^{b}	16.4^{b}	1.95	0.004
96	11.66^{c}	20.73^{a}	17.49^{b}	15.67^{b}	1.5	0.003
120	11.3^{c}	21.49^{a}	16.77^{b}	13.1^{c}	1.21	0.034
144	10.93^{c}	21.51^{a}	15.3^{b}	13.46^{c}	1.22	0.002

Means in the same row with different superscripts were significantly different from each other.
***Standard error.**
RP, RPSC, RPAS and RPSCAS represent rice polishing, rice polishing plus *Saccharomyces* culture, rice polishing plus ammonium sulphate and rice polishing plus ammonium sulphate and *Saccharomyces* culture, respectively.

Table II. Nutrient profile of rice polishing after fermentation.

Nutrients (%)	RP	RPSC	RPAS	RPSCAS	SE*	*p*-value
Ether extract	14.54^{c}	15.85^{b}	14^{d}	16.96^{a}	0.35	0.001
Ash	10.43^{ab}	10.74^{a}	9.85^{b}	11.11^{a}	0.16	0.005
Neutral detergent fiber	36.59^{a}	34.36^{ab}	33.83^{b}	32.64^{b}	0.51	0.008
Acid detergent fiber	18.75	18.15	17.88	17.09	0.25	0.094

Means in the same row with different superscripts were significantly different from each other.
***Standard error .**
RP, RPSC, RPAS and RPSCAS represent rice polishing, rice polishing plus *Saccharomyces* culture, rice polishing plus ammonium sulphate and rice polishing plus ammonium sulphate and *Saccharomyces* culture, respectively.

CONCLUSION

It is concluded that *Saccharomyces cerevisiae* has potential to improve the mineral and protein contents of rice polishing by fermentation process with or without addition of nitrogen source.

Acknowledgments

We gratefully acknowledge the financial support by the Higher Education Commission, Pakistan for financing this project study (Project No. 20-4592/NRPU/R&D/HEC).

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How to cite

SHARIF M, ZAFAR MH, RAHMAN SUR, ANAM S, ASHFAQ K & AQIB AI. 2020. Isolation and identification of Saccharomyces cerevisiae for fermentation of rice polishing in Livestock feeding. An Acad Bras Cienc 92: e20191140. DOI 10.1590/0001-3765202020191140.

Manuscript received on September 20, 2019;
accepted for publication on February 3, 2020

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Author contributions

All the team members have contributed in conceiving and designing the study, Muhammad Sharif prepared the research proposal and got financial support from Higher Education Commission (Project No. 20-4592/NRPU/R&D/HEC). Pakistanfor this project. He also supervised all the experiment. Muhammad Hammad Zafar performed fermentation part of experiment including chemical and statistical analysis. Moreover, he wrote first version of this manuscript. Sajjad UR Rehman was responsible for optimizing the protocol of this study. Sidra Anum performed the isolation of *Saccharomyces cerevisiae*. Khurram Ashfaq and Amjad and Islam Aqib assisted in proof reading the manuscript.