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BIOCHEMICAL CHANGES IN STARCH—ASPARAGINE, GLUCOSE—YEAST EXTRACT AND MALTOSE—YEAST EXTRACT MEDIA DURING THE FERMENTATION OF *AGARICUS TRISULPHURATUS, RHODOCYBE SUBGLIVA* AND *AGROCYBE PRAECOX*

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Studies were made to investigate the utilization of carbon and nitrogen sources and the subsequent changes in the *p*H and titrable acidity of the respective optimal media during the fermentation *Agaricus trisulphuratus*, *Rhodocybe subgliva* and *Agrocybe praecox*. From the analytical experiments, it might be concluded that total sugar content as well as nitrogen concentration of the media decreased gradually during the fermentation of the test - fungi. Furthermore, the pattern of fluctuation of *p*H and tritrable acidic contents of the three media analyzed correspond directly with the utilization of the different components of the respective media.

INTRODUCTION

Gray and Bushnell (1955) have studied the biosynthetic activities of a number of Basidiomycetes which included various species of mushrooms. Szuecs (1956) and Brock (1951) have reported that *Morchella esculenta* grows well in both glucose and maltose media. Pedersen and Lindberg (1970), during their studies of *Boletus variegatus* in surface and shake cultures have observed that the carbon source is exhausted and growth ceases after 9 days in the agitated cultures and after about 3 weeks in the stationary ones.

Some preliminary physiological experiments have been conducted on *Omphalia flavida* (= *Mycena citricolor*) by Wassink (1974) and he has reported that growth, glucose consumption and acidification of the medium in cultures with yeast extract or thiamine addition are strongly related. The initial carbohydrate (lactose) content has been observed to have reduced from 5 to 0.4% at the end of the submerged cultivation of morel mushroom mycelium in cheese whey (Kosaric and Nabuo, 1981). Hong *et al.* (1981) more recently have investigated the mycelial growth of *Agaricus bitorquis* and *Pleurotus ostreatus* in synthetic media and found that *p*H, total nitrogen and glucose contents of media decrease gradually during the culture period, although the yield of mycelium increases.

In the present study, it was considered desirable to investigate the utilization of the carbon and nitrogen sources and the subsequent changes in the *p*H and titrable acidity of the respective optimal media during the fermentation of the test mushrooms.

MATERIALS AND METHODS

Test Organisms

The tissue cultures of *Agaricus trisulphuratus* Berh. *Rhodocybe subgliva* (Berk, & Br.). Pegler and *Agrocybe praecox* (Pers. ex Fr.) Fayod were used in the study and were maintained on 3% malt extract-agar medium

Growth medium and conditions

For analytical experiments, optimal synthetic liquid media were used. During experimental studies on nutritional requirements of carbon and nitrogen by the mycelia of the test mushrooms, starch, glucose, maltose (40 g liter each) and asparagine yeast-extract, yeast-extract (2, 3, 1g liter each) have been found to be optimum carbon and nitrogen sources for *A. trisulphuratus*, *R. subgliva* and *A. praecox* respectively for the maximal production of protein by these species. The *p*H values of the optimal media of *A. trisulphuratus*, *R. subgliva* and *A. praecox* were adjusted to their respective optimum values of 5.5, 5.0 and 5.0 using appropriate volume of 0.2 M potassium phosphate buffer before sterilization. 50 ml of the medium was dispensed in each 250 ml Erlenmeyer flask, plugged and sterilived at 10 p.s.i. for 20 minutes. The media were inoculared separately with 1.0 ml of cell-suspension of each test mushroom and incubated on a shaking incubator (120 r.p.m.) in complete darkness at 25°, 35° and 35°C (±0 5°C) respectively.

Analytical Procedures

Prior to inoculation (after autoclaving at 10 pounds pressure for 20 minutes), the initial *p*H values of the three different optimal media were determined by means of a Beckman glass electrode *p*H meter, their total carbohydrates by the method of Dubois *et al.* (1956), total nitrogen by the method of Folin and Wu (1919) and Vogel (1961) and initial titrable acidity by titrating 5 ml aliquot with 0.IN NaOH using 1%phenolphthalein as indicator. All analytical experiments were conducted at room temperature, which varied from 28° to 30°C. During the incubation period, at intervals of 4 days, 3 flasks of each type of culture medium were removed from the incubator to make the following analyses. All results given are averages of triplicate determination.

A. *Residual carbohydrate concentration* was estimated according to the method of Dubois *et al.* (1956). The standard curve was prepared with D-galactose and the total carbohydrates present in the culture medium was calculated accordingly.

B. *Residual nitrogen concentration* was determined by the colorimetric methods of Folin and Wu (1919) and Vogel (1961).

C. *p*H of tne culture medium was measured with a Beckman glass elecrode *p*H meter at intervals of 4 days.

D. *Titrable acidity* : 5 ml aliquot of the culture medium was titrated with O 1N NaOH using 1% phenolphthalein as indicator. This measurement as well as *p*H measurement were made in order to gain some insight as to which way the fermentation was progressing.

After the above analyses were made, carbohydrate and nitrogen utilized were determined by calculating the difference between initial and final values.

RESULTS AND DISCUSSION

The analytical data in Tables 1, 2 and 3 represent the biochemical changes occurring in the starch-asparagine, glucose - yeast extract and maltose yeast extract media during the growth of *A. trisulphuratus*, *R. subgliva* and *A. praecox* respectively. These changes have been studied in order to find out the proper conditions for fermentation.

Table 1. *Data (mean)+ showing the biochemical changes in Starch - asparagine medium during the fermentation of* Agaricus trisulphuratus

Incubation period (Days)	Residual Starch* (mg/ml)	Residual Nitrogen** (Asparagine) (mg/100 ml)	*p*H	Titrable Acidity (ml of O. IN NaOH per 5 ml)	Dry wt. of mycelium (g/1)
4	29.02 ±0.10	37.29 ±0.01	5.4	0.66 ±0.03	3.57 ±0.09
8	24.27 ±0.13	31.65 ±0.03	5.7	0.53 ±0.01	6.21 ±0.12
12	19 95 ±0.09	26.44 ±0.03	6.6	0.36 ±0.01	8.40 ±0.01
16	16.06 ±0.11	22.10 ±0.01	6.8	0.33 ±0.02	10.91 ±0.13
20	14.10 ±0.12	18.82 ±0.02	7.0	0.31 ±0.03	11.18 ±0.12
22	13.29 ±0.12	17.44 ±0.03	6.5	0.38 ±0.02	11.92 ±0.11

+ Values are averages of triplicate determinations.
* Starch and ** nitrogen were added to the medium at 40 mg / ml and 42.4 mg / 100 ml respectively.

Table 2. *Data (mean)+ showing the biochemical changes in glucose-yeast extract medium during the fermentation of* Rhodocybe subgliva

Incubation period (Days)	Residual glucose* (mg/ml)	Residual nitrogen** (yeast-extract) (mg/100 ml)	*p*H	Titrable acidity (ml of O. 1N NaOH per 5 ml)	Dry wt. of mycelium (g/l)
4	27.94 ±0.13	21 81 ±0.02	5.0	0.73 ±0.02	3.98 ±0.12
8	22.60 ±0.11	18.09 ±0.03	5.4	0.68 ±0.01	4.72 ±0 01
12	19 38 ±0.12	14.36 ±0.01	5.4	0.68 ±0.01	6.31 ±0.14
16	16.25 ±0.10	10.17 ±0.02	5.5	0.66 ±0.02	9.58 ±0.12
20	11.87 ±0.09	8.50 ±0.02	5.5	0.66 ±0 02	12.84 ±0.14
22	10.13 ±0.11	8.08 ±0.01	5.0	0.73 ±0 01	12.09 ±0.11

+ Values are averages of triplicate determinations.
* Glucose and ** nitrogen were added to the medium at 40 mg/ml and 26.1 mg/100 ml respectively.

Utilization of carbohydrate

The change in starch utilization during the entire fermentation period by *A. trisulphuratus* was marked by steady fall from 40 mg/ml to 13.29 mg/ml (Table 1). The rate of starch utilization was greatly accelerated from 2nd day of fermentation and was maximum till the 16th day (the period of maximum mycelial yield) after which the rate declined The change in glucose utilization during the entire fermentation period by *R subgliva* was marked by a steady fall from 40 mg/ml to 10.13 mg/ml (Table 2). The rate of glucose utilisation was greatly accelerated from 2nd day of fermentation to 20th day. In case of *A. praecox*, however, the rate of utilization of maltose (Table 3) had been observed to be greatest upto the 16th day (period of maximum mycelial and protein yields) of fermentation. The increased rate of utilization of starch, glucose and maltose by the respective test mushrooms can be correlated with the fast rate of synthesis of mycelial materials which falls after the respective optimum incubation periods were reached.

Wassink (1974) also reported that growth and glucose consumption were strongly related in the mediнm used for the growth of *Omphalia flavida* while Hong *et al.* (1981) observed that total glucose contents of media decreased gradually during the culture period of *Agaricus bitorquis* and *Pleurotus ostreatus* in synthetic media.

Table 3. *Data (mean)+ showing the biochemical changes in maltose-yeast extract medium during the fermentation of* Agrocybe praecox.

Incubation period (Days)	Residual maltose* (mg/ml)	Residual nitrogen** (yeast-extract) (mg/100 ml)	*p*H	Titrable acidity (ml of O. 1N NaOH per 5 ml)	Dry wt. of mycelium (g/1)
4	29.37 ±0 13	8.10 ±0.02	5.0	0.73 ±0.03	3.06 ±0.11
8	25.74 ±0.11	7.22 ±0.01	5.4	0.69 ±0.03	5.18 ±0.14
12	23 13 ±0.12	6.00 ±0.03	5.5	0.66 ±0.01	7.63 ±0.12
16	20.88 ±0.12	4.05 ±0.02	5.5	0.66 ±0 01	10.04 ±0.13
20	19.30 ±0.10	3.68 ±0 02	5.0	0.74 ±0 02	9.82 ±0.09
22	18.74 ±0.11	3.29 ±0 01	4.8	0.76 ±0 01	9.05 ±0.10

+ Values are averages of triplicate determinations.
* Maltose and ** nitrogen were added to the medium at 40 mg/ml and 8.7 mg/100 ml respectively.

Utilisation of nitrogen : In all the three optimal media analysed it has been found that the total nitrogen concentration decreased slowly but steadily during the fermentation period. The rate of utilization of nitrogen by the test fungi was, however, found to be greater till the maximum mycelial growth was attained.

pH and titrable acidity :

While no attempt to determine the nature of the various metabolic products was made during the course of this work, changes in *p*H and titrable acidity of the optimal media were measured at regular intervals. The values obtained provide some index as to whether or not a considerable portion of the metabolic products are acidic. In all the media analysed, it was observed that *p*H of the medium rises during the initial 15 days of fermentation and then starts declining. It had also been noted that when *p*H of the medium rises the value for titrable acidity decreases and vice versa. The pattern of fluctuation of *p*H and titrable acidic contents of the three media correspond directly with the utilisation of the different components of the respective media.

Considering the respective optimum incubation periods of fermentation, it can be stated, that the three test mushrooms are not strong acid formers.

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