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Short Communication

Screening of agricultural wastes for Glutaminase biosynthesis via Solid-state fermentation

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Abstract

An incredible increase in the world's population has led to the generation of million tons of agro-industrial wastes, which are in turn a great source of several bioactive metabolites such as therapeutic enzymes namely glutaminases. The enzyme has got immense potential applications across various industries varying from pharmaceutical to food. With the onset of scientific innovations, the usage of these nutritionally rich agricultural wastes for the synthesis of many valuable compounds significantly minimizing the production cost and pollution load on environment. The current investigation is aimed to explore and evaluate these inexpensive agro-wastes for glutaminase production under solid-state fermentation using Aspergillus wentii NCIM 661 and Fusarium oxysporum NCIM 1008. All the agro-wastes supported good microbial growth with better enzyme productivity. But among them, the maximum enzyme yield was noticed with wheat bran (9.36U/gds) using Aspergillus wentii NCIM 661, and sesame oil cake (10.27U/gds) with Fusarium oxysporum NCIM 1008, leaving the other substrates as noteworthy alternatives for the synthesis of glutaminase enzyme. This work had established the economical use of agro-industrial wastes into valuable metabolites which has considerable promising economics and environmental meaning.

Keywords: *Aspergillus wentii*, *Fusarium oxysporum,* glutaminase, solid-state fermentation.

Introduction

India is an agricultural country with more than sixty percent of its population depending on agriculture and its allied sectors it for their principal source of income. During their processing and disposal, it has been supposed that around 700-800 million tons of agro-wastes are being generated in India annually. These wastes mainly include straw, husk, peel, stover, peel, oil cakes, etc. So, over the past few years, continuous efforts are being taken towards their effective utilization. Using these agro-wastes as an alternate source of raw material, many bioprocesses are developed to produce many vital compounds of biological importance[1].

L-glutamine amidohydrolase (E.C 3.5.1.2) most commonly known as L-glutaminase, belongs to hydrolytic enzymes group and basically catalyses the L-glutamine deamidation reaction[2,3]. In recent years, due to its vast potential uses, it has gained appreciable consideration in various fields of pharmaceutical, food and chemical industry. Main applications of glutaminase include: as a potent therapeutic agent in treating acute lymphocytic leukaemia[4,5] and HIV[6], as a bio-sensing agent[7], and also as an aroma and flavour enhancing agent[8,9].

Glutaminase activity is broadly distributed in all living systems including microorganisms especially in bacterial and fungal species. Production of glutaminase under submerged fermentation (SmF) conditions was extensively studied using several microbial genera[10-13]. To overcome the SmF drawbacks[14], solid-state fermentation (SSF) has received prominent significance since it has many advantages. The main features of SSF are better product characteristics, requirement of less energy, simple downstream processing steps, low effluent generation[15,16]. Another major feature of SSF is its ability to use cost-effective agro-wastes for metabolites production[17]. From the documented data, only a few microbial genera are engaged for the production of glutaminase[18-21].

In this work, different locally available, low-cost agricultural wastes for glutaminase production were exploited and assessed using the potential fungal strains under SSF.

Materials and methods

Chemicals: Used chemicals are of analytical grade and were purchased from Sigma Aldrich, Bangalore, India.

Substrates: Various agricultural residual wastes namely coconut oil cake, fruit waste, rice bran, sesame oil cake, sugarcane bagasse, wheat bran and vegetable waste procured from the nearby markets of New Delhi, were employed as substrates in this study. Before use, all the substrates were sun-dried and processed accordingly without any pre-treatment.

Microorganisms: In this work, *Aspergillus wentii* NCIM 661 and *Fusarium oxysporum* NCIM 1008 received from NCIM, Pune were used all through the research. Both the fungal strains were grown on Potato Dextrose agar (PDA) medium slants. These were stored at 4°C and sub-cultured monthly. Under aseptic conditions, conidial suspension of both the fungal cultures was prepared from their freshly raised slant cultures by dislodging them in 10 ml of sterile saline solution (0.85%). The obtained conidial suspension is used as inoculum for the subsequent SSF experiments.

SSF of Substrates: Every substrate (5g) was dispensed individually into 250 ml of cotton-plugged Erlenmeyer flasks and saturated with moistening media[20]. To these autoclaved flasks, about 1 ml of the concerned fungal conidial suspension was inoculated aseptically. The constituents in the flasks were blended uniformly and allowed to ferment in a static incubator at 28°C for about five days (fermentation time) respectively.

Enzyme extraction and assay: Crude enzyme was extracted as per Sabu *et al.*[18]. Glutaminase activity was obtained by measuring the amount of ammonia released by Nesslerization method[11,18]. One unit (U) of glutaminase is described as the amount of enzyme required to liberate one µmol of ammonia under specific optimal assay conditions, and communicated as the activity of L-glutaminase attained per grams of dry substrate (U/gds). All the experiments and assays were run in three sets and the mean value was noted for better results.

Results and discussion

Choosing a potential substrate in SSF: All the solid-substrates utilised in solid-state fermentation are mostly water-insoluble and play an influential role in the metabolite synthesis, as they render both nutrients and anchorage to the growing microbial cells[15,22]. So, choosing a desirable substrate for the fermentation process is one of the most influential factors in SSF, and hence involves an intensive screening of various substrates for better microbial growth and improved product formation.

Among the entire micro flora investigated, fungal species fit better for cultivation in SSF because of their biochemical, enzymological and physiological properties. The two important features of fungi that make them efficient and competent enough in the entire microbial genera are their capability to tolerate low moisture requirement (A_w) and high osmotic pressure conditions. Moreover, fungal species are very much capable in penetrating into the solid substrates for the efficient utilisation of all the available nutrients within solid substrate particles[16]. So, based on the criteria, *Aspergillus wentii* NCIM 661 and *Fusarium oxysporum* NCIM 1008 were chosen as potent fungal strains for the production of glutaminase enzyme.

From the results (Figure-1), all the solid-substrates supported the fungal growth with better enzyme productivity in terms of yield. Among the various agro-wastes evaluated, wheat bran (9.36 U/gds) with *Aspergillus wentii* NCIM 661; sesame oil cake (10. 27 U/gds) with *Fusarium oxysporum* NCIM 1008 has given the maximum enzyme yield. The pattern of the enzyme synthesis typically varied with the nature of agricultural wastes utilized. This variation might be greatly associated with the reason that each substrate has unique physical and mechanical properties[20] and nutritional composition in terms of carbon, hydrogen, nitrogen and other growth stimulators[21].

Effect of Nutritional sources: At first, the effect of nutritional sources was examined to assess the substrate nutritional capabilities on microbial growth and enzyme activity. For this, various available carbon sources (fructose, glucose, maltose, soluble starch, sucrose and xylose) and nitrogen sources (corn steep liquor, L-glutamine, malt extract, peptone, urea and yeast extract) were incorporated at 1 % (w/v) to the agro-waste based production medium. The utilized substrates supported the enzyme productivity as they acted as a tremendous source of all the essential nutrients that are necessary for the growth of fungal culture and synthesis of enzyme, which is a positive indication in minimizing the production costs of the process drastically. The usage of nutritionally rich substrates enhances good microbial cell growth and form metabolites of exceptional characteristics.

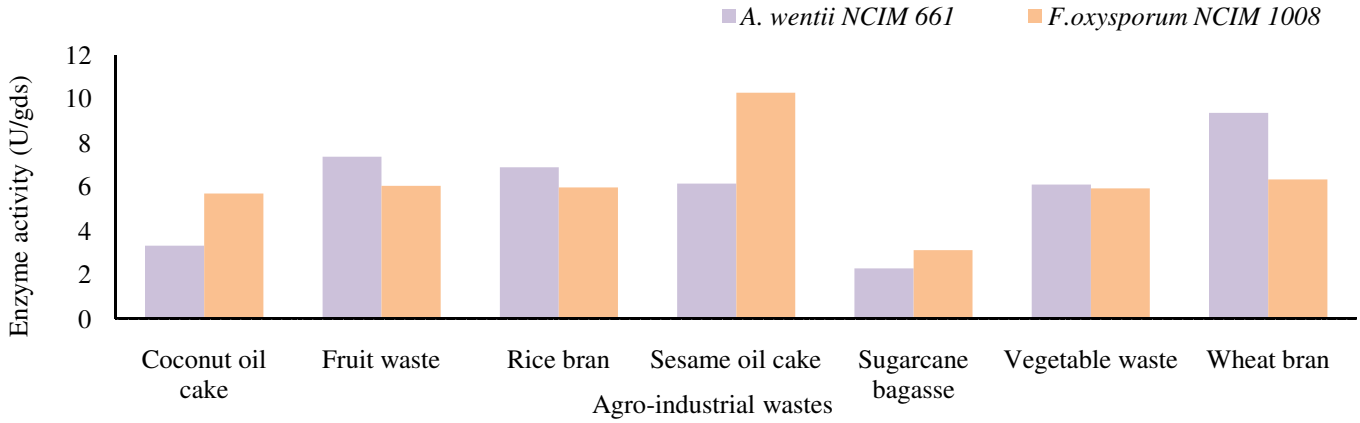

Figure-1: Screening of Agro-industrial wastes for glutaminase production using fungal strains

Conclusion

From the above findings, it is evident that utilization of inexpensive agro-wastes can be used for the production of glutaminase with the chosen potent fungal strains, *Aspergillus wentii* NCIM 661 and *Fusarium oxysporum* NCIM 1008 under solid-state fermentation. The abundant availability of agricultural wastes in India can be productively utilized for the effective solid-waste management thus, reducing the burden of production costs and environmental hazards.

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