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Research Article
Production of Polysaccharides and Single-Cell Protein by Some Local Isolates of *Trichoderma* spp.

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Abstract

Background and Objective: Polysaccharides and Single-cell protein are one of the best essential natural products of microorganisms, they are excreted by different microorganisms such as yeast, fungi, bacteria and algae. This study was carried out to detect the ability of four local fungal isolates of *Trichoderma* spp. to produce polysaccharides and Single-cell protein. **Materials and Methods:** Standard Czapek Dox Broth Medium was used to detect the ability of fungal isolates to produce polysaccharides and Single-cell protein, with modified the components of medium for improved production using banana peels as a source of carbon and different nitrogen sources at different concentrations and the factorial experiment was carried out using a completely randomized design **Results:** The highest dry weight and polysaccharides production and protein content have been achieved for the fungus *T. reesei* with rates of (2.15, 0.276 and 0.94) g/100 mL, respectively, in comparison with the other treatments, the use of ammonium phosphate at concentration 0.6 g L^{-1} has given the highest dry weight and production of polysaccharides and protein content with rates of (3.75, 0.364 and 2.77) g/100 mL, respectively, also the use of banana peels extract at concentration 40 mL L^{-1} has given the highest dry weight and production of polysaccharides and protein content with rates of (5.21, 0.539 and 3.63) g/100 mL, respectively. **Conclusion:** The possibility of using the local isolate of *T. reesei* in the production of polysaccharides and Single-cell protein using some cheap agricultural waste such as banana peels as a carbon source instead of throwing them as waste and pollutants for the environment.

Key words: Intracellular polysaccharides, single-cell protein, *Trichoderma* spp., banana peels, peptidoglycan, teichoic acid

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The increase in world population, food shortages and the difference in living standards have a major impact on the fact that recent studies are moving towards the use of fermentation methods to produce different foodstuffs from inexpensive agricultural and industrial wastes[1]. These methods were not limited to animal feed supports production but rather developed to produce foodstuffs that are suitable for human consumption, among the most important of these materials are microbial polysaccharides and Single-cell protein[2,3]. Polysaccharides are one of the best essential natural products of microorganisms and these compounds are found either in or outside the cells and their presence provides the basis for their classification as they are either included in the cell wall synthesis and thus are called Structural polysaccharides and they are specific to some microorganism such as Peptidoglycan, Lipopolysaccharide and Teichoic acid in the bacterial cells and Mannan in yeast or they are sugars stored inside the cells and thus are called Intracellular polysaccharides or they are excreted outside the cells and thus are called Extracellular polysaccharides[4]. The number of these sugars amounts to some 200 different types depending on the species of the organism that produces them[5]. Microbial polysaccharides contain different polymeric monosaccharides like D-glucose, D-galactose, D-mannose, L-rhamnose and L-fucose. In their composition, these compounds may contain amino sugars such as N-acetyl-D-glucosamine and sugar acids such as D-glucuronic acid and D-galacturonic acid, they thus gain a negative charge[6]. Unicellular proteins are also non-traditional foods that are used with high efficiency to meet part of the nutritional needs of humans and animals. These proteins are produced from the cultivation of microorganisms including bacteria, fungi, yeasts and some types of algae on the culture media from agricultural and industrial wastes, with the addition of some growth supports[7,8]. The term Single-cell protein was first used by the scientist Carol Wilson and its first production began in World War II in Germany[9]. This type of protein can be produced at low economic costs using large and diverse quantities of agricultural and industrial wastes as basic materials, this means ridding the environment of the accumulation of these wastes and using them to obtain products of economic benefit[10,11]. This study was carried out to detect the capability of some local fungal isolates, which are *Trichoderma harzianum*, *T. viride*, *T. reesei* and *T. hamatum* to produce polysaccharides and Single-cell protein and the impact of using banana peels and different nitrogenous sources to improve production.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biology, College of Science , University of Qadisiyah, Iraq from August-November, 2020.

Microorganism: The fungal isolates under study (*T. hamatum*, *T. viride*, *T. reesei* and *T. harzianum*) were obtained from the Ministry of Science and Technology, Directorate of Agriculture Research, Department of Biotechnology, Iraq. They were preserved inside sterile glass bottles at 5°C, containing the slant PDA medium.

Culture media

Potato dextrose agar medium (PDA): This medium was used to isolate, preserve and stimulate the fungal isolates under study, it has been prepared from 200 g of potatoes, 20 g of glucose and 20 g of Agar. Then filled to 1000 mL with distilled water and sterilized using autoclave at 121°C for 20 min.

Czapek-dox broth medium: This standard medium was used to grow the fungal isolates under study during the experiments to detect their ability to produce polysaccharides and Single-cell protein, this medium consists of the following materials:

- About 0.3 g $NaNO_3$, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 1 g K_2HPO_4, 5 g Yeast extract, 30 g Sucrose and 1000 mL distilled water, then the medium was sterilized using autoclave at 121°C for 20 min[12]. The antibiotic Chloramphenicol was added at a concentration of 25 (mg L^{-1}) to the above culture media after sterilization to prevent the growth of contaminated bacteria

Preparation of the fungal inoculum: The fungal inoculum was prepared from pure 7 days old fungal colonies under sterile conditions, sterile distilled water was added to the growing fungal colonies in sterile glass bottles containing the slant PDA medium. Afterwards, the fungal culture was scraped separately using an L-shaped glass rod[13].

Detecting the capability of fungi to produce polysaccharides and single-cell protein: A total 100 mL of pre-prepared Czapek-Dox Broth Medium was added to sterile 250 mL flasks and the initial pH of the medium was set at 6.0, then the fungal inoculum prepared in the previous step was added at a concentration of 1% at a rate of three replicates per treatment separately, Then the flasks were

placed in the incubator at 28°C for 6 days, then the flasks were removed from the incubator, the dry weight of the fungi. Polysaccharides and the amount of protein in the culture media were estimated as follows:

Estimation of dry weight: After the incubation period ended, the centrifugation process of the content of each flask was conducted at a rate of 6000 rpm for 10 min, after which the clear solution was taken for the estimation of polysaccharides while the fungal cells were collected in small plates of known weight and were dried in the oven at a 70°C for 24 hrs, afterwards the dry weight was estimated using a sensitive balance and the biomass was measured by the difference of both weights[14].

Estimation of polysaccharides: A total of 10 mL of the clear solution that resulted from the centrifugation process was taken and 4 volumes of the organic solvent were added to it (ethanol 70% volume/volume) and the mixture was vigorously stirred to precipitate the polysaccharides, then the centrifugation process was conducted at a rate of 9000 rpm for 30 min, After that the polysaccharides were collected in small plates of known weight and were dried in the oven at 70°C for 24 hrs, afterwards the weight of polysaccharides was estimated by the difference of both weights[15].

Estimation of protein: The amount of protein in the dry weight of all the experiments above was estimated using the method of Mondal *et al.*[16], then the most efficient isolate at production was selected and then the use of different nitrogenous sources for Czapek-Dox Broth Medium was tested instead of using sodium nitrate $NaNO_3$ to improve the production and those sources are: urea CH_4N_2O, ammonium sulfate $(NH_4)_2SO_4$, ammonium phosphate $(NH_4)_3PO_4$ and ammonium chloride NH_4Cl, different concentrations from the best nitrogenous source were also tested.

Banana peels medium: After determining the optimal isolation and conditions for the production of polysaccharides and Single-cell protein, the possibility of using different concentrations of banana peels extract (10, 20, 30, 40 and 50) mL L^{-1} as a carbon source instead of using sucrose in the standard Czapek-Dox Broth Medium was tested. The banana peels extract was prepared by collecting 500 g of banana peels and washing them well with water and then with sterile distilled water, they were cut into small pieces and then soaked in water and blended with a mixer in the ratio of 1:4, then banana peel extract was obtained with the help of cheesecloth[17]. After that, the dry weight, polysaccharides and the amount of protein produced were measured as in the above-mentioned methods, with the same production methods and conditions.

Statistical analysis: The factorial experiment was carried out using a completely randomized design, the results were statistically analyzed and tested using the Duncan method test[18].

RESULTS

Detecting the dry weight, polysaccharides and protein content: The results are shown in Table 1 that there is a clear significant difference in growth and production rates between the types of the fungus *Trichoderma* spp, that are cultivated in Czapek-Dox Broth Medium, it was found that the fungus *T. reesei* has given the highest dry weight and polysaccharides production with rates of (2.15, 0.276) g/100 mL, respectively. Regarding protein content, the results have shown that there were no significant differences in production for the two fungi *T. reesei* and *T. viride* with rates of (0.94, 0.92) g/100 mL, respectively, in comparison with the other treatments. Accordingly, *T. reesei* has been selected for other experiments.

Nitrogenous sources effect on the dry weight, polysaccharides production and protein content: When using different nitrogenous sources for the cultivation of *T. reesei*, the results are shown in Table 2 that there are significant differences in growth and production rates, it was noted that the nitrogenous source of ammonium phosphate gave the highest dry weight and production of polysaccharides and protein content in comparison with the other treatments with rates of (2.83, 0.293, 1.05) g/100 mL, respectively. The control treatment (without adding a nitrogenous source) has given the lowest dry weight and production of polysaccharides and protein content, in comparison with the other treatments with rates of (1.45, 0.163 and 0.56) g/100 mL, respectively.

Ammonium phosphate concentrations effect on the dry weight, polysaccharides production and protein content: The results indicated that concentration 0.6 g L^{-1} gave the highest dry weight and production of polysaccharides and protein content with rates of (3.75, 0.364 and 2.77) g/100 mL, respectively with significant differences in comparison with the other treatments, followed by concentration 0.4 g L^{-1} with rates of (2.58, 0.295 and 1.19) g/100 mL, respectively (Table 3).

Table 1: Detecting the dry weight, polysaccharides and protein content

Fungal species	Dry weight (g/100 mL)	Polysaccharides (g/100 mL)	Protein content (g/100 mL)
T. harzianum	1.64±0.20^{d}	0.180±0.01^{c}	0.72±0.02^{c}
T. viride	1.88±0.15^{c}	0.147±0.01^{d}	0.92±0.03^{a}
T. reesei	2.15±0.10^{a}	0.276±0.02^{a}	0.94±0.01^{a}
T. hamatum	1.96±0.20^{b}	0.192±0.03^{b}	0.81±0.02^{b}

Values followed by vertically different letters indicate that there are significant differences between them at the probability of level 5%. Values are means average of three replicates±standard deviation

Table 2: Effect of different nitrogenous sources on the dry weight, the production of polysaccharides and protein content for *T. reesei*

Nitrogenous sources	Dry weight (g/100 mL)	Polysaccharides (g/100 mL)	Protein content (g/100 mL)
Control 0.0	1.45±0.10^{d}	0.163±0.01^{e}	0.56±0.04^{d}
Urea	2.46±0.30^{c}	0.242±0.03^{c}	0.81±0.02^{c}
Ammonium sulfate	2.57±0.25^{b}	0.269±0.02^{b}	0.94±0.02^{b}
Ammonium phosphate	2.83±0.10^{a}	0.293±0.02^{a}	1.05±0.01^{a}
Ammonium chloride	2.45±0.20^{c}	0.214±0.03^{d}	0.83±0.03^{c}

Values followed by vertically different letters indicate that there are significant differences between them at the probability of level 5%. Values are means average of three replicates±standard deviation

Table 3: Effect of different ammonium phosphate concentrations on the dry weight, the production of polysaccharides and protein content for *T. reesei*

Ammonium phosphate concentration (g L^{-1})	Dry weight (g/100 mL)	Polysaccharides (g/100 mL)	Protein content (g/100 mL)
Control 0.0	1.40±0.20^{e}	0.166±0.02^{e}	0.53±0.03^{e}
0.2	2.46±0.20^{c}	0.235±0.02^{c}	0.72±0.03^{d}
0.4	2.58±0.20^{b}	0.295±0.01^{b}	1.19±0.01^{b}
0.6	3.75±0.20^{a}	0.364±0.02^{a}	2.77±0.01^{a}
0.8	2.03±0.30^{d}	0.237±0.01^{c}	0.86±0.02^{c}
1.0	1.39±0.30^{e}	0.213±0.03^{d}	0.73±0.01^{d}

Values followed by vertically different letters indicate that there are significant differences between them at the probability of level 5%. Values are means average of three replicates±standard deviation

Table 4: Effect of using different concentrations of banana peels on the dry weight, the production of polysaccharides and protein content for *T. reesei*

Banana peels concentration (mL L^{-1})	Dry weight (g/100 mL)	Polysaccharides (g/100 mL)	Protein content (g/100 mL)
Control 0.0	1.21±0.20^{e}	0.113±0.04^{e}	0.21±0.02^{e}
10	2.47±0.30^{d}	0.244±0.02^{d}	0.62±0.01^{d}
20	2.92±0.25^{c}	0.389±0.03^{c}	1.38±0.02^{c}
30	4.46±0.20^{b}	0.454±0.01^{b}	2.87±0.01^{b}
40	5.21±0.30^{a}	0.539±0.02^{a}	3.63±0.01^{a}
50	1.42±0.20^{e}	0.242±0.01^{d}	0.65±0.02^{d}

Values followed by vertically different letters indicate that there are significant differences between them at the probability of level 5%. Values are means average of three replicates±standard deviation

Different concentration of banana peels effect on the dry weight, polysaccharides production and protein content: The results showed that concentration 40 mL L^{-1} gave the highest dry weight and production of polysaccharides and protein content in comparison with rates of (5.21, 0.539 and 3.63) g/100 mL, respectively, with significant differences in comparison with the other treatments, followed by a concentration of 30 mL L^{-1} with rates of (4.46, 0.454 and 2.87) g/100 mL, respectively (Table 4).

DISCUSSION

The results showed the ability of the local fungal isolates understudy to produce polysaccharides and Single-cell protein with varying amounts using the standard Czapek Dox broth medium. The discrepancy in the growth and production rates among the fungal isolates might be due to the difference in enzymatic capacity and thus exploiting the culture medium components and synthesizing it[19]. Several studies have indicated that the properties of productive polysaccharides and the Single-cell protein vary according to the microorganism, carbon source, nitrogenous source, nutrients and the utilized cultivation method[20,21]. Creating a state of balance between growth and carbon source concentration is one of the important things that must be taken into consideration when those microorganisms are intended for production without biomass, it is known that carbon compounds play an important role in building cells aside from the fact that they are a source of energy, therefore the culture medium used for production must be prepared

in balanced quantities from the carbon source to obtain the best production[22]. Accordingly, the nitrogenous source adopted ammonium phosphate as the best nitrogenous source and different concentrations of it were to increase production. As for the nitrogenous source, it affects the fermentation process. The process of adding easily consumed sources by the microorganism in specific concentrations leads to the promotion of primary metabolism processes and the speed of cellular growth at the expense of the production of secondary metabolites[23]. Ammonium phosphate is one of the most important inorganic nitrogenous sources because it contains a good percentage of nitrogen which is one of the essential components for cell building and its growth, in addition to that, it is included in the synthesis of proteins and nucleic acids[24]. The results obtained conform to several studies that indicated the possibility of using different types of fungi (molds and yeasts) in the production of polysaccharides and Single-cell protein, it has been produced Single-cell protein from medium containing banana peel waste using local isolate *T. harzianum* which produced a significant level of biomass yield in the presence of different carbon and nitrogen sources[25]. It also produced a mixture of extracellular polysaccharides from the fungus *Penicillium vermiculatum* which is Proteoglycan[26]. De Gregorio *et al*.[27] used lemon residues for the production of Single-cell protein by the two fungi *Aspergillus niger* and *T. viride*. In another study, it was produced Single-cell protein through fermenting pineapple wastes and banana peels by *T. viride* and this protein was used to feed animals[28]. Moreover, in two modern studies, it was found that *T. reesei* can produce Single-cell protein in high amounts using solid-state fermentation cultures containing rice straw pulp and urea as a carbon and nitrogen source, respectively[29,30]. The production of polysaccharides and Single-cell protein depends mainly on the type of fungal isolates used in production and on providing the best sources of carbon, nitrogen, mineral elements and nutrients, in addition to providing the appropriate environmental conditions, such as temperature, pH and incubation period[31,32]. Therefore, the use of cheap agricultural waste, such as banana peels as an example in this study, instead of throwing them as harmful pollutants to the environment, is one of the most important steps in achieving the aims of biotechnology applications in the production of useful, important and non-toxic materials from cheap and abundant waste and preserving the environment from pollution by using various microorganisms and developing production by using special larger fermenters and other fermentation methods and improving production conditions such as the size of the inoculum, temperature, pH and incubation period.

CONCLUSION

This study demonstrates the possibility of using the local isolate *T. reesei* in the production of polysaccharides and Single-cell protein using Czapek-Dox Broth Medium with Banana peels extract as a carbon source at a concentration of 40 mL L^{-1} instead of using Sucrose and ammonium phosphate as a nitrogenous source at a concentration of 0.6 g L^{-1} instead of using sodium nitrate with production conditions that were represented by a temperature of 28°C, the incubation period for 6 days and an initial pH of the medium at 6.0.

SIGNIFICANCE STATEMENTS

This study discovered the possibility of using the local isolate *T. reesei* in the production of polysaccharides and Single-cell protein which has great importance in the food and industrial field using some cheap agricultural waste such as banana peels instead of throwing them as waste and pollutants for the environment. This study will help the researcher to uncover the possibility of using *Trichoderma* spp. especially *T. reesei* in important biotechnological processes in converting various agricultural and industrial wastes that are harmful to the environment into useful and important materials on the food, medical or commercial levels, which have not been extensively studied previously compared to the study of their use in biological control.

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REFERENCES

1. Ayala-Zavala, J.F., V. Vega-Vega, C. Rosas-Dominguez, H. Palafox-Carlos and J.A. Villa-Rodriguez *et al*., 2011. Agro-industrial potential of exotic fruit byproducts as a source of food additives. Food Res. Int., 44: 1866-1874.

2. Aksnes, A., B. Hope, Ø. Høstmark and S. Albrektsen, 2006. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, Gadus morhua. Aquaculture, 261: 1102-1110.
3. Lee, K.J., M.S. Powell, F.T. Barrows, S. Smiley, P. Bechtel and R.W. Hardy, 2010. Evaluation of supplemental fish bone meal made from Alaska seafood processing byproducts and dicalcium phosphate in plant protein based diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 302: 248-255.
4. Lovegrove, A., C.H. Edwards, I. De Noni, H. Patel and S.N. El *et al*., 2017. Role of polysaccharides in food, digestion and health. Crit. Rev. Food Sci. Nutr., 57: 237-253.
5. Huang, W.C. and I.C. Tang, 2007. Bacterial and Yeast Cultures-Process Characteristics, Products and Applications. In: Bioprocessing for Value-Added Products from Renewable Resources, Yang, S.T. (Ed.)., Elsevier B.V., pp: 185-223.
6. Shintani, T., 2019. Food industrial production of monosaccharides using microbial, enzymatic and chemical methods. Fermentation, Vol. 5. 10.3390/fermentation5020047.
7. Upadhyaya, S., S. Tiwari, N.K. Arora and D.P. Singh, 2016. Microbial Protein: A Valuable Component for Future Food Security. In: Microbes and Environmental Management, Singh, J.S. and D.P. Singh (Eds.)., Studium Press, New Delhi, pp: 21.
8. Jones, S.W., A. Karpol, S. Friedman, B.T. Maru and B.P. Tracy, 2020. Recent advances in single cell protein use as a feed ingredient in aquaculture. Curr. Opin. Biotechnol., 61: 189-197.
9. Schultz, N., L. Chang, A. Hauck, M. Reuss and C. Syldatk, 2006. Microbial production of single-cell protein from deproteinized whey concentrates. Appl. Microbiol. Biotechnol., 69: 515-520.
10. Nasseri, A.T., S. Rasoul-Amini, M.H. Morowvat and Y. Ghasemi, 2011. Single cell protein: Production and process. Am. J. Food Technol., 6: 103-116.
11. Nangul, A. and R Bhatia, 2013. Microorganisms: A marvelous source of single cell proteins. J. Microbiol. Biotechnol. Food Sci., 3: 15-18.
12. Deshmukh, A.M., 2007. Handbook of Media, Stains and Reagents in Microbiology. Oxford Book, Pages: 240.
13. Mitchell, T.G., 2000. Overview of basic medical mycology. Otolaryngologic Clin. North Am., 33: 237-249.
14. Hendricks, K.E., M.C. Christman and P.D. Roberts, 2017. A statistical evaluation of methods of *in-vitro* growth assessment for *Phyllosticta citricarpa*: Average colony diameter vs. area. PLOS ONE, Vol. 12. 10.1371/journal.pone.0170755.
15. Francois, J.M., 2006. A simple method for quantitative determination of polysaccharides in fungal cell walls. Nat. Protoc., 1: 2995-3000.
16. Mondal, A.K., S. Sengupta, J. Bhowal and D.K. Bhattacharya, 2012. Utilization of fruit wastes in producing single cell protein. Int. J. Sci. Environ. Technol., 1: 430-438.
17. Oshoma, C.E., S.O. Eguakun-Owie and I.S. Obuekwe, 2019. Utilization of banana peel as a substrate for single cell protein and amylase production by *Aspergillus niger*. Afr. Sci. 18: 143-150.
18. Armitage, P., G. Berry and J.N.S. Matthews, 2008. Statistical Methods in Medical Research. 4th Edn., John Wiley & Sons, New Jersey, United States, Pages: 832.
19. Hernández-Chávez, M.J., R.J. González-Hernández, J.E. Trujillo-Esquivel, A. Hernández-Cervantes and H.M. Mora-Montes, 2014. The Secretory Pathway in the Filamentous Fungus *Trichoderma.* In: Biotechnology and Biology of *Trichoderma*, Gupta, V.K., M. Schmoll and M.G. Tuohy (Eds.)., Elsevier B.V., pp: 115-121.
20. Özcan, E. and E.T. Öner, 2015. Microbial Production of Extracellular Polysaccharides from Biomass Sources. In: Polysaccharides, Ramawat, K.G. and J.M. Mérillon (Eds.)., Springer, Cham, pp: 161-184.
21. Pfau, F. and J. Hummel, 2019. Microbial protein formation of different carbohydrates *in vitro*. J. Anim. Physiol. Anim. Nutr., 103: 1739-1746.
22. Wang, X., K. Xia, X. Yang and C. Tang, 2019. Growth strategy of microbes on mixed carbon sources. Nat. Commun., Vol. 10. 10.1038/s41467-019-09261-3.
23. Parrondo, J., L.A. García and M. Díaz, 2009. Nutrient balance and metabolic analysis in a *Kluyveromyces marxianus* fermentation with lactose-added whey. Braz. J. Chem. Eng., 26: 445-456.
24. Dodds, W.K. and M.R. Whiles, 2010. Nitrogen, Sulfur, Phosphorus and Other Nutrients. In: Freshwater Ecology: Concepts and Environmental Applications of Limnology A volume in Aquatic Ecology, Dodds, W.K. and M.R. Whiles (Eds.)., Elsevier Inc. pp: 345-373.
25. Sankar, N.R., V.K. Kumar, R. Shailaja, K. Saritha and N.V. Naidu 2011. Single cell protein production by *Trichoderma harzanium* using banana peel. Int. J. Microb. Res., 2: 78-81.
26. Kogan, G., M. Matulová and E. Michalková, 2002. Extracellular polysaccharides of *Penicilliumver miculatum*. Z. Naturforsch. C, 57: 452-458.
27. De Gregorio, A., G. Mandalari, N. Arena, F. Nucita, M.M. Tripodo and R.B.L. Curto, 2002. SCP and crude pectinase production by slurry-state fermentation of lemon pulps. Bioresour. Technol., 83: 89-94.
28. Anichebe, C.O., B.O. Uba, E.L. Okoyec and C.C. Onochie, 2019. Comparative study on single cell protein (SCP) production by *Trichoderma viride* from pineapple wastes and banana peels. Int. J. Res. Publ. Forthcoming. Vol. 23. 10.2139/ssrn.3448990.

29. Zaki, M. and S.D. Said, 2018. *Trichoderma reesei* single cell protein production from rice straw pulp in solid state fermentation. IOP Conf. Ser.: Mater. Sci. Eng., Vol. 345. 10.1088/1757-899x/345/1/012043.
30. Said, S.D., M. Zaki, E. Novita and T.M. Asnawi, 2019. Production of single cell protein by a local *Trichoderma reesei* in solid state fermentation: Effects of process variables. J. Phys.: Conf. Ser., Vol. 1376. 10.1088/1742-6596/1376/1/012043.
31. Mahapatra, S. and D. Banerjee, 2013. Fungal exopolysaccharide: Production, composition and applications. Microbiol. Insights, Vol. 6. 10.4137/MBI. S10957.
32. Gervasi, T., V. Pellizzeri, G. Calabrese, G.D. Bella and N. Cicero and G. Dugo, 2018. Production of single cell protein (SCP) from food and agricultural waste by using *Saccharomyces cerevisiae*. Nat. Prod. Res., 32: 648-653.