ABSTRACT

Due to increasing demand of energy and decreasing availability of fossil fuels, emphasis has been given to solve the liquid fuel crisis by the bioconversion of renewable sources into ethanol. Since bioconversion is achieved mainly by bacteria, fungi and actino-mycetes and they are easily available from their natural habitat, efforts have been made for the isolation of a hyper active starch hydrolyzing as well as ethanol producing amylolytic strain.

In search of a new hyper active Amylomyces for the biodegradation of starchy waste materials, one mold was isolated from the soil of the campus of IIT, Kharagpur. Because of the presence of rhizoids the soil isolated fungus was identified as Rhizopus and for the presence of not quite straight, often branched with swelling sporangiophores, the species was identified as oryzae. But due to some differences in morphological physiological and biochemical characteristics, it is considered as a new strain and designated as Rhizopus oryzae IIT KG-1.

In presence of 2% malt extract agar medium germination of its spores occurs after 3-4 h. The organism showed optimum growth at pH 5.0, temperature 40^{o}C, 120 h incubation period and at 7% substrate concentration.

It was observed that the organism could grow in presence of all types of carbon source (1%) except cellulose. Beside this the organism was able to assimilate both inorganic and organic nitrogen compounds except urea.

The organism was very much sensitive in presence of polyene antibiotics. Except fungizone, 100 µg/ml concentration was the minimum concentration for the inhibition of its growth.

Except cellulase, the organism was able to synthesize various industrially important enzymes like lipase, amylase, protease, etc. The organism was also able to produce industrially

important acids like lactic acid, citric acid but not fumaric acid. It was also able to produce ethanol from starch in single step.

Regarding amylolytic enzyme production it was observed that biosynthesis of α-amylase enzyme was maximum at pH 4.5, temperature 30°C and 72 h incubation period and among various carbon sources, arabinose, xylose, sucrose, maltose were suitable for its synthesis whereas sodium nitrate and peptone were effective nitrogen sources for the production of the same .

Regarding concentration of carbon and nitrogen sources it was observed that individually 1% maltose and 0.1% peptone concentration was the best for the synthesis of α-amylase production. But following the EVOP optimization technique it was observed that combination of 1% maltose and .01% peptone was the best concentration for the synthesis of α-amylase.

Again among various forms of inoculum, one day mycelial mat was the best inoculum for the production of α-amylase. Regarding effect of metal ions, in absence of Mendel and Reese trace solution, the organism showed better efficiency for α-amylase production. Regarding effect of vitamins, in presence of 0.2% v/v Vogel vitamin solution the organism showed better efficiency in α-amylase production. Surfactant had great influence on the enhancement of enzyme synthesis. Among Tween "80" and Triton X-100, Tween "80" was superior to Triton X-100.

Regarding effect of starchy agro residues, enzyme production was maximum in presence of wheat followed by rice, potato , tamarind seed, wheat bran, rice bran. Between shaking and stationary culture, enzyme production was maximum in latter condition.

The α-amylase produced by Rhizopus oryzae showed highest activity at pH 5.0 and stability from acidic to alkaline pH range (i.e. 4.0 - 9.0). At 70°C it was most active and also stable upto 60°C. The enzyme further showed maximum activity at 50 minutes incubation period and at 5% substrate concentration.

For the isolation of mutants from the wild strain i.e. Rhizopus oryzae IIT KG-1, 0.15% survival percentage was selected. The initial screening was based on the ratio (R) of the diameter of starch clearance zone to the diameter of agar block. The colonies with high values of R were assayed for their amylase activity in liquid medium at stationary condition. Out of 200 colonies about 30 mutants were producing higher degree of clearance on starch agar plate than wild. Among these only 10 mutants were selected and of these only 4 mutants i.e. R.O. IIT M I 13, R.O. IIT MIII 5, R.O. IIT MIII 8, R.O.IIT MIV 22 showed the highest amylase activity.

Both wild and mutant strains of Rhizopus oryzae IIT KG-1 were able to produce ethanol from starchy agroresidues. Among them R.O. IIT MIV 22 produced maximum amount of ethanol from rice, R.O. IIT M III 5 in presence of wheat and R.O. IIT KG-1 produced highest amount of ethanol in presence of potato.

As Rhizopus oryzae IIT KG-1 had the capability of converting starchy agroresidues into ethanol, this organism could therefore synthesise both amylase and alcohol dehydrogenase. On the other hand yeast can synthesise alcohol dehydrogenase but not amylase. So to convert starchy agroresidues into ethanol studies were carried out with mixed culture of both R.O. IIT KG-1 and yeast. From these studies interesting results were obtained.

Based on laboratory data scale up studies can be under taken in future for ethanol production from starchy agroresidues. It would be worth to put effort on the preparation of genomic library of Rhizopus oryzae IIT KG-1.

KEY WORDS

Aseptate, α-Amylase, α-Amylase activity, Baker's yeast, Columella, Chlamydospore, Coenocytic, Dinitro salicylic acid (DNS), Doubling time (td), Evolutionary Operation Technique (EVOP), Inoculum, Genome, Growth Kinetics, International Unit(IU), Mold, Mycelium, Mycelial mat, Mutant, Mutagenic agent, Optimization, Rhizopus oryzae IIT KG-1, (R.O. IIT KG-1) Reducing Sugar (RS) Rhizoid, Relative activity, Sporangiophore, Sporangium, Spore Suspension, Surfactant, Specific growth rate (μ), Single Step Ethanol Production, Specific amylase activity, Ultraviolet (UV) irrediation, Zygospore