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Studies on Methanol-assimilating Yeasts

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ABSTRACT

The distribution of methanol-assimilating yeasts on three different sources (elm bark, soil and fresh-water mud) and the growth conditions of a new strain of *Candida boidinii* (SlO) were examined. From 150 samples, 91 methanol yeasts were isolated through enrichment culture; they were identified as 77 strains of *Candida boidinii* including four new strains, 5 isolates of *Torulopsis pinus,* 3 strains of *Hansenula polymorpha* and one strain of *Pichia pastoris* respectively. The comparison of these yeasts with three sources indicated that decaying bark of elm tree *(Ulmus glabra)* was more successful in isolating methanol-assimilating yeasts than the other two, and that *Candida boidinii* was most frequently distributed in all three sources. Four new strains of *Candida boidinii* were freshly isolated and their taxonomical properties were discussed. Of them, SlO strain was selected and characterized for its growth on methanol. This yeast could grow well on less than 1%(v/v) methanol. However, its growth was inhibited at 10% methanol. The cell yield was 3.1g (dry weight) per 1000ml of mineral medium containing 1%(v/v) methanol as well as 0.1% yeast extract as additive. The concentration of 0.1% yeast extract appears to be effective for the biomass production. Optimum conditions for growth on methanol was found to be: 28°C, NH_4+ as nitrogen sources, thiamine as vitamin, and pH 4.5 to 6.0. The cell composition was as follows: crude protein and nucleic acids were 54% and 7% respectively. The amino acids were also described.

INTRODUCTION

Since Ogata *et al.* reported methanol-assimilating yeasts in 1969, a number of such yeasts and new species have been isolated and described from various kinds of natural sources (Sahm and Wagner, 1972; Oki *et al.* 1972; Kato *et al.*, 1974; Van Dijken and Harder, 1974; Volfava and Pilat, 1974; Yokote *et al.*, 1974; Henninger and Windish, 1975; Tezuka et al., 1974; Henninger and Windish, 1975; Tezuka *et al.*, 1975; Asai *et al.*, 1976; Lee and Komagata, 1980[a] and 1980[b]). Most of them are in nature particularly in soil and water rich in organic matters, tree exudates and decomposing wood (Ramirez, 1953; Santa Maria, 1968; Asthana *et al.*, 1971; Kato *et al.*, 1974; Van Dijken, 1976; Mimura *et al.*, 1978; Minami *et al.*, 1978). On the other hand, Hazeu *et al.*, (1972) examined methanol-assimilation by the type strains preserved at the Culture Collection of Centraal-

bureau voor Schimmelcultures, and suggested that the number of methanol-utilizing yeasts might be increased in the bark of tree with lignin, a compound rich in methoxy group. However, a comparative study about the occurrence of methanol-assimilating yeasts among the decomposing bark, soil and mud has not been studied yet. On the other hand, there have been a considerable increase of attention to the studies of methanol-utilizing yeasts for the production of single cell protein (Levine and Cooney, 1973; Tezuka *et al.*, 1974; Yokote *et al.*, 1974; Minami *et al.*, 1978; Mimura *et al.*, 1978) because yeasts are in principle not only cheaper to harvest cells from spent medium but also easier to handle them than bacteria (Kaneda and Rouxburgh, 1956; Tye and Willetts, 1973); in addition, yeasts in general contain a lower content of nucleic acid than methanol-utilizing bacteria (Van Dijken, 1976). However, the protein yield of yeasts is lower than that found for bacteria. Therefore, it is appropriate to isolate methanol-assimilating yeasts with a high content of protein from natural habitats.

This paper deals with a comparative occurrence of methanol-assimilating yeasts among three different sources (decomposing bark of elm tree, soil and fresh-water mud), and also with the growth conditions of freshly isolated new strain of *Candida boidinii* with a comparably high content of protein.

MATERIALS AND METHODS

Samples. Sampling was done from October through November, 1980. All samples were taken in a sterilized vinyl bag from 10 sites along the River Forth in Scotland, U.K. 50 samples were collected from the bark of *Ulmus glabra*, soil and fresh-water mud respectively. The bark samples from the bottom of tree were dark and brittle because they were almost decomposing. Soil, rich in organic matters, was collected from just below the trunk of tree, from which bark samples were taken. Sampling of fresh-water mud was made at a shallow water level that was in horizontal line to the sampling sites of soil.

Isolation medium. The enrichment medium used had the following composition: $(NH_4)_2SO_4$, 0.5g; $MgSO_4.7H_2O$, 0.2g; KH_2PO_4, 3.0g; yeast extract, 1mg; biotin, 2μg; thiamine, 400μg; 0.2ml of trace salts solution. Trace salts solution had the following components (gram per liter): $CaCl.2H_2O$, 4.0; $MnCl.4H_2O$. 4.0; $FeCl_3.6H_2O$, 2.8; $CoCl.6H_2O$, 0.2; $(NH_4)_2.MoO_3$, 0.2; $ZnSo_4.7H_2O$, 0.1; $SrCl.6H_2O$, 0.2; $NiCl.6H_2O$. 0.2; boric acid, 0.2; and KI, 0.1. Yeast extract, thiamine and methanol were seperately filter-sterilized, and aseptically added to the mineral medium after sterilization at 15 Lb/in^2 and cooling to room temperature. The concentration of methanol only as carbon and energy source was 0.5% (v/v). In order to retard the growth of bacteria, chloramphenicol (200mg per liter) was directly added to the above mineral medium, and the pH of the medium was adjusted to 4.5 with NaOH.

Isolation procedure 250ml conical flasks containing 50ml of enrichment medium were inoculated with approximately 1g of samples. Incubation was on rotary shaker (140rpm per min) at 25C. Such temperature seemed to be suitable for the isolation of methanol-assimilating yeasts from natural sources because most of them may grow well at 25C. Yeasts were examined with microscope at the end of first 7 days enrichment culture. The flasks positive for the growth of yeasts in first culture were transferred to second flasks containing the fresh medium without antibiotics. 6 days after transfer, most of positive enrichment in first culture showed a good growth in second flasks. 0.1ml of the culture broth from the second flasks was plated on Saboraud agar. Pure culture of the yeasts was obtained using conventional techniques. Methanol utility was defined by a good growth of isolates after shaking culture for 3 days in 300ml conical flasks containing 50ml of enrichment medium without yeast extract. After the establishment of methanolutilities, the isolates were maintained on yeast introgen base agar containing 0.5% of methanol only as carbon and energy sources.

Identification. Identification of the isolated

Table 1. Distribution of methanol-assimilating yeasts on three different sources

Species	No. of isolates	% of total no. isolates	Sources		
			Bark	Soil	Mud
Candida boidinii	76	84	32	28	16
Torulopsis pinus	5	5	2	3	–
Hansenula polymorpha	3	2	1	1	–
Pichia pastori	1	2	1	1	–
Total No.		93	36	33	16

yeasts was done by the usual method (Lodder, L970).

Cell dry weight analysis. The dry weight was determined for two samples. 10ml suspension of culture broth was filtered through membrane filter (Oxoid, 0.4μm), and washed twice with distilled water. It was dried at 80°C to a constant weight. The weight of dried pellet was calculated to the cell dry weight per liter of broth.

Methanol analysis: Methanol concentration was analyzed by gas chromatography with a flame ionizing detector (Perkin Elmer, Model F11). A column packed with PEG 1500 was used for the estimation of methanol. Nitrogen gas was used as carrier gas. An injection temperature of 270°C, column temperature of 70°C, flow rate of 20 Lb/in^2 were operating conditions. An internal standard of ethanol was used, and sample size was 5μ liter. Injection samples were used after the filtration of culture broth through membrane filter (Oxoid, 0.2μm) under pressure.

Protein analysis. The protein content of whole cell was determined by the biuret method as described by Layne (1957). Bovine albumine (Shigma Co.) was used as a standard.

Nucleic acids analysis. Ribonucleic acid was determined by the method of Schneider (1960). Deoxy-ribonucleic acid was assayed by the diphenylamine reaction of Burton (1955).

Amino acids analysis. The amino acids content of cells was estimated in an amino acid analyzer (Locart Ltd. in England). All samples were acid hydrolyzed with6N-HC1under vacuum for 24 hr. at 105C.

Growth studies. All growth experiment were carried out in 300ml flask with 500ml of mineral-salts-methanol medium with vitamins and/or with yeast extract. Methanol, vitamins and yeast extract, all filter-sterilized, were added aseptically to autoclaved mineral medium. Duplicated flasks were inoculated either from a slant or with 0.1ml of culture suspension of log phase. Growth rate was determined by measuring broth optical density on Unicam Ultra-visible spectrophotometer since this instrument may be used to follow cell growth over a smaller range of cell densities.

RESULTS AND DISCUSSION

Ecological study. The methanol-assimilating yeasts which were isolated from elm bark, soil and fresh-water mud (each of 50 samples) are given in Table 1. As shown in Table 1, 91 isolates were identified as 76 strains of *Candida boidinii* including four new ones, 5 strains of *Torulopsis pinus*, 3 strains of *Hansenula polymorpha*, and one isolates of *Pichia pastoris* respectively. *Candida boidinii* was most frequently isolated and represented 84 per cent of a total isolates, and also dominantly distributed in all three sources examined. This is in almost accordance with the result of Lee and Komagata (1980[b]), who reported that this yeast was accounted for 20 strains of the 31 isolates, amounting approximately 65 per cent of a total isolates.

Amongst the 76 isolates of *Candida boidinii*,

Table 2. Morphological and biochemical characteristics of a new strain of *Candida boidinii*

Growth in glucose-yeast extract-peptone water: After 3 days at 25°C, the cells are short ovoid to elongate, often slightly curved, (1.5-2 x 6-13) um occur singly, in pairs or in short chains of 4-6 cells. A little thick, yellowish coloured pellicle is formed.

Psedomycelium formation on corn meal agar: An abundant pseudomycelium is formed and consists of branched chains and pseudohyphae with plastoconidia.

Ascospore formation: No sporulation on Kleyn's acetate agar, Gorodkowa agar, cornmeal agar, and yeast extract - malt extract agar.

Fermentation			
Glucose	+	Galactose	–
Sucrose	–	Maltose	–
Raffinose	–	Melibiose	–
Lactose	–	Trehalose	–
Melezitose	–	Cellobiose	–
Starch	–		

Assimilation			
Glucose	+	L-Rhamnose	–
Galactose	–	Ethanol	+
L-Sorbose	–	Glycerol	+
Maltose	–	i-Erythritol	+
Sucrose	–	Ribitol	+
Cellobiose	–	Galactitol	–
Trehalose	–	D-Mannitol	+
Lactose	+ (W)	D-Glucitol	+
Melibiose	–	a-Methyl Glucoside	–
Inulin	–	Salicin	–
Soluble starch	–	DL-Lactic acid	+
D-Xylose	–	Succinic acid	–
L-Arabinose	–	Citric acid	–
D-Arabinose	–	Inositol	–
D-Ribose	–	Arbutin	–

Assimilation of potassium nitrate: Positive
Growth in vitamin free medium: Positive, with a thiamine requirement
Growth on 50% (w/w) glucose-yeast extract agar: Negative
Growth at 37 C: Positive
Liquefaction of gelatin: Negative
Production of extracelluar starch: Negative
Cycloheximide resistance (100 ug/ml): Positive
NaCl tolerance (%): 9-10

+ = positive; – = negative; w = weak.

32 strains were isolated from elm bark, 28 from soil just below the bark of elm, and 16 from fresh-water mud 20m away from the sampling sites of bark respectively. This shows that the number of isolates decreases with the distance from bark to mud. In view of sampling all done on the horizontal line, it is predictable that this yeast was originally developed on elm bark, and dispersed into soil and mud, following the diminution in its number. As far as the author knows, this species have not been isolated from *Ulmus glabra*. Therefore, the fact that a large number of this yeast were obtained from above tree is of interest.

Torulopsis pinus was originally isolated from pine tree in Sweden by Lodder and Kreger van Rij (1952), and its methanol assimilation has been reported by Hazeu *et al.* (1972). In the present study, three strains of this yeast were found in soil, and two isolates were obtained from bark. Although *Pichia pastoris* and *Hansenula polymorpha* have been isolated from *Ulmus fulva* and reported by Miller *et al.* (1960). *Torulopsis pinus* has not been isolated from *Ulmus glabra*. This yeast is considered to be freshly isolated from above tree. On the other hand, three strains of *Hansenula polymorpha* and only one isolate of *Pichia pastroris* were obtained from bark and soil respectively. The former was frequently found in various kinds of trees (Lodder, 1970), and recently isolated from soil by Levine and Cooney (1973), who reported the methanol assimilation of this yeast. While the latter was frequently obtained from *Ulmus fulva* by Miller *et al.* (1960). The few isolates of above two yeasts obtained here may indicate more or less accidental contamination with soil and bark from other sources. According to the Yeasts (Ed, Lodder, 1970), however, the majority of these yeasts were isolated from the insect living on trees. Thus, these two species appears to be associated with deciduous tree or soil near to such trees.

When comparing three sources with number, variety and recovery, elm bark listed 36 isolates belonging 4 species, and soil has 33 comprising 4 species. Whereas 16 strains of *Candida boidinii* were isolated from mud samples. The recovering percentage of yeasts from elm bark, soil and mud was 38, 36 and 20 respectively. This result shows that the number in both isolates and species was increased in soil and particularly in decaying bark. Furthermore, the recovery was significantly higher in bark as well as in soil than in mud. Most of methanol-assimilating yeasts so far reported have been frequently isolated from soil and exudates of tree although they are found only sporadically in nature (Van Dijken and Hardern, 1974; Volfova and Pilat, 1974; Lee and Komagata, 1980b). On the other hand, Hazeu *et al.* (1972) suggested that the number of methanol-utilizing yeasts might be increased in the bark of tree, rich in a lignin which may produce methanol during decomposing processes. The more increasing trend in both number and recovery from decomposing bark than the other two samples (Table 1) indicates that decaying bark of elm tree may be a favorable habitat to harbour methanol-assimilating yeasts, proving the suggestion of Hazeu *et al.*, (1972). One interesting fact is that yeasts were obtained from 20 to 36%. This result shows somewhat higher value when compared with the result obtained by Van Dijken (1976), who found enrichment for methanol-assimilating yeasts positive in 15-25% from several hundred case. Although there is a difference in the number of samples, such discrepancy is probably due to the difference in selecting a possible niche for methanol yeasts. However, it is recognized that the differences in a composition of enrichment medium, in a culture condition and in the kinds of samples collected from worker to worker may produce different results. More studies are required before some degree of generlization is suggested.

Taxonomical study. During the identification of methanol yeasts, four new strains of *Candida boidinii* were found The biochemical and physiological characteristics of these strains are almost in accordance with those of the system of Lodder (1970). However, some differences were found between these strains and the known yeast strains (CBS 3092, CBS 5325, CBS 5777.

Table 3. Vitamin requirement for growth of a new strain of Candida boidinii (S10)

Vitamin	Concentration (μg per L)	Growth (OD at 610 nm)
None	0	0.12
Thiamine-HCl	400	0.29
Biotin	2	0.25
Riboflavin	200	0.13
Pyridoxine-HCl	400	0.23
Noctotinic acid	1000	0.16
P-Aminobenzoic acid	200	0.17
Ca-Pantothenate	400	0.10

Shaking culture in test tubes was carried out in the mineral medium without yeast extract at 25°C for 72 hr. 1.0 % (v/v) methanol was added to 0 hr.

Table 4. Growth of a new strain of *Candida boidinii* on various nitrogen compounds

Nitrogen sources	Concentration (%)	Growth (OD at 610nm)
$(NH_4)_2SO_4$	0.05	0.63
	0.10	1.08
NH_4NO_3	0.05	0.86
	0.10	1.27
NH_4Cl	0.05	0.80
	0.10	0.87
$(NH_4)\ H_2PO_4$	0.05	0.41
	0.10	0.42
Urea	0.05	0.46
	0.10	0.49

Shaking culture was conducted at 25 C for 72 hr. in 1.0% (v/v) of methanol medium without yeast extract.

CBS 2428, and Sl 70) in vitamin requirement, a strong growth in vitamin free medium and in a weak assimilation of lactose as shown in Table 2. In addition to the minor morphological differences, the following physiological character should be stressed; the growth of these strains was stimulated by thiamine rather than by biotin while any known strains showed that a biotin stimulated their growth. However, these stains were identified as a new strains of *Candida boidinii* since there are always the minor phiological and biochemical differences between known strains as have been indicated by Craver *et al.* (1976).The other strains of *Candida boidinii*, and four species; *Torulopsis pinus*, *Hansenula polymorpha* and *Pichia pastoris*, were in agreement with the description of type species (Lodder, 1970).

Growth characteristics. Of the four new strains of *Candida boidinii* the strain S10 exhibited a good growth on methanol, and an attempt was made to examine some growth conditions.

Witamin requirement. Table 3 shows that thiamine was the most efficient growth factor in methanol medium. This indicates that there

is a difference in vitamin requirement between the strain S10 and the other strains of *Candida boidinii*. The latters were found to require biotin as growth factor rather than thiamine in vitamin free medium as well as in methanol medium (Sahm and Wagner, 1972; Craver *et al.*, 1976).

Effect of nitrogen compounds. The effects of nitrogen compounds on the growth of S10 are shown in Table 4. It shows that $(NH_4)_2SO_4$ or NH_4NO_3 is suitable for the growth of S10 as nitrogen sources. This result is in agreement with the result of Sahm and Wagner (1972).

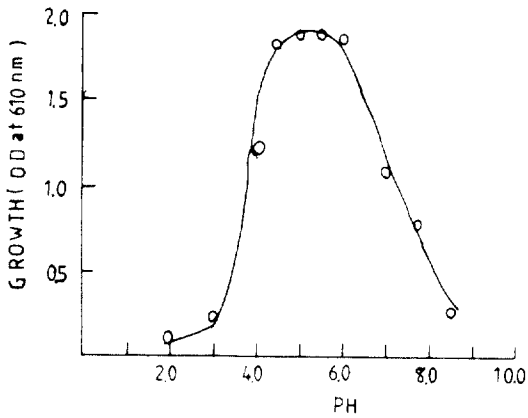

Fig. 1 Effect of pH on the growth of a new strain of *Candiad boidinii* (S10)
Shaking culture in flask was carried out for 72 hr. 1% (v/v) methanol was added at 0 hr.

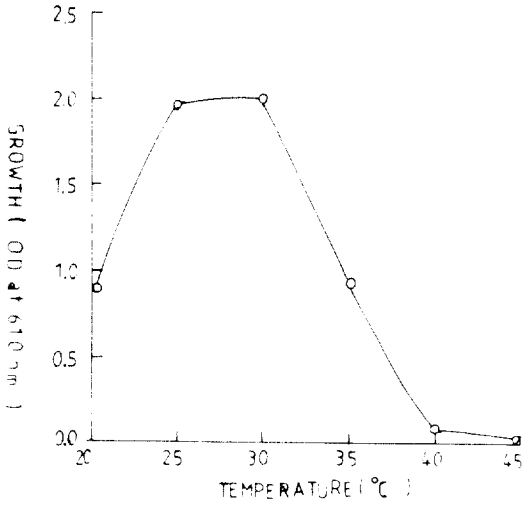

Fig. 2. Effect of temperature on the growth of a new strain of *Candida boidinii* (S10).
Shaking culture in flask was carried out for 72 hr. 1% (v/v) methanol was added at 0 hr.

Effect of pH on the growth of S10. The strain S10 was able to grow in the range of 3.0 to 8.0 but the optimum pH was between 4.5 and 6.0 (Fig. 1).

Effect of temperature on the growth of S10, This yeast was able to grow in the range of 20 to 37°C, and the optimum temperature for the growth was between 25 and 30°C (Fig. 2). It was found that this strain was also in the similar range of other strains (Sahm and Wagner, 1972 and Cardini *et al.*, 1975).

Effect of methanol concentration was determined by culturing the S10 in 300ml flask filled with 50ml of isolation medium without yeast extract but with various concentration of methanol (0.5--10%). The temperature was controlled at 25°C and the initial pH was set at 4.5 (Fig. 3). The strain S10 was able to grow in as much as 8% methanol concentration. When the concen-

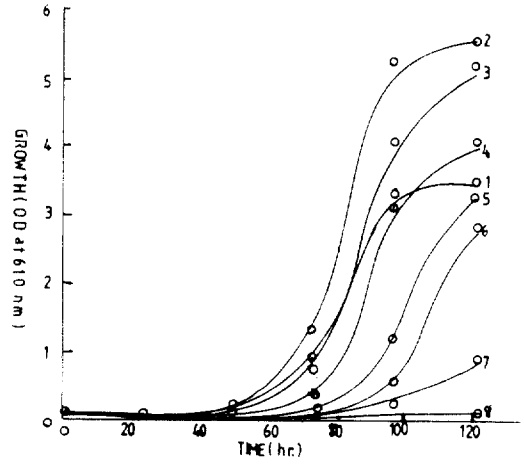

Fig. 3. Effect of methanol concentration on the growth of a new strain of *Candiad boidinii* (S10).

Curve 1: 0.5% (v/v) methanol
Curve 2: 1.0% (v/v) methanol
Curve 3: 2.0% (v/v) methanol
Curve 4: 3.0% (v/v) methanol
Curve 5: 4.0% (v/v) methanol
Curve 6: 5.0% (v/v) methanol
Curve 7: 8.0% (v/v) methanol
Curve 8: 10.0% (v/v) methanol

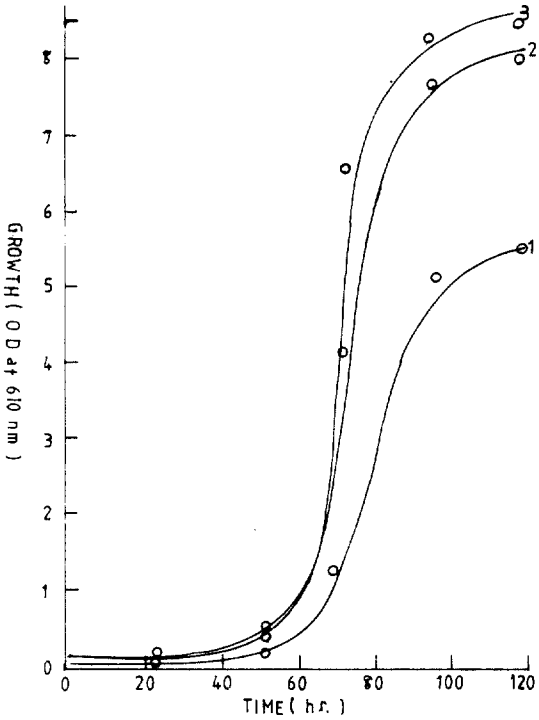

Fig. 4. Effect of yeast extract on growth of a new strain of *Candida boidinii* (S10).
Curve 1: 0.01% yeast extract concentration
Curve 2: 0.05% yeast extract concentration
Curve 3: 0.1% yeast extract concentration

tration of methanol was higher than 8%, it appears to inhibit growth. However, subsequent transfer to fresh medium containing less concentration of methanol than this showed that the cells were not killed even at 10% methanol. This result indicates that the strain S10 may grow at a higher concentration of methanol than other yeast isolates examined so far. The growth of both *Torulopsis grabrata* (Asthana et al., 1971) and *Candida boidinii* (Sahm and Wagner, 1973) were inhibited at 3% methanol, and *Schizosaccharomyces* 11 Bh (Volfova and Pilat, 1974) and *Candida methanophila* SY-97 *(Candida boidinii* Lee et Komagata, 1980b) were not grown at 8 and 7% respectively.

Effect of yeast extract on the growth of S10. When following the growth of cells on the addition of yeast extract ranging from 0 to 0.1% to isolation medium, it was found that the growth rate and cell yield increases while the lag phase decreases (Fig. 4). The specific growth rate of cells with u = 0.08 in the absence of yeast extract increases with increasing concentration of yeast extract, reaching u = 0.12 at 0.05% of yeast extract concentration and u=0.14 at 0.1% yeast extract respectively. As shown in Fig. 4, higher yeast extract concentration increased the

Table 5. Amino acid composition of a new strain of *Candida boidinii* (S10) grown on methanol

Amino acids	FAO reference	g amino acid per 100 g dry cells	g amino acid per 100 g crude protein
Lysine	5.5	3.32	6.14
Threonine	4.0	2.62	4.85
Cystine		0.83	1.54
Methionine	3.5	0.68	1.26
Leucine	7.0	3.30	6.11
Valine	5.0	2.67	4.94
Isoleucine	4.0	2.61	4.83
Aspartic acid		5.56	10.29
Serine		2.38	4.37
Glutamic acid		7.02	12.99
Proline		1.71	3.26
Alanine		2.72	5.03
Tyrosine		2.30	4.26
Phyenlalanine	6.0	2.08	3.85
Glycine		2.27	4.20
Histidine		1.17	2.16
Arginine		2.40	4.44

phase of logarithmic and linear growth while the lag phase was reduced. 1-2 hour of lag was more shorter in the addition of 0.05%-0.1% yeast extract than in the absence of yeast extract in medium. The cell yield was 0.23g dry weight per 1% methanol at 0.05% yeast extract, 0.27g dry weight per 1% methanol at 0.05% yeast extrast and 0.31g per 1% methanol at 0.1% yeast extract respectively. The production of cell biomass appears to be more efficient in the medium with 0.1% yeast extract as additive than in the medium without it since preliminary experiment showed that methanol utilities were somewhat reduced at over 0.5% yeast extract concentration. Although there is a possibility that a commercial yeast extract may contain a trace amounts of other elements capable of utilizing as carbon and energy sources as has been indicated by Van Dijken (1976), however, it might be suggested that other growth factors except vitamins might be included in yeast extract. Further study is required in the future.

Composition of cell. S10 has a protein content of 54%, and nucleic acid was 7%, divided into 0.5% DNA and 6.5% RNA. A crude protein content of methanol-grown cells have been reported from 35% for *Candida boidinii* (Sahm and Wagner, 1972) to 61% for L1 70 strain (Cardini *et al.*, 1975). In comparison to above two strains, S10 does not have high protein content as that of Cardini *et al.* (1975) at 54%, while being higher than the 45.7% obtained by Sahm and Wagner (1972). The amino acid compositions of cells of S10 grown on methanol are given in Table 5. A crude protein content and the total cellular amino acid except tryptophan were 54% and 45.7% respectively. All essential amino acids in S10 strain were considered to have more or less the same profile obtained by other reporters (Ogata *et al.*, 1970; Sahm and Wagner, 1972; Levine and Cooney, 1973; Mimura *et al.*, 1978; and Cardini *et al.*, 1975) except tryptophan which was not obtained. Methionine, cystine and leucine are a little below the FAO reference level. However, they are not so low as to be unattractive as protein sources. Although animal feeding experiment and pepsin digestibility, which are the important criteria for the evaluation of animal protein feed, remains to be examined, this yeast could be used as another single cell protein for domestic animals.

摘 要

相異한 세가지 分離源(느릅나무 樹皮, 土壤 淡水 진흙)上의 메탄올 資化 酵母菌의 分布와 새로운 菌株인 *Candida boidinii*(S10)의 生長條件을 調査하였다. 150個 sample에서 91個 菌株의 메탄올 資化 酵母菌을 集積培養法으로 分離 하였는데 이들을 同定한 結果 새로운 4 菌株를 包含한 77菌株의 *Candida boidinii*와 5 菌株의 *Toulopsis pinus*, 3 菌株의 *Hansenula polymorpha* 그리고 1 菌株의 *Pichia pastoris*이었다. 이들 酵母를 分離源別로 比較해본 結果 느릅나무의 腐敗中의 樹皮가 다른 두 分離源보다 메탄올 資化 酵母菌을 分離하는데 成功的이었으며 *Candida boidinii*가 세가지 分離源 全部에서 가장 頻頻히 分布하고 있음을 보여 주었다.

4 個의 새로운 菌株인 *Candida boidinii*가 새로이 分離되어 分類学的 特性을 論考 하였으며 이들 새 菌株中 S10 菌株를 選定하여 이의 메탄올上에서의 成長 特性을 調査하였다. 이 菌株는 1% (v/v) 메탄올 濃度 以下에서 成長이 좋았으나 10% 메탄올 濃度에서 그 成長이 抑制 되었다. 細胞 收率은 1% (v/v) 메탄올과 添加済로서 0.1% yeast extract를 含有한 1000ml 無機物 培養液当 3.1g(乾燥量)이었으며 0.1% yeast extract가 生体生産에 效果的인것 같았다. 메탄올上에서 生長 最適条件은 温度가 28℃ 窒素源은 NH_4, 바타민은 thiamine, 그리고 pH는 4.5~6.0임을 알았다. 細胞 成分은 蛋白質이 54% 核酸이 7%이었으며 아미노酸도 記述하였다.

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