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Production of Oil Containing Rose like Aroma By ASPERGILLUS TERREUS

KEYWORDS: A terreus, oil, aroma constituents

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ABSTRACT *A laboratory maintained fungus Aspergillus terreus grown on glucose nitrate medium was found to produce maximum yield of oil containing rose like aroma. Standardization of parameters like varying concentration of glucose and different nitrogen sources for maximum growth and oil yield of A terreus was carried out. A 21 day old culture was found to be an ideal inoculum. Oil subjected to TLC analysis revealed the presence of four aroma constituents, such as nerol, geraniol, citronellol and anisaldehyde.*

Introduction

Filamentous fungi display an important potential for the production of natural aromas. Many have been studied and several authors have shown great diversity of aroma constituents which can be produced by different fungal sp; fruity aroma by Penicillium decumbens (Halim 1975)[1] or rose like aroma by Osmoporos odorata,, almond like aroma by Myoacia uda(Sastry KSM, et al (1980 a,b)[2]. The widely studied genus Ceratocystis produced a large diversity of fruit aroma like peach, banana, pear, rose or citrus depending upon the strain and environmental conditions, (Lanza and Palmer 1976)[3] and (Hanssen 1981)[4] and Pineapple like aroma by Pseudomonas cepacia (Bhoosreddy 1985)[5]. The production of coconut like aroma by fungi are produced by the fungus Ceratocystis (de Alberto AA et al 2002)[6] and Trichoderma viridae (Ralph P. Collins and Halim 1972)[7], (S. G. Prapulla et al 1992)[8], and (Yong and Lim 1986)[9].

The exploitation of microorganisms is still continuing as a potential source for natural aroma constituents but, oil containing aroma constituents by fungi have been less exploited. The potential of using A tererus producing oil containing rose like aroma is being investigated in the present study.

Materials and Methods

The fungi which were used in the present investigation were laboratory stock cultures. The chemicals used were of analytical grade and the aroma chemicals like nerol, geraniol, citronellol and anisaldehyde were purchased from Sigma Chemicals.

Screening of the fungus

A large number of fungi were screened to produce oil containing aroma constituents from the stock cultures maintained in the laboratory. All fungal strains were maintained on PDA slants and grown on mineral salt medium containing glucose-nitrate and incubated at 28°C for 7 days using orbital shaker. After incubation, the cultures were subjected to sensory revelation of aroma. Of the various fungal cultures tested one fungus was shown to produce rose like aroma. The fungal mat was separated from the medium, homogenized and subjected to solvent extraction using ether at 60°C to obtain oil containing rose like aroma (Guenther 1958) [10].Further experiments were carried out in triplicate.

Effect of duration on aroma production

The culture was incubated ranging from 7 days to 15 days for maximum growth and for production oil containing rose like aroma. The separated mycelia mat subjected to oil recovery after 7th day was found to contain rose like aroma. In subsequent experiments the fungus was incubated for seven days.

Effect of glucose concentration

Twenty one day old culture was used as an ideal inoculum. The culture was grown on glucose-nitrate medium. Glucose in varying concentrations was tested for maximum growth and oil yield. Growth was determined on dry weight basis gm^{-l} and oil % lipid gm/100 gm biomass (Table 1). The effect of carbon sources on aroma production has been reviewed (Christen and Maurice Raimbault 1991)[12]

Effect of various nitrogen sources

As reviewed by (Yong, FM, and Lim G, (1986) [9]nitrogen sources have also found to influence the aroma production. Various nitrogen sources were tested (equivalent to $5gm^{-l}$ of N) for growth and oil containing rose like aroma (Table 2).

Thin layer chromatographic analysis

The oil obtained was subjected to TLC analysis using standards such as nerol, geraniol, citronellol and anisaldehyde and solvent system 5% ethyl acetate in hexane for separation of aroma constituents (E Stahl).[11]

Results and Discussion

The laboratory fungal strains screened revealed that one fungus A terreus produced oil containing rose like aroma. The culture was maintained on PDA slants for further experiments. Various sugars like glucose, starch, sucrose, lactose and maltose was tested as an ideal carbon source. Of these sugars tested glucose was satisfactory carbon and resulted in rose like aroma production. Also, of the various nitrogen sources tested, sodium nitrate was found to support growth and aroma production. Thus glucose-nitrate medium supplemented with mineral salts like $NaHPO_4$, KH_2PO_4, K_2SO_4 and $MgSO_4$. $7H_2O$ was formulated. The formulated medium was used for scale up fermentation to produce large amount of biomass and recovery of oil. Further oil containing rose like aroma subjected to TLC analysis revealed the presence of four separate aroma constituents. These were eluted using 5% hexane and their Rf values determined were similar to nerol, geraniol, citronellol and anisaldehyde.

Effect of various glucose concentrations:

Of the various carbon sources tested, glucose was responsible for rose like aroma. Similar Fruity notes were observed with Ceratocystis fimbriata using glucose (Christen and Maurice Raimbault 1991).[12] Of the various glucose concentrations tested to obtain maximum growth and oil yield revealed that 15gm/l was optimum (Table 1).

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Table: 1 Effect of different glucose concentration

S. No	Glucose Concentration (gm-l)	Growth Dry wt basis (gm-l)	Oil yield (%gm/100 gm biomass)
1	5	10.57	3.46
2	7	20.37	4.70
3	9	20.98	4.20
4	11	20.17	4.20
5	13	20.17	4.20
6	15	20.28	5.26
7	20	20.00	5.15

Effect of various nitrogen sources:
From the various nitrogen sources tested, sodium nitrate was the best to support greater yield of growth and oil containing rose like aroma production (Table 2).Similar results were noted by use of nitrogen sources on aroma production (Christen'" and Maurice Raimbault 1991).[12]

Table: 2 Effect of various nitrogen sources

S. No	Nitrogen	Growth Dry wt basis ($^{gm-l}$)	Oil yield (%gm/100 gm biomass)
1	Potassium nitrate	15	4.30
2	Ammonium Chloride	6.5	13
3	Ammonium nitrate	12.6	4.5
4	Sodium nitrate	14.2	1.2
5	L-leucine	5.2	1.2
6	L-asparagine	15	2.27
7	l-valine	15	-

Effect of duration for aroma production:
The culture was incubated ranging from 7 days to 15 days for maximum growth and oil containing aroma. However, after 7th day of incubation the aroma concentration in oil was most.

Thin layer chromatographic analysis:
The oil obtained was subjected to TLC analysis using standards such as nerol, geraniol, citronellol and anisaldehyde and solvent system 5% ethyl acetate in hexane. The presence of the four aroma constituents with Rf values 0.3, 0.6, 0.66, and 0.75, respectively were similar to the above compounds for rose like aroma. The aroma production by fungi is reported [1, 9], but the fungus Aspergillus terreus is hitherto unreported for rose like aroma production.

Conclusion
The review of literature reveals about microorganisms as important and potential sources of aroma constituents. But, the production of containing aroma will find greater application in cosmetics as they are biodegradable and non-toxic and no possible side effects of their application. However, the major bottleneck is in the greater yield productivity of oil produced by fungi.

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