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PRODUCTION OF YEAST PROTEIN BY *CANDIDA UTILIS* FROM PINEAPPLE JUICE
I. SHAKE FLASK STUDY

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Summary

This study was undertaken to determine the possibility of cultivating ***Candida utilis*** *on pineapple juice as substrate. Investigation of the desirable composition of medium for growth of the yeast was first studied with the emphasis on yeast yield and its protein content. The growth of* ***C. utilis*** *on pineapple juice supplemented with various sources of nitrogen such as peptone, urea, and ammonium sulfate, and other nutrients, i.e., phosphorus and growth factors, was examined in shake flask study. The concentration of sugar in pineapple juice was diluted to 2% (w/v). The pH during cultivation was kept at 4.0. Yeast growth was measured by determining yeast dry weight or absorbance of yeast culture at 500 nm. It was found that pineapple juice supported good grawth of* ***C. utilis*** *with the additions of* $(NH_4)_2SO_4$ *and* KH_2PO_4 *at 0.5% (w/v) each as the extraneous sources of nitrogen and phosphorus. The yeast yield and its protein content after 64 hours of cultivation were 42.1 and 55.2%, respectively.*

Introduction

Protein deficiency exists in about 60% of the populated areas of the world, particularly the tropical countries. And it will become more and more difficult to supply the necessary protein by conventional agriculture. Consequently, foods containing protein from unconventional sources will become increasingly important. Much attention has been given to the production of single-cell protein[1-5]. Among microorganisms considered as possible food sources, yeast has attracted perhaps the greatest interest. It represents, in fact, the only sort of microbial protein that has in the past been used as a food to a great extent[6-8]. Yeasts *(Candida, Saccharomyces)* have had a well established place for many decades as animal food. The technology for yeast production is well known, as are its composition and nutritive value. So, yeast may be one of the few types of microbial cell substances that could, in the near future, be used as food to an extent large enough to be of consequence in the world food situation.

Candida utilis has been found most suitable for the production of a food yeast from most liquors, i.e., molasses[9-13], wood hydrolyzates[14-16], and potato starch wastes[17-19]. Low cost raw materials suitable for yeast cultivation have so far been restricted principally to sulfite waste liquors, wood hydrolyzates and molasses. In Thailand, one industry which contributes significantly to water pollution is pineapple processing. Approximately 1000 cubic meters of waste water are being produced daily from each canned pineapple factory[20]. The waste water disposed during the canning processes can be very beneficial to utilize for yeast protein production and simultaneous decrease its pollution load. The present work was undertaken to determine the growth of *C. utilis* for protein production on pineapple juice.

Material and Methods

A strain of *Candida utilis* was obtained from Department of Science, Ministry of Industries. The stock cultures were maintained on potato dextrose agar slants[21], and transferred regularly to maintain viability. The fresh pineapple juice was filtered through course filter paper and diluted with water to the desired sugar concentration. The clear solution was sterilized by autoclaving at 121°C for 15 minutes. Individual or combinations of ingredients, as listed in Table I, were supplemented in pineapple juice. These ingredients were separately prepared by dissolved in a small amount of distilled water, autoclaved at the same condition, and added to the pineapple juice substrate solution in order to obtain the desired composition. The initial pH of the medium was between 4-5.

The culture slants were removed from the refrigerator and kept at room temperature (25°C) for 48 hours. They were then transferred to new potato dextrose agar slants twice at 24 hours interval and kept at the same temperature. The inoculum was prepared by transferring the yeast cells from agar slants into 500 ml-Erlenmeyer flasks containing 50 ml medium. The flasks were stoppered with sterile cotton and shaken on a reciprocal shaker at the vibrating speed of 240 rpm. After 12 hours of shaking, additional medium (50 ml) was added to each flask and shaking continued for another 12 hours. The absorbance at 500 nm (Bausch and Lomb spectrophotometer, VWR Scientific Inc. N.Y.) was followed to determine the concentration of yeast cells in the inoculum[22]. Concentrated cell suspensions were diluted with distilled water to an absorbance between 0.2 and 0.6 prior to measurement. The prepared inoculum was used to inoculate 200 ml of the medium in 500 ml-Erlenmeyer flasks stoppered with sterile cotton. Twenty percent of inoculum, based on the final volume of the medium, was inoculated into the medium to obtain a final absorbance of about 1. Duplicates were made on each run for each medium studied. The flasks were shaken on a reciprocal shaker at the vibrating speed of 240 rpm. and at 25°C. The pH during cultivation was maintained at 4.0 by the addition of 1 N sodium hydroxide over 4 hours interval. The yeast growth was determined both by measuring absorbance of the cultures as described above, and by determining dry weight of yeast cells at every 4 hours cultivation. A quantity of centrifuged yeast cells was washed with distilled water, recentrifuged, dried in oven

TABLE I: FORMULATION OF NINE MEDIA IN THE SHAKE-FLASK EXPERIMENTS

Ingredients	Media								
	1	2	3	4	5	6	7	8	9
Pineapple juice, 2% invert sugar	+	+	+	+	+	+	+	+	+
$(NH_4)_2SO_4$, 0.5% w/v	+		+		+		+	+	
HK_2PO_4, 0.5%	+	+	+	+	+	+	+		
Peptone, 0.5%		+				+	+		
Urea, 0.03%				+	+		+		
Yeast extract, 0.1%			+		+	+	+		

at 100°C, and kept for determination of protein content. The supernatant obtained from the determination of dry weight was collected for analysing sugar consumption in the medium. Fresh medium as added to compensate for the sample take and loss due to evaporation.

The concentration of sugar in the pineapple juice was analysed by the method of Lane-Ennon[23], and expressed as percent invert sugar. The sugar content in the media during cultivation was measured as percent invert sugar by the method of Stiles *et al.*[24]. The Kjeldahl nitrogen was determined by the AOAC method[25] and yeast protein was calculated using a factor of 6.25[1].

Results and Discussion

In Fig. 1, the growth curves of *C. utilis* in media 1, 3, 8 and 9 are compared. It appeared that *C. utilis* growth in medium 1 was better than the other three media while medium 9 gave the poorest result. This indicated that the pineapple juice contained insufficient amounts of nitrogen and phosphorus for yeast to grow. The results agree with the work of Shannon and Stevenson[26], and Burrow[11] who reported that the addition of nitrogen to carbohydrate substrate increased the growth of yeast. The needed nitrogen is utilized principally as building materials for synthesis of protein[27-29]. Ammonium sulfate does not provide only the source of nitrogen but the yeast can take the sulfur from the sulfate too. Most yeasts take up the sulfur they needed from inorganic sulfate[30]. Hence, the supplement of ammonium sulfate to pineapple juice provides both the nitrogen and sulfur sources. Ammonia and ammonium salts, particularly ammonium sulfate and diammonium phosphate, have been found to be the most suitable sources of nitrogen on account of their availability, low cost, and rapid assimilation[29, 31]. Phosphates have been found to serve several purposes for yeast growth. They supply phosphorus necessary for synthesis of nucleoprotein which the yeasts elaborate during the growth. They are also assisted in buffering the wort during propagation[27-30]. This is very important to produce yeast of

TABLE II: COMPARISON OF YIELD AND PROTEIN CONTENT OF *CANDIDA UTILIS* GROWN IN VARIOUS MEDIA WITH SHAKE-FLASK EXPERIMENTS

	Media			
	1	3	4	7
Yeast dry weight, g/l	8.23	8.23	7.76	6.80
Protein content, %	55.20	54.10	53.30	53.32
Yield, %	42.10	41.70	39.10	38.20

good quality and to obtain high yield of yeast from raw materials used. Phosphorus and potassium are the principal mineral requirements of the yeast organism[18, 28, 32]. The growth of *C. utilis* in media 1 and 3 are not much different (see Fig. 1). However, the yield of yeast obtained from medium 1 is slightly higher than that of medium 3 (see Table II). The difference of the yields may come from the variation in sugar consumption of both media (see Fig. 4). Hence, it appeared that the production of yeast cell mass on the pineapple juice supplemented with ammonium sulfate and potassium dihydrogenphosphate at the cultivation condition employed did not require yeast extract for good growth. The result obtained in this study agrees well with that of Synder[33], and Bunker[34] who reported that in cultivating *C. utilis* no additional growth-promoting factor was necessary. Vitamin or yeast extract did not have any positive effect on the growth of *C. utilis*[35]. These results may be explained in two ways. First, sufficient growth factors may already be present in the medium because the substrate is pineapple juice which is a complex medium containing many known and unknown nutritional factors[36]. Second, the presence of yeast extract may not be necessary because some microorganisms are capable of synthesizing growth factors for their own metabolic needs, with the result that they are independent of the exogenous supply[35]. *Candida utilis* produce, in general, a sufficient amount of growth factors in synthetic medium[37], and hydrocarbon medium[38]. It is able to synthesize vitamin B complex, nicotinic acid, and glutathione from sugar and mineral salts in the nutritive medium[28, 39].

The effect of supplementation of other nitrogen sources for growth of yeast was further studied. The addition of ammonium sulfate, peptone and urea in media 1, 2, 4 and 6 showed different effects (Fig. 2). The growth of *C. utilis* in medium 1 is better than others. Hence, ammonium sulfate was the preferred source of nitrogen for *C. utilis* when grown on pineapple juice. The ability of *C. utilis* to utilize ammonium nitrogen in addition to organic nitrogen was recognized[31, 33]. The assimilation of peptone and urea is more complicated than ammonium sulfate[40]. Another disadvantage of peptone is that it might not provide enough sulfur for good growth of the yeast. The sulfur content in pineapple juice may not be enough to cover the requirement by the yeast. Furthermore, the disadvantage of urea was found to be due to the limit of concentration used in the medium[41]. An exceedingly high

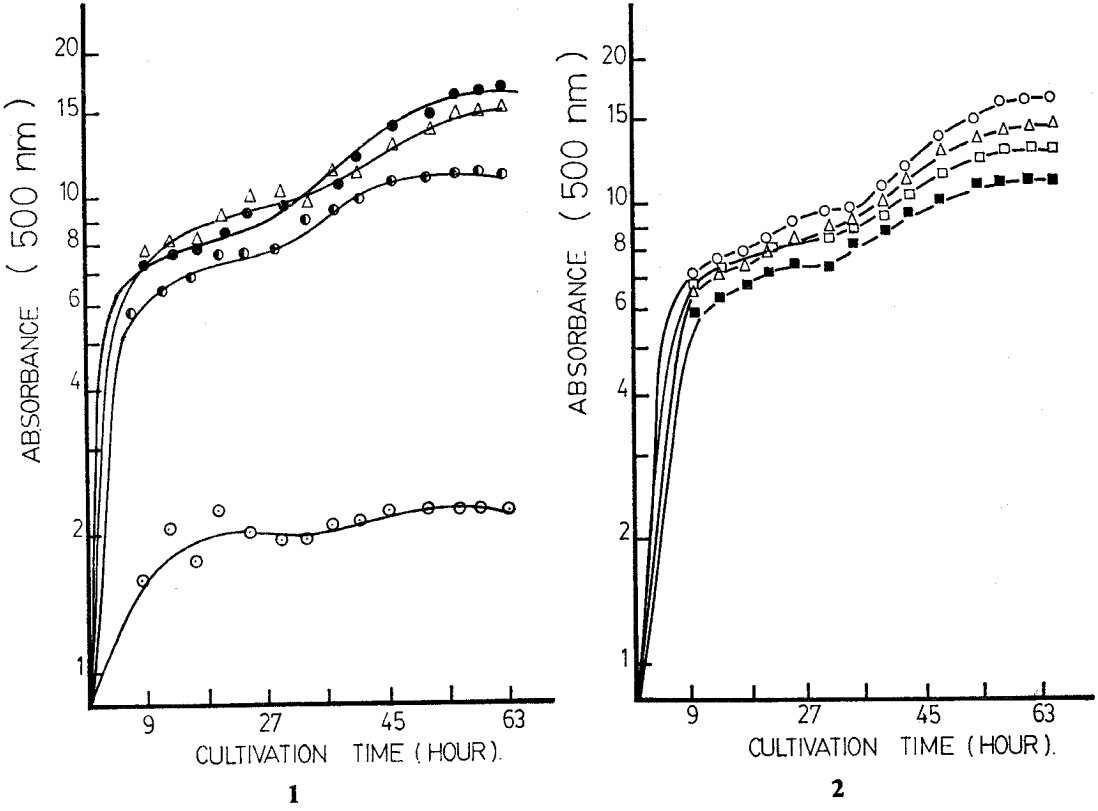

Fig. 1. The growth curves of *C. utilis* in media 1, 3, 8 and 9 for shake-flask experiments. Cultivation condition: pH = 4.0, temperature = 30°C, shaking speed = 240 rpm, cultivation time = 64 hours. The medium composition—see Table I. (● – medium 1, △ – medium 3, ◐ – medium 8, ○ – medium 9)

Fig. 2. The growth curves of *C. utilis* in media 1, 2, 4 and 6 for shake-flask experiments. The cultivation condition and medium composition—see Fig. 1 and Table I respectively. (○ – medium 1, ■ – medium 2, △ – medium 4, □ – medium 6)

concentration of urea was found to retard the growth of yeast[31, 41]. Yeast extract was added to provide the necessary growth factors for the assimilation of some nitrogen sources (peptone and urea). It appeared that the growth of yeast in medium 6 is slightly better than that from medium 2 (see Fig. 2). It is not certain whether this is due to the presence of yeast extract, since this has no beneficial effect when ammonium sulfate and urea are used as nitrogen sources (Fig. 3). The yeast grown in medium 4 is better than in medium 5 especially near the end of cultivation (after 42 hours). Additional ammonium sulfate was intended to solve the problem of urea. Yeast extract was added to provide biotin which was required for assimilation of urea[30]. The poor growth in medium 5 could be explained as follows: (a) the concentration of ammonium sulfate and urea were not in optimum proportion, (b) two nitrogen sources showed a competitive effect, and (c) urea might exert a toxicity effect. The result does not agree with the report of Nickerson and Rose[27] that an increase in growth accompanies an increase of nitrogen sources. In the present work, a mixture of three nutrient nitrogens, i.e., ammonium sulfate, peptone and urea together with potassium dihydrogenphosphate and yeast extract were used. These nutrients were shown to fulfil the requirement of the growth of yeast[30, 31, 42]. The growth of yeast in medium 1 was slightly better than medium 7 over the entire period of study (Fig. 3). The yeast yield obtained from medium 1 and its protein content was also higher than that from medium 7 (see Table II). These might be due to antagonistic effect in medium 7 that sometimes results from growing yeast in the presence of a mixture of compounds eventhough the individual component of the mixture was beneficial to the organism[29]. The consumption of sugar during the cultivation of yeast in media 1, 3, 4 and 7 is shown in Fig. 4. The percentage of sugar consumption was relatively high, exceeding 50% after 24 hours of cultivation, and increased rapidly until the end of 57 hours. After that the rate of sugar consumption leveled off as the cultivation proceeded. The amount of sugar consumed was about 95% for all media studied except medium 7. Comparison of yield and protein content of *C. utilis* grown in media 1, 3, 4 and 7 are also tabulated (Table II). Yeast grown in medium 1 gave the highest yield as well as the protein content. The protein content of the yeast grown on medium 1 was found to be 55.2 %. Hence, medium 1, which has a simple composition suitable for commercial practice, was selected for further study of yeast protein production among nine media chosen in this first series of experiments.

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บทคัดย่อ

ได้ศึกษาวิธีการที่เหมาะสมในการเพาะ *Candida utilis* โดยใช้น้ำสับปะรดเป็นน้ำเลี้ยง โดยได้หาส่วนประกอบที่จะให้ได้ผลผลิตยีสต์มากที่สุด และมีโปรตีนสูงที่สุด ได้ลองใช้ส่วนประกอบที่เป็นตัวให้ไนโตรเจน ได้แก่ เปปโตน ยูเรีย และอัมโมเนียมซัลเฟต และส่วนประกอบอื่น ๆ เช่น ฟอสฟอรัส และสารที่จำเป็นในการเติบโต การทดลองนี้ใช้ระบบขวดเขย่า (shake flask) เมื่อปรับความเข้มข้นของน้ำตาลในน้ำสับปะรดให้เป็น 2% pH เป็น 4.0 และวัดการเจริญเติบโตจากน้ำหนักแห้ง หรือ แอบซอร์แบนซ์ ที่ 500 nm ปรากฏว่ามีการเจริญเติบโตดีที่สุด เมื่อเติม $(NH_4)_2SO_4$ และ KH_2PO_4 อย่างละ 0.5% ให้เป็นแหล่งไนโตรเจน และ ฟอสฟอรัสตามลำดับ ผลผลิตและปริมาณโปรตีน หลังจากเพาะ 64 ชั่วโมง เท่ากับ 42.1% และ 55.2% ตามลำดับ