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Bio-ethanol production from sweet potato using co-culture of saccharolytic molds (Aspergillus spp.) and Saccharomyces cerevisiae MTCC170

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ABSTRACT

In the present study, sweet potato which are in abundance and do not interfere with food security was subjected to simultaneous saccharification and fermentation process by co-culture of Aspergillus spices and Saccharomyces cerevisiae.

Aims:

The aim of this work was to study the optimization of co-culturing of Aspergillus spp. and Saccharomyces cerevisiae on sweet potato (Ipomoea batatas L.) flour (SPF) for the production of bio-ethanol using solid-state fermentation (SSF).

Materials and Results:

Aspergillus spices were tested for their amylase activity on 1% starch agar medium. Clear zone formed by on all strains used but the largest zone was formed by A. niger, A. niger MTCC-104, A. niger RKS104, A. oryzae and A. sulphureus strains. The co-culture experiment was conducted by using these five strains with S. cerevisiae MTCC170. Optimal ethanol yields were obtained in the pH range of 5.0 to 6.0. S. cerevisiae MTCC170 and selected Aspergillus spp. used in co-culture for the simultaneous saccharification and fermentation (SSF) were also analyzed for starch (dry sweet potato powder) utilization from 1% to10% (w/v) to ethanol. The medium containing 4% sweet potato starch showed maximum ethanol yield i.e. 4.02%.Similarly maximum ethanol yield was observed on the 4th day of fermentation.

Conclusion:

The results of the study clearly showed that simultaneous saccharification and fermentation of sweet potato starch to ethanol by a mixture of starch digesting fungus Aspergillus spp and a non-starch digesting but sugar fermenting S. cerevisiae is feasible. Agricultural wastes that contain fermentable sugars can no longer be discarded into the environment, but should be converted to useful products like bio-ethanol.

Indexing terms/Keywords

Saccharomyces cerevisiae MTCC 170; Solid-state fermentation; Aspergillus spp.; co culture and ethanol

Academic Discipline and Sub-Disciplines

Microbial biotechnology

Subject Classification

Biotechnology

Type (Method/Approach)

Quasi-experimental

INTRODUCTION

In developing countries, energy depends on imports and more than 90% of total energy comes from non-renewable fuel sources like coal, petrolieum etc. This will causes pressure on oil supply, emission of CO_2 to the atmosphere, inducing environmental pollution [1]. Therefore, bioethanol is considered as one of the key renewable energy resources in the future with economic and environmental benefits [2, 3, 4, and 5]. Global biofuel demand is projected to grow 133% by 2020[6]. However, the biofuel supply is estimated to deficit by more than 32 billion litres over the same period and the deficit is worse for ethanol than biodiesel. Further the demand for ethanol has been increasing due to its various uses such as, chemical feedstock and more importantly as an alternative source of liquid fuel for automobiles. Worldwide bioethanol production is dominated by Brazil and the USA. In recent years, the development and application of bioethanol from sweet potato is the main goal of Taiwan Renewable energy policy, as the advantages of sweet potato are its easy growth, adaptation to many farming conditions and prices are more stable than other agricultural major energy crops [7] and [8]. Due to ability to increase in size until harvested and presence of high starch content, sweet potato is considered as one of the most capable crop for ethanol production from biomass (Wu and Bagby, 1987) [9]. According to report of FAO Statistics Division 2011 (www.faostat.fao.org), the worldwide production of sweet potato and the total area harvested are 102,297,894 tones and 8,216,124 hactares respectively. The ethanol fermentation processes from starchy materials commonly involves two stages (i) liquefaction of starch by α-amylase and enzymatic saccharification of the low molecular weight liquefaction products such as dextrin to produce glucose; (ii) fermentation of glucose to ethanol [10,11,12]. The

development of a process for simultaneous liquefaction, saccharification and fermentation of starch would reduce the energy input and increase the efficiency of substrate utilization [13]. Many researchers have been attempted to combine the two stage fermentation process in a single-step [14, 15]). Initial studies aimed to eliminate the enzymatic liquefaction and saccharification step by using symbiotic co-culture of amylolytic and sugar-fermenting organisms 16]. For example, in the "Symba" process for single-cell protein production from potato-processing wastes, eliminated the enzymatic liquefaction and saccharification step by using a coculture of *Endomycopsis fibuligera* amylolytic yeast and *Candida utilis* (a non amylolytic sugar utilizer) [17,18]).Co-culture system of *Rhizopus sp.* and *Saccharomyces cerevisiae* and obtained 6% ethanol after 72h also studied by [19] . In this present study, starch hydrolysing fungi were used in co-culture with *S. cerevisiae* MTCC170 to develop and evaluate a simultaneous single-step system for bioethanol production from sweet potato starch.

MATERIALS AND METHODS

1- Micro-organisms

Amylase-producing fungi, *A. niger* RKS104, *A. niger*, *A. oryzae* and *Aspergillus sulphureus,* were obtained from the Department of Biotechnology, CDLU, Sirsa and were maintained on potato dextrose agar slants. *S. cerevisiae* MTCC170 and *Aspergillus niger* MTCC170 was obtained from Microbial Type Culture Collection, Chandigarh. *S. cerevisiae* MTCC170 was maintained on slants of sterile yeast malt agar medium which contained: yeast extract, 3.0 g; malt extract, 3.0 g; peptone, 5.0 g; glucose, 10.0 g and agar, 20.0 g per litre.

2- Sweet potato starch

Sweet potato starch containing 63.04% starch on dry weight basis used in this investigation was obtained from air dried and pulverized sweet potato chips and stored at room temperature. This dried sweet potato starch contained 98.6% (w/w) sugar based on total carbohydrate estimation on an enzymatic hydrolyzed sample.

3- Media

The growth medium used for preparing the fungal inocula contained: potato starch, 1.0g; peptone, 0.1g; malt extract, 0.1g; yeast extract, 0.2g; $MgCl_2.6H_2O$, 0.1g; $CaCO_3$, 0.2g; $(NH4)_2PO_4$, 0.2g and $FeSO_4.7H_2O$, 0.001g per 100 ml. The fermentation medium used for ethanol production from starch was identical to the growth medium except that the starch concentration varied from 1.0 to 10.0 g in different experiments. For testing the effect of pH on fermentation, 1N HCl or 1N NaOH was added to this medium to obtain the desired initial pH.

4- Preparation of inocula

Fungal inocula were prepared by using slant cultures to inoculate 20 ml of sterile growth medium (mentioned above) in 50 ml foam-stoppered Erlenmeyer flasks. The flasks were incubated with shaking (200 rpm) at 30°C for 5 days. *S. cerevisiae* MTCC 170 inoculum was prepared in the same way as the fungal inoculum except that YM broth (pH 5.5) was used (instead of growth medium) and incubated for 24h.

5- Fermentation procedures

Ethanol fermentation was carried out in 500 ml Erlenmeyer flasks containing 200 ml of medium. The flasks were sterilized by autoclaving at 121°C for 30min and 5.0 % (v/v) inoculum of a given amylolytic fungus or yeast was used, unless otherwise mentioned. Cultures were incubated under shaking (200 rpm) at 30 ^{0}C for 7 days.

6-Analytical procedures

Samples (10.0 ml) were collected from a given flask and centrifuged at 4°C for 20 min at 5,000 × g to remove cells and the supernatant fluid was used for determining ethanol and the concentration of reducing sugar. The theoretical ethanol yield was calculated assuming complete conversion of glucose, obtained from starch hydrolysis, to ethanol, whereby 180 g of glucose (1.0 mol) yields 92 g of ethanol (2.0mol). This value was then used to calculate the percentage of ethanol produced in the experimental flask.

7- Ethanol concentration

Ethanol concentration was determined after distillation using the standard method described by AOAC (1990) [20].

8- Statistical analysis

All experiments were carried out in a completely randomized design and in triplicates. The results were subjected to analysis of variance (one-way ANOVA) and the treatment means were compared using the least significant difference (LSD) values at a significance level of $P < 0.05$. Simple ANOVA were evaluated using SPSS 16.0 software (SPSS, O.P. Sheoran Programmer, Computer Section, CCS HAU, Hisar).

RESULTS

1- Screening of fungal isolates for amylolytic activity

Total fourteen strains of *Aspergillus* spp. (*A. niger* MTCC 104, *A. candidus*, *A. flavus* 873, *A. flavus* RKS 108, *A. terrus*, *A. ocraceous*, *A. niger* RKS 104*, A. sulphurous, A. flavus*, *A. niger*, *A. oryzae*, *A. allahabadi, A. niger* 1) were screened for the production of amylase using starch agar plate method [21, 22). Based on maximum zone of hydrolysis i.e. 26, 24, 22,

22 & 22 mm after iodine treatment, five strains i.e. *A. niger*, *A. niger* MTCC 104, *A. niger* RKS 104, *A. sulphureus, A. oryzae,* respectively were selected for further study (Table 1)

Table: 1 Primary screening of various strains of *Aspergillus* for amylolytic activity.

Sr. no.	Fungal strains	Iodine test on medium	Width of halos (mm)
1	*A. candidus*	-	14
2	*A. oryzae*	+	22
3	*A. allahabadi*	-	15
4	*A. sulphurus*	+	22
5	*A. terrus*	-	19
6	*A. flavus*	±	13
7	*A. flavus* MTCC873	+	17
8	*A. niger*	+	26
9	*A. niger* RKS104	+	22
10	*A. niger* MTCC 104	+	24
11	*A. ocraceous*	-	12
12	*A. flavus* RKS 108	±	14
13	*A. nidulans*	-	09
14	*A. fumigates*	-	11

+ Amylolytic - Non amylolytic ± Ambigious

2-Fermentation by co-culture

Co-culture based SSF between starch decomposing microbe and ethanol producing microbe is an advanced route for ethanol production from starch as it reduces cost of process and save energy. Enzymatic hydrolysis and fermentation can also be performed in a combined step so-called simultaneous SSF. Co-culture was single-step process for ethanol production from soluble starch using co-culture of amylolytic fungus (*Aspergillus* spp.) and *Saccharomyces cerevisiae* MTCC170.

3-Effect of starch

Kinetics of ethanol production at increasing concentrations of starch (1 to 10% w/v) using co-cultures of *Aspergillus* spp. and *S. cerevisiae* MTCC 170 was determined. *Aspergillus* spp. and *S. cerevisiae* inoculums were used as 5% (v/v). The results showed that ethanol yields were comparable at each of the starch concentrations tested. The effect of increasing level of ethanol production in the co-culture was proportional to the starch utilized. As the starch concentration was increased from 1% to 4% ethanol production increased from 1.38% to 3.42% (v/v) when *A. niger* and *S. cerevisiae* was used as co-culture and above 4% starch concentration, ethanol production was decreased (Fig.2). Similar results were obtained for *A. niger* RKS-104, *A. niger* MTCC-104, *A. oryzae* and *A. sulphureus* when used as co-culture with *S. cerevisiae* MTCC170. (Figure1).

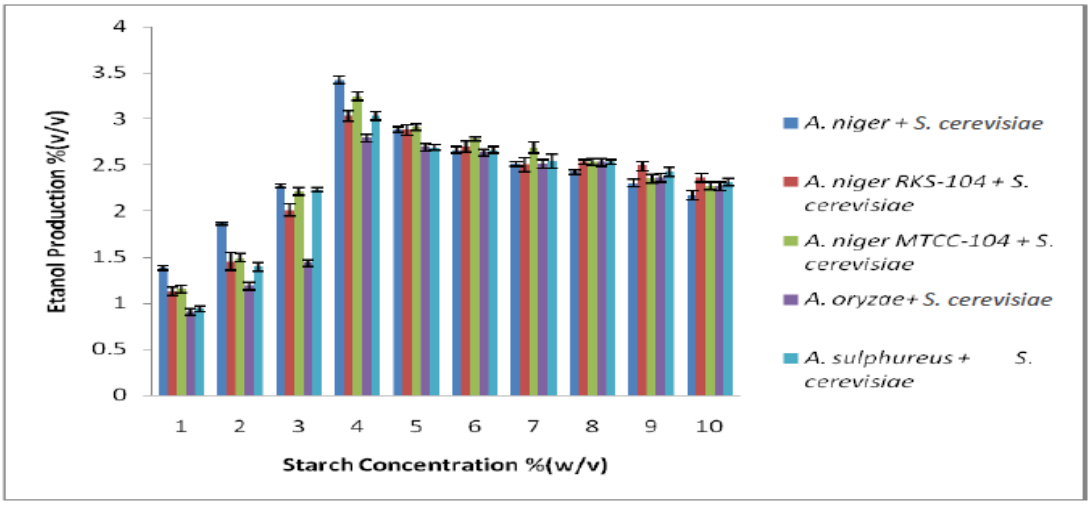

Fig 1: Effect of starch concentrations on ethanol production by co-cultures of *Aspergillus* spp. and *S. cerevisiae* MTCC170

4-Effect of pH

The effect of initial pH on direct fermentation of starch to ethanol by co-cultures of *Aspergillus* spp. and *S. cerevisiae* MTCC170 was determined by monitoring ethanol concentration. Co-cultures of *Aspergillus* spp. and *S. cerevisiae* was carried out at pH range of 4.0-9.0 and ethanol production was determined. Maximum ethanol production i.e. 4.02% (v/v) was observed at pH 6.0 by co-culture of *A. niger* and *S. cerevisiae* MTCC170. Similarly maximum ethanol production i.e. 3.51% (v/v), 3.90% (v/v), 3.51% (v/v) and 2.98% (v/v) was observed for *A. niger* RKS104, *A. niger* MTCC104, *A. oryzae* and *A. sulphurous,* respectively at pH 6.0 when co-culture with *S. cerevisiae* MTCC170 (Fig.2). So the optimum pH 6.0 was used in rest of the experiments.

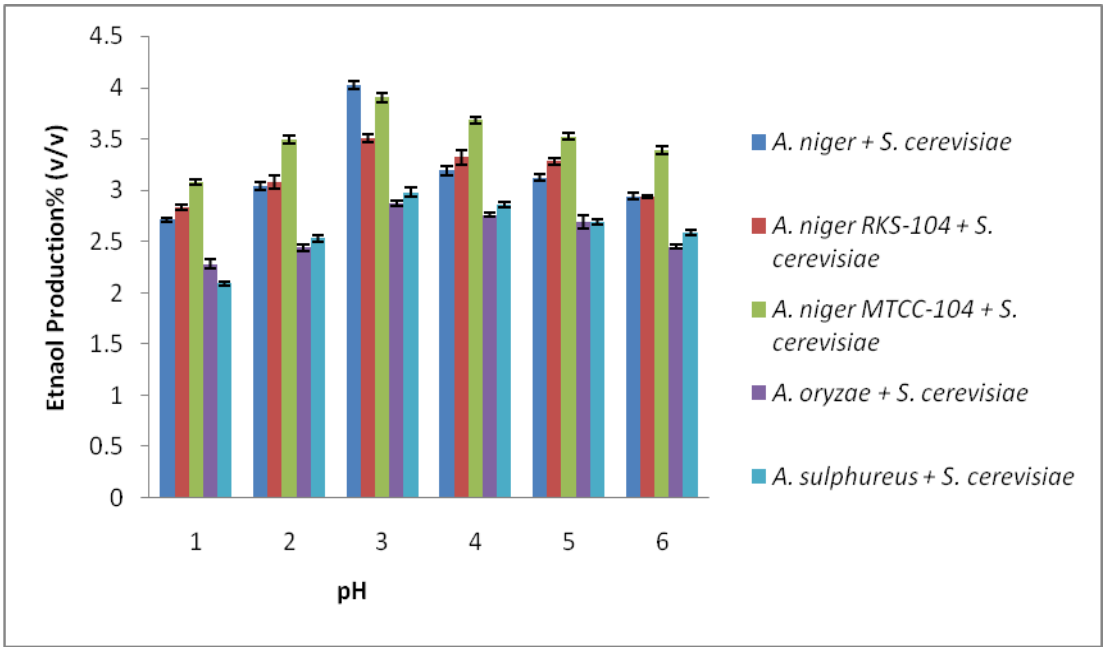

Fig 2: Effect of pH on ethanol production by co-culture of Aspergillus spp. and S. cerevisiae MTCC -170

5-Effect of incubation period

To examine the effect of incubation period ethanol production by co-culture, *Aspergillus spp.* and *S. cerevisiae* MTCC170 were incubated for different periods of time from 1-7 days and ethanol production was determined. Data from the figure 3 showed that maximum ethanol production i.e. 3. 27% (v/v) was observed on the fourth day of incubation of *A. niger* and *S. cerevisiae* MTCC -170. Similarly maximum ethanol production i.e. 3.03% (v/v), 3.24% (v/v), 2.74% (v/v) and 3.03% (v/v) was observed for *A. niger* RKS-104, *A. niger* MTCC-104, *A. oryzae and A. sulphureus* respectively on fourth day of incubation when co-culture with *S. cerevisiae* MTCC170.

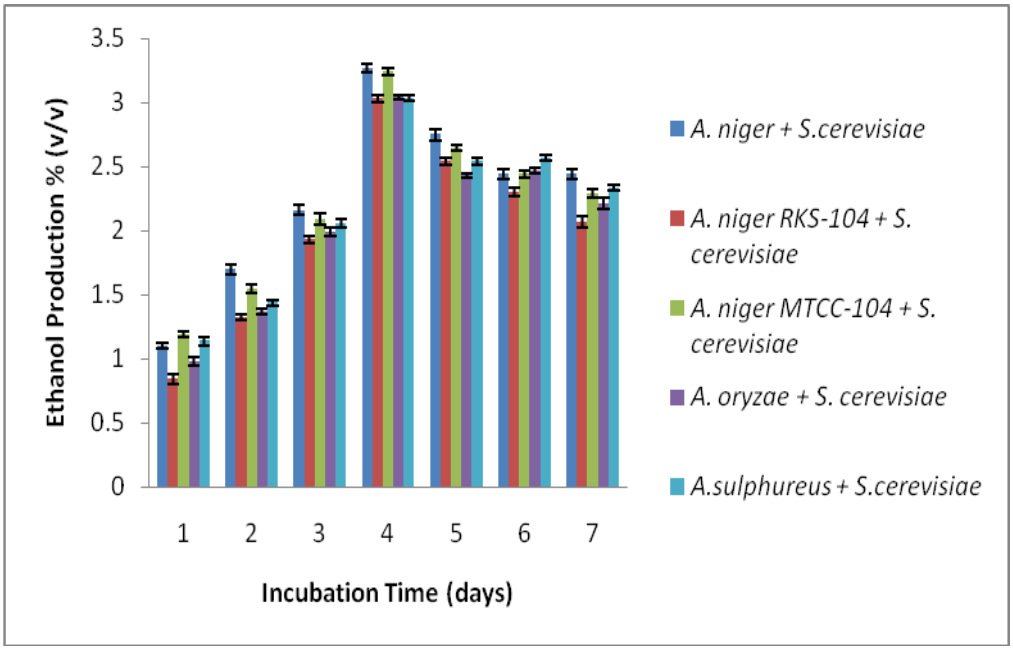

Fig 3: Effect of incubation period on ethanol production by co-culture of Aspergillus spp. and S. cerevisiae MTCC170

DISCUSSION

Kumar et al. [23, 12]) isolated, characterized and measured the performance of *Aspergillus* strains in terms of hydrolysing zone formation and found similar results. The maximum ethanol concentrations and amylolytic activity in co-cultures of *A. niger* and *S. cerevisiae* has been observed at 5% starch concentration [24]. Manikandan and Viruthagiri [25] worked on co-culture of starch digesting *A. niger* and non starch digesting and sugar fermenting *S. cerevisiae* in a batch fermentation and observed that optimum values of pH and temperature were found to be 5.5, 30°C, respectively for ethanol production. Lee et al. [26] studied the effect of the initial pH on ethanol production by co-culture of *A. oryzae* and *S. cerevisiae* and observed that the initial pH 4.0 gave the highest ethanol production of 3.17% (v/v), followed by 3.11% (v/v) at pH 3.0 and 2.66% (v/v) at pH 6.0. The highest ethanol yield and production rate were achieved at an initial pH 4.0.The effect of initial pH on direct fermentation of starch to ethanol by co-cultures of *A. niger* and *S. cerevisiae* was determined by monitoring amylolytic activity and ethanol concentration. Ethanol production was optimal in the pH range 5.0 to 6.0. [24]. Ado et al. [27] observed that ethanol yield from the synthetic medium containing glucose and complex medium containing corn cobs gave a maximum ethanol yield of 3.45% and 6.23%, respectively at 72 h of fermentation period. Similar results were obtained by Lee et al. [26] Azmi et al. [28] worked on co-culture of *Ragi tapai* and *S. cerevisiae* and observed that the maximum ethanol production took place after 72h.

CONCLUSION

The production of bio-ethanol from sweet potato is a mature technology that is not likely to see significant reduction in the production costs. Substantial cost reductions may be possible if starch based agricultural wastes such as sweet potato is used. The results of the study clearly showed that simultaneous saccharification and fermentation of sweet potato to ethanol by a mixture of starch digesting fungus and a non-starch digesting sugar fermenter S*. cerevisiae* is feasible. The results of this study suggest that agricultural wastes that contain fermentable sugars can no longer be discarded into our environment, but should be converted to useful products like bio-ethanol. The substrates at 4% concentrations supported higher yield of ethanol and ethanol production increased with fermentation time and peaked at 96 hours. The efficiency of starch conversion to ethanol was > 92% of the theoretical maximum expected. Use of such a combination of organisms allows elimination of enzymatic starch hydrolysis.

Competing of interest

The author(s) declare that they have no competing interests.

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