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Research article

MUTATIONAL STUDIES OF *SPORIDIOBOLUS SALMONICOLOR* FOR THE PRODUCTION OF γ – DECALACTONE

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ABSTRACT: γ- Decalactone was an aroma compound present naturally in many fruits and fermented products. In this study we have collected *Sporidiobolus salmonicolor* samples and preserved. Using these cultures we aimed to develop a better yeast producing γ-Decalactone in high quantity. For this purpose we have mutated the strains under UV light for few min (3-10min). First the cultures were precultured and grown in γ-Decalactone producing media like YM agar media. Later these were subjected to UV light for mutations. The mutated colonies were preserved and studied for γ-Decalactone production.
Key words: γ-Decalactone, *Sporidiobolus salmonicolor,* Mutations, YM agar media

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INTRODUCTION

A lactone is a cyclic ester which can be seen as the condensation product of an alcohol group -OH and a carboxylic acid group -COOH in the same molecule [1,2]. It is characterized by a closed ring consisting of two or more carbon atoms and a single oxygen atom, with a ketone group =O in one of the carbons adjacent to the other oxygen. Lactones are widely distributed in foods and beverages [3]. They have been found in the aromas of more than 120 foodstuffs, which explain their importance in the aroma industry. γ Lactones are widely distributed in nature; this moiety is present in around 10% of all natural compounds. Most display a broad biological profile including strong antibiotic, antihelmetic, antifungal, antitumour, antiviral, anti-inflammatory and cytostatic properties which make them interesting lead structures for new drugs [4]. An increasing demand for natural products has resulted in the use of biotechnological processes for the production of these lactones. This has led to numerous patents being taken out, and nowadays the biotechnologically produced lactone family is mainly represented by γ-decalactone, but also to a smaller extent by γ-dodecalactone and γ–octalactone [5, 6]. Traditional biotechnologies enable the flavour characteristics of food products to be modified and improved, and the causal microbial activities are the basis of current biotechnological methods for the production of non-volatile flavours, such as lactones acidulants, amino-acids, and nucleotides [7]. By contrast, the generation of volatile flavours on an industrial scale is in its infancy and is still largely carried out using complex and very tedious empirical procedures [8-10]. However, an increasing demand for natural products has resulted in the use of biotechnological processes for the production of these lactones. This has led to numerous patents being taken out, and nowadays the biotechnologically produced lactone family is mainly represented by γ –decalactone [11, 12].

MATERIALS AND METHODS

Microorganisms

Sporidiobolus salmonicolor (MTCC 485) was obtained from the microbial type culture collection and Gene bank, Institute of microbial technology sector 39-A, Chandigarh, 160036, INDIA.

Maintenance of Microorganism

The Yeast *Sporidiobolus salmonicolor* (MTCC 485) was maintained in YM agar media slants at 25^0C. After growing three days Yeast spores was stored in refrigerator.

Media for γ – Decalactone Production

The media used for γ – decalactone production were YM agar media, Glucose media and MR media and the compositions were

YM AGAR MEDIA: Yeast extract 3.0 gm; Malt extract 3.0 gm; Peptone 5.0 gm; Dextrose 10.0 gm; Agar 20.0 gm; Distilled water 1000 ml; PH 7. Medium was sterilized at 121^0C for 20 minutes.

GLUCOSE MEDIA: Glucose 15 gm; Tryptone 0.5 gm; Yeast extract 1 gm; Malt extract 1gm; Casamino acids 2 gm; Potassium dihydrogen ortho phosphate 2gm; Calcium chloride 0.13 gm; Ferrous sulphate 0.01 gm; Magnesium sulphate 3 gm; Distilled water 1000ml; PH = 6. Medium is sterilized at 121^0c for 20 minutes.

MR media: Glucose media + methyl Recinoleate (castor oil).

PRECULTURE

Precultured samples were transferring into 250 ml screw-capped Erlenmeyer flask which contain Glucose medium. Incubated with shaking at 160 rpm and at 25^0C. Cells were pre cultured in a glucose medium for 19 hours to the logarithmic phase.

Mutagenesis and Mutant isolation

For the analysis of survival rates by UV mutagenesis, cells were grown on YM agar slants for 18 hours at 25^0C. After incubation culture was collected and suspended in sterile distilled water. After that cell concentration was determined by counting cells was spread on YM agar plates. The plates were placed under a UV lamp at a distance of 55 cm and were irradiated for various periods of time (3, 5, 7 and 10 min). Following irradiation, the plates were kept in dark for 1 hour before incubation at 25^0c for 3 days. The number of colonies on plates was then counted to determine survival rates. Mutant preculture was transferred into 250 ml screw-capped flask which contain 100 ml Glucose medium incubated with shaking at 160 rpm and at 25^0c. Cells were precultured in a Glucose medium for 49 hours to the logarithmic phase.

RESULTS AND DISCUSSION

The parent cultures of *Sporidiobolus salmonicolor* were procured from MTCC, Chandigarh and were maintained in the Department of Biotechnology, for the past 2 years and they were maintained at refrigerated condition (5- 7^0C) (Fig. 1).

Fig 1: The Yeast *Sporidiobolus salmonicolor* (MTCC 485) on YM Agar media slants at 25^0C.

The effect of incubation period, substrate variation, temperature and moisture on γ - Decalactone production was studied.

Fig 2: 250 ml screw-capped Erlenmeyer flask which contain Glucose medium.

Sporidiobolus salmonicolor was subcultured on YM agar medium using the parent culture under aseptic conditions the culture was maintained in dark room at ambient temperature. On first day no growth was observed. By 2nd day, slight blackish mycelial growth was observed and on 3rd day it turned to total black colour with slight sporulation. By the end of 7th day, deep/dark black colour with very good sporulation was observed (Fig. 3).

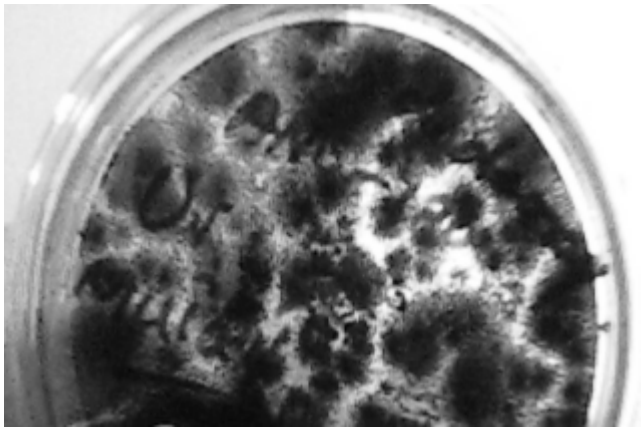

Fig 3: Superior UV mutant *Sporidiobolus salmonicolor* growing on YM agar media (5min.)

Table 1: Time period for mutation studies

Mutant Name	Time of irradiated
Sporidiobolus salmonicolor UV-1	3min
Sporidiobolus salmonicolor UV-2	5min
Sporidiobolus salmonicolor UV-3	7min
Sporidiobolus salmonicolor UV-4	10 min

CONCLUSION

An in vitro trial was conducted to develop the technology for production, isolation, purification and standardization of γ-Decalactone reference standards. γ-Decalactone was produced on different media like YM agar media, Glucose media and MR media at different moisture levels, different incubation periods and at different temperature levels by inoculating with *Sporidiobolus salmonicolor* (MTCC 485) using BOD incubator. The maximum growth of organism was obtained at 7 days of incubation with a moisture level of 35% and temperature maintained at 30^0C.

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