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MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 5.238
PR #2

Attachment

5.062

PROGRESS REPORT No.2

Hybridization of Halotolerant Yeast for the Production of Alcohol Fuels

A RESEARCH PROJECT

USAID/PSTC PROGRAM

Grant No. 936-5542-G-00-5080-00

SUBMITTED BY

Asst. Prof. SAVITREE LIMTONG

PROJECT LEADER

Kasetsart University

Bangkok Thailand

Project Profile

Country	:	Thailand
Grant No.	:	936-5442-G-00-5080-00
Program	:	Program on Science and Technology Cooperation
Project Title	:	Hybridization of Halotolerant Yeast for the Production of Alcohol Fuels
Project Leder	:	Asst.Prof. Savitree Limtong
Organization	:	Kasetsart University
Co-investigators	:	Mr.Wichien Yongmanitchai Mr.Pramote Tammarate Miss Manee Tantirunjkit
Project Advisor	:	Dr.Jaroon Kumnuanta
Project Consultants	:	Prof.Dr.G.R. Fink Prof.Dr.J. Hicks
Authorized Officer	:	Pro.Dr.Sutharm Areekul , Rector
Total Project Budget	:	150,000 US $ Dollars
Project Duration	:	3 years, August, 1985 to November, 1988
Reporting period	:	March 1, 1986 to August 31, 1986
Budget Allocation for This Period	:	657,653.98 Baht

Background/Introduction

In Thailand and other LDCs alcohol distilleries are small and scattered all over the country. It is being considered as one of the few local agro-industries which has economic impact on the community in terms of employment and utilization of local raw materials, since 75-80% of the production cost comes from raw material. From our own investigation and personal communication with local alcohol distilleries in northeastern and southern areas where fresh water is scarce and brackish water is used in the mashing process, fermentation proceeds slowly and efficiency is reduced by about half. This is due to many complications such as the intolerance of the yeast strain employed to high salt concentrations which sodium chloride is included, and the high salt concentration of the brackstrap molasses itself, the combined effects of which are detrimental to fermentation activities. The inhibiting effect on yeast growth enables other microorganisms present in the mash to over grow and retard fermentation by yeast, since the main mash is unpasteurized.

To solve such problem of alcohol distilleries improvement of yeast strain employ in mashing process to be halotolerant yeast is one promising method. Protoplast fusion technique is a novel technique in constructing hybrids of microbial strains currently used in industry, especially for yeasts. Athough the concept and techniques are well known,

hybridization of yeasts from the two distinct characteristics of halotolerance and high ethanol production has not yet been reported.

Protoplast fusion has proved to be a highly efficient procedure for accomplishing genetic recombination in microorganisms, including yeast. Successful fusion has been reported in many industrial strains of yeast, for example, by Spencer et al. (1980) on Saccharomyces diastaticus, which improved starch hydrolysis ability. Wilson et al. (1982) obtained hybrids from Schwanniomyces alluvious and Saccharomyces spp. which ferment starch directly to ethanol. Morishita and Yamaguchi (1983) fused Torulopsis halonitratophila, a salt-tolerant yeast which required vitamins for growth, with Prototheca zopyfii, a salt sensitive fungus and obtained a hybrid which grew in 1.5 M NaCl and did not require vitamins for growth. Limtong et al. (1983) obtained high ethanol producing and flocculated hybrids from a flocculated strain of S. cerevisiae isolated in Thailand and an industrial ethanol producing strain from Japan.

Halotolerant yeast, a kind of osmophilic yeast, is usually present in various kinds of fermented food where high salt concentrations are used in the process, such as soysauce, miso etc. Predominant strains usually found in these products are S. rouxii which can tolerate 20-22 % salt concentration (Lee and Lee, 1978). However, the efficiency of alcohol fermentation by these strains is very low; S. rouxii var. halomembranis, for example, produced only 1.9% ethanol in the presence of 18% NaCl and 3.2% without

salt (Onishi, 1963). Yamagata and Fujita (1970) isolated and identified several strains of halotolerant yeasts varied widely between 0-22% NaCl. Wickerham and Burton (1960) reported heterothallism in S. rouxii, and various strains of this species are either diploid or haploid with opposite mating types. Heterothallism may be useful for the purpose of mating desirable strains of opposite mating types by conventional method. Mori and Onishi(1967) were successful in breeding two strains of S. rouxii and obtained a diploid hybrid of high salt tolerance. Dhevahuti (1978) investigated S. rouxii in fermenting soy mash and found that this species was involved in soysauce fermentation in Thailand as well. A single mating type was predominant in most of the soysauce factories in Bangkok, while opposite mating type was isolated from only one sample in Uthai-Thani province, north of Bangkok.

In the first six months (August, 1986 to February, 1986) 333 strains of halotolerant yeasts had been isolated from various sources of brine fermented products, products of high salt or sugar content and molasses collected from different parts of Thailand. Two hundred sixty one strains from 693 strains obtained from stock yeast cultures showed ability to grow on salted molasses medium were considered as halotolerant yeast and are being included in screening. Six from nine strains of yeast for industrial ethanol fermentation produced more than 8% by weight of ethanol in molasses medium, at 35°c were selected.

In this reporting period, some additional samples were collected and isolated for halotolerant strains. All halotolerant yeasts obtained in last and this reporting periods are being screened for potent halotolerant strains to be used as parent for protoplast fusion. The screening is being divided into 2 systems. First is to obtain strains which can ferment ethanol at high rate and yield in 5 % sodium chloride containing molasses medium. Second is to obtain halotolerant strains which can grow in high concentration of sodium chloride, 17 %, although ethanol production is relatively low. And 6 strains of yeast for industrial ethanol fermentation were screened in molasses medium without and with sodium chloride for highest ethanol producing strain to be used as another parent for protoplast fusion.

Objectives

Overall objectives

The project is aimed at constructing a new hybrid from two different foundation strains, namely halotolerant strain and an industrial ethanol production strain, through a protoplast fusion technique. The halotolerant strain of *S. rouxii*, currently used for fermentation of soysauce, which tolerates salt concentrations over 18 % but produces low ethanol concentration (usually less than 5% V/V) will be used as the parental strain. The industrial alcohol production strain, *S. cerevisiae*, which can ferment cane molasses at high rate and produce high ethanol concentration will be used as the other parental strain. The hybrid

from both strains mentioned will incorporate the characteristics of producing high ethanol concentration in high salinity molasses mash, due to recombination of desirable characteristics from the parental strains. This result is impossible by conventional mating techniques because the parental strains required are from different yeast species, but a protoplast fusion technique can be used successfully for the combining of two different species , as reported by many authors. The performance of new hybrid strains obtained will be tested under laboratory conditions.

It is expected that the selected hybrid will be used for industrial alcohol production, especially for distilleries where brackish water or high salinity well water is the only available resource. There will be no doubt that the new hybrid can be used in other LDCs under similar environmental conditions. Furthermore, the new hybrid will be useful for fuel alcohol production from cellulose-based feed stock using an acid treatment process which also generates high-salinity mash and requires a special yeast strain for that purpose.

Objectives of this reporting period

1. To select potent halotolerant yeast to be used as parental strain for protoplast fusion.

2. To select highest ethanol producing strain to be used as another parent for protoplast fusion.

Material and Methods

I. Isolation of halotolerant yeast Isolation procedure was same as in first report. It was divided into 2 steps. First, sample was enriched in enrichment medium A and B. Medium A consisted of molasses of 10% sucrose equivalent sugar concentration, 0.05% of ammonium sulfate and 5% of sodium chloride. Medium B consisted of molasses of 5% sucrose equivalent sugar concentration, 0.05% ammonium sulfate and 17% sodium chloride. Cultivation was performed at 37 and 40°c for 3 days. Second step, the fermented mash of both A and B medium was streak on molasses agar A and B, respectively, and incubated at 37 and 40°c. After colonies appeared on molasses agar they were restreaked on same medium to purify the cultures and kept in molasses medium A as stock cultures. The purpose of using medium A is to isolate the good ethanol fermenting yeasts while medium B is to isolate salt tolerant yeasts. The 5% salt in medium A is relatively high for ethanol fermentation while in the real practic sodium chloride in the mash will be less than 2%. Also fermentation at 37 and 40°c for required for selection of yeast strains for ethanol fermentation in the tropics.

II. Screening for potent halotolerant yeasts to be used as parental strains.

A. First system Medium being used in this system consist of molasses of 20% sucrose equivalent sugar concentration, 5% sodium chloride and 0.05% of ammonium sulfate. Procedure

of screening was divided into 3 steps as follows. First 2 steps were already performed.

1. Standing tube culture In this step, fermentation was carried out in test tube. One loop of cells which cultivated for 2 days on molasses agar slant, at 37°c was inoculated into molasses fermentation medium. Cultivation was performed by standing for 48 h, at 40°c.Culture broth was analysed for concentration of ethanol and cell.

2. Flask culture In this step fermentation was conducted in 200 ml of molasses medium contained in 500 ml Erlenmeyer flask. Inoculum was prepared by cultivation of cells in 5% glucose YPD medium (5% glucose, 2% polypeptone and 1% yeast extract) on rotary shaker at 37°c for 15-18 h. Initial cell concentration in fermentation was adjusted to optical density at 660 nm approximately 0.5. After inoculation flask was stood at 40°c for 48 h and culture broth was taken at 0,6,12,24,36 and 48 h and analysed for concentration of ethanol and cell.

3. Fermentor culture In this step fermentation will be carried out in 5 l fermentor (microferm fermentor, New Brunswick Scientific Co.Inc.,U.S.A). Working volume of molasses medium will be 3 l and cultivation temperature will be controlled at 40°c. Inoculum will be prepared by cultivation of cells in 5% glucose YPD medium on rotary shaker,at 37°c for 15-18 h. Culture broth will be analysed for concentration of ethanol and cell at every 4-6 h.

B. Second system Medium being used in this system consist of molasses of 5% sucrose equivalent sugar concentration, 17% of sodium chloride and 0.05% of ammonium sulfate. The screening procedure was also divided into 3 steps as follows. First 2 steps were already performed.

1. Standing tube culture Method for cultivation of this step was same as of first system. Culture broth was analysed for concentration of cell and ethanol after incubation for 96 h, at 40°c.

2. Flask culture The cultivation was carried out in 200 ml of medium contained in 500 ml Erlenmeyer flask . Inoculum was prepared by cultivation of cells in 5% glucose YPDmedium same as in flask culture of first system. Initial cell concentration was adjusted to optical density at 660 nm approximately 1.0. Cultivation was performed on rotary shaker, at 70-80 rpm, at 40°c. Culture broth was analysed at 0 and 72 h for concentration of ethanol and growth.

3. Fermentor culture Cultivation will be carried out in 5 l fermentor with fermentative conditions. Working volume of medium will be 3 l and temperature will be controlled at 40°c throughout cultivation. Inoculum will be prepared by cultivation of cells in 5% glucose YPD medium on rotary shaker, at 37°c for 17-18 h. Culture broth will be analysed for concentration of cell and ethanol at every 4-6 h.

III. Screening for highest ethanol production strains from industrial yeasts to be used as another parent.

Six strains which produced more than 8% by weight of

ethanol in molasses medium (molasses of 20 % sucrose equivalent sugar concentration, 0.05% ammonium sulfate). at 35°c were tested for ethanol fermentation ability in molasses medium without and with 5% sodium chloride, at 40°c. Fermentation was carried out in 5 L fermentor with working volume of 3 L. Inoculum was prepared by cultivation of yeast in 5 % glucose YPD medium on rotary shaker, at 37°c for 15-18 h. Fermentation conditions were; initial pH was 4.5, temperature was controlled as 40°c and agitation speed was 250 rpm. Sample was taken and analysed for concentration of ethanol, cell and sugar every 4 h from 0 to 24 h and every 6 h from 24 to 48 h of cultivation.

Analysis Ethanol was analysed by using liquid gas chromatograph (Shimudzu GC-9A) under the following conditions: packing material, polyetnylene glycol 20 M in glass column, 1 m length and 3 mm inner diameter; carrier gas ,nitrogen at 40 ml/min; temperature of column and injector 70° and 125°c, respectively, propanol was used as initial standard. Cell concentration was measured as optical density of cell suspension at 660 nm after washing and resuspendiregin 0.1N HCl. Sugar concentration was determined as sucrose by phenol sulfuric acid method.

Results/Discussion

1. Collection of samples and isolation of halotolerant yeasts

In addition, 69 samples were collected from some other provinces in Thailand as shown in Figure 1. The samples consisted of 11 samples of shrimp paste, 10 samples of pickle, 8 samples of fermented fish, 6 samples each of palm sugar

and fish sauce, 5 samples of salted crab, 4 samples of fermented soybean (miso), 3 samples each of fermented peach and fermented pork, 2 samples each of soysauce and sufu and 1 sample each of tamarind, salted lettuce, salted fish, pineapple, shell in fish sauce, soybean and honey. (Table 1)

Eighty strains of halotolerant yeasts were isolated from these 68 samples as shown in Table 2. It was summarized in Table 1 that 13 strains each were isolated from fermented fishes and soysauce, 10 strains from pickles, 2 strains each from fermented peach, tamarind, fish sauce and shell in fish sauce, 5 strains from fermented soybean, 4 strains each from sufu, fermented pork, 3 strains each from salted crab and pineapple and 1 strain each from salted fish, honey and soybean.

II. Screening for potent halotolerant yeasts to be used as parental strains.

Totally 674 strains of halotolerant yeast obtained by isolation and selection in last and this reporting periods were screened.

A. First system In this system the strains which ferment ethanol at high rate and yield in 5% sodium chloride containing molasses medium is expected to obtain. The screening procedure was divided into 3 steps as mentioned previously. In order to obtain strains which ferment ethanol at high rate, 48 h of cultivation time was selected for the test. Result of first step of screening, tube culture was shown in Table 3 (p25 to p46). Fifty two strains which did not

show characteristic of film forming on surface of medium was not included, produced ethanol more than 2.5% by weight at 48 h in tested conditions were selected. They were strain no 016, 101, 165, 166, 167, 236, 246, 251, 266, 274, 277, 292, 293, 307, 308, 309, 310, 313, 314, 339, 402, s002, s003, s005, s006, s009, s010, s013, s014, s015, s019, s020, s021, s022, s036, s037, s065, s075, s076, s077, s078, s079, s081, s082, s083, s084, s085, s086, s089, s090, s107 and s108. These 52 strains were further screened by standing flask culture and results showed in Figure 2 (p 74 to p85). In this step 7 strains which produced ethanol more than 2.0 % by weight, at 40°c, after 48 h of cultivation were selected. They were strain no. 016, 251, s013, s019, s037, s065 and s090 which produced 3.35, 2.80, 2.34, 2.73, 2.46, 2.33 and 2.00 % by weight of ethanol, respectively. These 7 strains will be tested in fermentor.

B. <u>Second system</u> To obtain halotolerant yeasts which can grow at high concentration of sodium chloride, they were tested ability to grow in 17% sodium chloride containing molasses medium, at 40°c. The screening was also divided into 3 steps as mentioned perviously. Since cultivation conditions were very stress because of high concentration of sodium chloride and temperature were used, therefore cultivation period was prolonged to 96 h in first step of screening. The result of first step, tube culture, was shown in Table 4 (p 47 to p 67). Fifty one strains which could grow and gave cell concentration as OD 660 nm more than 2.5 were selected to test in flask culture. They were

strain no. 031, 062, 065, 086, 090, 092, 116, 126, 130, 131, 149, 167, 174, 192, 229, 246, 252, 254, 257, 270, 288, 290, 294, 295, 326, 344, 346, 352, 356, 357, 365, 366, 370, 387, 394, 396, 399, 402, 403, 405, 406. 407, 408, 409, s010 s011, s068, s069, s205, s233 and s244. These 51 strains were tested in slowly shaking flask culture and cultivation period was reduced to 72 h inorder to obtain fast growing halotolernat strains. Result of shaking flask culture was shown in Table 5. Twelve strains which gave higher growth, OD 660 nm more than 3.0, than others were selected for further selection. Twelve selected strains were strain no. 062, 065, 090, 092, 126, 130, 229, 246, 356, 366, 370 and 394. Since strain no 346 produced ethanol 1.72% by weight in this condition, it was selected for further screening.

III. <u>Screening for highest ethanol production strains from industrial yeasts to be used as another parent</u>

To select high ethanol producing strains from industrial strains to be used as another parent for protoplast fusion, 6 from 9 strains which produced more than 8% by weight of ethanol in molasses medium, at 35°c were test for ethanol production in molasses medium without and with sodium chloride, at 40°c in fermentor. The results of ethanol production in molasses medium without addition of sodium chloride were shown in Figure 3,4,5 and 6 <u>S. cerevisiae</u> M30 , AM12 and Sc90 produced ethanol 7.83, 7.52 and 6.23% by weight, respectively. While <u>S. cerevisiae</u> 191, B120 and 281 could not grow in this high temperature. Therefore <u>S. cerevisiae</u> M30, AM12 and Sc90 were tested for ethanol production ability in high salinity molasses medium.

The result of S. cerevisiae M30 in molasses medium contained 5% sodium chloride, at 40°c showed that cell concentration was very low increase and maximal ethanol produced was 1.64% by weight at 60 h (Table 6). Of S. cerevisiae AM12 cell concentration obtained in this cultivation was very low and maximal ethanol produced was 0.72% by weight at 72h (Table 7). Same as of S. cerevisiae M30 and AM12 almost no growth and ethanol production was observed by S. cerevisiae Sc90(Table 8).

From these results S. cerevisiae M30, AM12 and Sc90 was selected and will be used as parent for protoplast fusion with halotolerant yeast.

Conclusion

The work that has been carried out in last 6 months (March 1986 to July 1986) could be summarized as follows:

1. In addition, 80 strains of halotolerant yeasts have been isolated from 69 samples of brine fermented products, fermented products, products of high salt and sugar content and fruits, newly collected in this reporting period.

2. All 674 strains of halotolerant yeast obtained in last and this reporting periods are being screened for potent strain to be used as halotolerant parent for protoplast fusion. The screening was divided into 2 systems. Each system consisted of 4 steps and first 2 steps of each system were already performed. In the first system strains which fermented ethanol at high rate and yield is expected to obtain . And by 2 steps of screening strains which produced

ethanol more than2.0% by weight in 5% sodium chloride containing molasses medium, at 40°c were selected for final screening in fermentor. In second system, by 2 steps of screening 12 strains showed higher growth in 17 % sodium chloride than other strains were selected for further screening.

3 In order to select high etanol production strain,6 strains of industrial yeast selected in last period were tested for fermentation ability in molasses medium without and with 5% sodium chloride, at 40°c. S. cerevisiae M30, AM12 and Se90 produced much higher ethanol than other 3 in molasses medium without sodium chloride were selected to be used as ethanol producing parent for protoplast fusion with halotolerant strains. These 3 strains were tested for ablity to grow in 5% sodium chloride containing molasses medium and found that they grew very low and produced ethanol in very small concentration in tested conditions.

Work plan for the next period

Work plan for the next period is scheduled as follows:

1. Final screen for halotolerant strains which can ferment ethanol at high rate and yield in molasses medium contain 5% of sodium chloride and which can grow in molasses contain 17% of sodium chloride.

2. Study characteristics of selected halotolerant and high ethanol producing strains, such as salt toleration.

3. Identification of selected halotolerant strains.

4. Auxotrophic mutation of potent strains inorder to construct selective marker.

5. Selection of mutant which show no or nearly no difference from wild type in fermentation rate and yield or in growth rate.

6. Preliminary study of protoplast fusion of selected strains.

References

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3. Morishita, H. and M. Yamaguchi, 1983. Transfer of salt resistance in yeast by protoplast fusion. Third inter-Congress Abstract, 562 p.
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Tables and pictures

Table 1 Summarization on number of halotolerant yeast isolated in the medium containing 5 and 17 % of sodium chloride

Product	Number of Sample	Number of isolates				Total
		5% NaCl		17% NaCl		
		37°C	40°C	37°C	40°C	
Fermented fish	10	4	6	3		13
Pla-ra	(7)	(3)	(6)	(3)	(-)	
Pla-chao	(1)	(1)	(-)	(-)	(-)	
Tai-pla	(2)	(-)	(-)	(-)	(-)	
Pickles	10	8	2			10
Bamboo shoot	(4)	(1)	(-)	(-)	(-)	
Lettuce	(4)	(4)	(2)	(-)	(-)	
Ginger	(1)	(1)	(-)	(-)	(-)	
Garlic	(1)	(2)	(-)	(-)	(-)	
Fermented peach (tor dong)	3	1	1	-	-	2
Fermented soy bean (Miso)	4	4	1	-	-	5
Soy sauce	2	13	-	-	-	13
Sufu	2	3	1	-	-	4
Fermented pork (nam)	3	1	3	-	-	4
Palm sugar	6	5	2	5	2	14
Tamarind	1	2	-	-	-	2
Salted crab	5	1	2	-	-	3
Salted lettuce	1	-	-	-	-	-
Salted fish	1	1	-	-	-	1
Shrimp paste (kapi)	11	-	-	-	-	-
Fish sauce	6	2	-	-	-	2
Pineapple (fermented)	1	3	-	-	-	3

Table 1 (Cont.)

Product	Number of Sample	Number of isolates				Total
		5% NaCl		17% NaCl		
		37°C	40°C	37°C	40°C	
Shell in fish sauce	1	2	-	-	-	2
Honey	1	1	-	-	-	1
Soybean	1	1	-	-	-	1
Total	69	52	18	8	2	80

Table 2 Lists of additional halotolerant yeasts isolated from various location in Thailand.

Sample No.	Sample Name	Sampling Place	Isolation method 5%NaCl 37°C	5%NaCl 40°C	17%NaCl 37°C	17%NaCl 40°C	Strain No.
217	Palm sugar	Chiang-Rai			+		334
217	" "	"			+		335
218	Palm sugar	Lamphun			+		336
218	" "	"			+		337
219	Pla-ra	"		+			338
219	" "	"		+			339
219	" "	"		+			340
220	Palm sugar	Chiang-Mai				+	341
220	" "	"				+	342
220	" "	"			+		343
220	" "	"		+			349
220	" "	"	+				364
220	" "	"	+				366
221	Miso	"			+		344
222	Pla-ra	Chiang-Rai			+		345
223	Pla-ra	"			+		346
223	" "	"		+			355
224	Lettuce pickle	Chiang-Mai		+			347
224	" "	"	+				360
225	Lettuce pickle	"		+			348
225	" "	"	+				361
226	Nam (Fermented pork)	"		+			350
226	" "	"	+				368

Table 2 (Continue)

Sample No.	Sample Name	Sampling Place	Isolation method 5%NaCl 37°C	5%NaCl 40°C	17%NaCl 37°C	17%NaCl 40°C	Strain No.
227	Nam(Fermented pork)	Chiang-Mai		+			351
228	Nam(Fermented pork)	Chiang-Rai		+			352
229	Salted crab	"		+			354
230	Fermented peach(Tor dong)	Chiang-Mai		+			356
231	Sufu	Songkhla		+			357
231	"	"	+				369
231	"	"	+				370
232	Palm sugar	Chiang-Rai	+				358
232	" "	"		+			359
233	Fermented peach(Tor dong)	Chiang-Mai	+				362
234	Ginger pickle	"	+				363
235	Sufu	"	+				365
236	Salted fish	"	+				367
237	Salted crab	Chiang-Rai					-
238	Salted crab	"					-
239	Shrimp paste (kapi)	Lampang					-
240	Shrimp paste (kapi)	"					-
241	Shrimp paste (kapi)	"					-
242	Pla-ra	Lamphun					-
243	Shrimp paste (kapi)	"					-

Table 2 (Continue)

Sample No.	Sample Name	Sampling Place	Isolation method				Strain No.
			5%NaCl		17%NaCl		
			37°C	40°C	37°C	40°C	
244	Shrimp paste (Kapi)	Lamphun					-
245	Shrimp paste (Kapi)	"					-
246	Bamboo shoot pickle	Chiang-Mai					-
247	Bamboo shoot pickle	"					-
248	Salted Lettuce	Chiang-Mai					-
249	Fermented peach(tor dong)	"					-
250	Tai-pla	"					-
251	Fish sauce (2-3 months)	"					-
252	Fish sauce (5-6 months)	Bangkok					-
253	Fish sauce (7 months)	"					-
254	Fish sauce (9-10 months)	"					-
255	Shrimp paste (Kapi)	Chiang-Mai					-
256	Shrimp paste (Kapi)	"					-
257	Shrimp paste (Kapi)	"					-
258	Shrimp paste (Kapi)	"					-
259	Palm sugar	Nakhon-sithammarat					-
260	Fish sauce	"					-

Table 2 (Continue)

Sample No.	Sample Name	Sampling Place	Isolation method 5%NaCl 37°C	5%NaCl 40°C	17%NaCl 37°C	17%NaCl 40°C	Strain No.
261	Shrimp paste (Kapi)	Nakhonsithammarat	Nakhon-sithammarat				-
262	Miso	"					-
263	Tai-pla	Songkhla					-
264	Salted crab	Nakhonratchasima		+			371
265	Salted crab	"	+				372
266	Pla-ra	"		+			373
266	"	"	+				374
267	Pla-ra	Nakhonratchasima		+			375
267	" "	"	+				376
267	" "	"	+				377
267	" "	"				+	383
268	Pla-ra	Saraburi					-
269	Miso	"	+				378
269	"	"	+				379
269	"	"	+				380
270	Bamboo shoot pickle	"					-
271	Fish sauce	Bangkok	+				381
			+				382
272	Soy sauce	Rajchaburi	+				383
272	" "	"	+				385
272	" "	"	+				386

Table 2 (continue)

Sample No.	Sample Name	Sampling Place	Isolation method 5%NaCl 37°C	5%NaCl 40°C	17%NaCl 37°C	17%NaCl 40°C	Strain No.
272	Soy sauce	Rajchaburi	+				389
272	"	"	+				390
272	"	"	+				392
272	"	"	+				393
273	"	"	+				384
273	"	"	+				387
273	"	"	+				388
273	"	"	+				391
273	"	"	+				394
273	"	"	+				395
274	Lettuce pickle	Nakhonrat-chasima	+				396
275	Pineapple (fermented)	Nakhonpathom	+				397
275	"	"	+				398
275	"	"	+				399
276	Bamboo shoot pickle	Petchabun	+				400
277	Garlic pickle	Petchabun	+				401
277	"	"	+				402
278	Lettuce pickle	Nakornsawan	+				403
279	Shell in fish sauce	Petchabun	+				404
279	"	"	+				405

Table 2 (continue)

Sample No.	Sample Name	Sampling Place	Isolation method 5%NaCl 37°C	5%NaCl 40°C	17%NaCl 37°C	17%NaCl 40°C	Strain No.
280	Pla-chao	Pitsanulok	+				406
281	Miso	Nakhonratchasima	+				407
282	Palm sugar	"	+				408
282	"	"	+				409
283	Honey	Chanthaburi	+				410
284	Tamarind	Pitsanulok	+				411
284	"	"	+				412
285	Boiled soybean	"	+				413

Table 3 Ethanol fermentation by halotolerant yeast in molasses medium contained 5% of sodium chloride in standing culture, at 40°c

Strain No.	Ethanol at 48h % W/V	Remark
001	0.55	film yeast
002	0.24	"
003	1.46	"
004	0.88	"
005	0.49	
006	NT	film yeast
007	1.64	"
008	0.25	"
009	0.52	"
010	0.49	"
011	1.28	"
012	0.63	
013	0.39	film yeast
014	1.41	
015	0.73	
016	3.20	film yeast
017	3.20	
018	1.29	film yeast
019	1.05	
020	NT	
021	1.14	
022	0.65	film yeast
023	0.40	"
024	0.33	"
025	0.27	"
026	0.22	"
027	0.54	"
028	0.30	
029	NT	
030	NT	

NT = non test

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
031	0.27	film yeast
032	0.25	"
033	1.21	"
034	0.26	"
035	0.21	"
036	0.27	"
037	0.90	
038	0.04	
039	0.39	
040	0.88	
041	0.03	
042	0.27	
043	NT	
044	0.02	
045	0.96	
046	0.24	film yeast
047	0.08	"
048	0.04	"
049	0.28	"
050	0.26	"
051	0.20	"
052	0.47	"
053	1.68	
054	0.11	
055	NT	
056	0.45	film yeast
057	1.34	
058	0.23	film yeast
059	0.35	
060	0.70	

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
061	0.52	
062	1.32	
063	0.97	
064	NT	
065	0.99	
066	1.37	
067	0.18	
068	0	
069	NT	
070	1.03	
071	0.94	
072	0.12	
073	0.47	
074	NT	
075	0.39	
076	0.29	
077	0.60	
078	0.67	
079	1.31	
080	0.64	
081	0.39	
082	NT	
083	0.32	
084	1.19	
085	0.50	film yeast
086	0.68	"
087	0.37	
088	0.72	film yeast
089	0.63	
090	0.61	film yeast
091	0.83	

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
092	0.68	
093	0.32	film yeast
094	0.36	"
095	1.11	
096	1.10	
097	0.57	film yeast
098	0.56	
099	0.85	
100	0.72	
101	0.71	
102	0.31	
103	0.40	film yeast
104	0.60	
105	0.30	
106	0.50	
107	0.38	film yeast
108	0.68	
109	0.76	
110	0.32	
111	0.22	
112	NT	
113	0.37	film yeast
114	0.45	"
115	0.79	
116	0.73	
117	0.34	film yeast
118	0.29	
119	0.21	
120	0.31	film yeast
121	1.13	"
122	NT	

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
123	0.68	
124	NT	
125	0.25	film yeast
126	0.90	
127	0.33	
128	0.33	film yeast
129	0.31	
130	1.00	
131	NT	
132	NT	
133	NT	
134	0.31	
135	0.24	
136	0.25	
137	0.40	
138	0.62	
139	0.71	
140	1.81	
141	0.85	
142	0.36	
143	0.31	
144	0.30	
145	1.17	
146	0.89	
147	0.89	
148	0.37	
149	0.91	
150	0.36	
151	NT	
152	1.12	

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
153	0.26	
154	1.03	
155	1.01	
156	NT	
157	1.17	
158	0.12	
159	0.55	
160	0.25	
161	0.39	
162	0	
163	0	
164	NT	
165	3.16	
166	2.63	
167	2.74	
168	0.16	
169	0	
170	NT	
171	NT	
172	0.71	
173	1.16	
174	1.11	
175	0	
176	0.17	film yeast
177	1.32	
178	NT	
179	0.96	film yeast
180	0.08	
181	0.94	film yeast
182	NT	
183	1.80	
184	0.08	film yeast

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
185	NT	
186	1.28	
187	NT	
188	0.10	
189	NT	
190	0	
191	0.01	
192	0	
193	0.55	
194	0.11	
195	0.03	
196	0.13	
197	0	
198	0	
199	0	
200	0.17	film yeast
201	0.02	
202	0.09	
203	0	
204	0.28	
205	0.1	
206	0	
207	0.01	
208	0	
209	0.08	
210	0.22	
211	0.38	
212	0.21	
213	0	
214	0.23	
215	0.29	film yeast

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
216	0.71	
217	0.03	
218	0.36	
219	0.26	
220	0	
221	0.09	
222	0.52	film yeast
223	NT	
224	NT	
225	0.10	
226	0.40	film yeast
227	NT	
228	1.20	
229	0.86	
230	0.60	film yeast
231	1.93	
232	0.14	
233	1.60	
234	1.23	
235	0.80	
236	2.29	
237	0.19	
238	0.42	
239	0.31	film yeast
240	NT	
241	0.59	film yeast
242	0.13	
243	0.98	
244	0.88	film yeast
245	1.64	
246	0.39	
247	NT	

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
248	1.20	
249	NT	
250	1.17	
251	3.12	
252	1.25	
253	0.26	film yeast
254	1.78	
255	NT	
256	NT	
257	2.45	
258	0	
259	0.96	
260	0.33	
261	NT	
262	0.23	
263	0.24	
264	1.09	
265	1.26	
266	2.16	
267	0.85	
268	0.20	
269	1.30	
270	1.11	
271	NT	
272	0.86	
273	0.25	
274	2.03	
275	0.30	
276	0.63	
277	2.12	
278	0.28	

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
279	1.56	
280	0.21	
281	0.66	
282	0.34	
283	0.29	
284	0.31	
285	0.29	
286	1.37	
287	1.37	
288	1.99	
289	0.62	
290	0.58	
291	0.91	
292	2.95	film yeast
293	2.40	
294	1.70	
295	0.73	
296	0.94	
297	0.22	
298	1.29	
299	0.31	
300	0.31	
301	1.13	
302	1.68	
303	0.28	
304	0.67	
308	2.06	
309	2.18	
310	2.19	
311	1.10	
312	1.89	

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
313	2.13	
314	2.00	
315	0.34	
316	1.03	
317	0.18	
318	NT	
319	0.63	
320	0.34	
321	0.20	
322	0.22	
323	0.57	
324	0.29	
325	0.28	
326	0.27	
327	0.25	
328	0.15	
329	0.65	
330	0.51	
331	1.59	
332	0.55	
333	0.26	
334	0.77	
335	1.00	
336	0.59	
337	0.59	
338	0	
339	0	
340	0	
341	1.67	
342	1.20	
343	0.93	

Table 3(continue)

Strain No.	Ethanol at 48h % W/V	Remark
344	0.09	
345	0.15	film yeast
346	0.12	
347	0	film yeast
348	0	"
349	0.61	
350	0.93	film yeast
351	0.41	
352	0	
353	0.77	film yeast
354	0	
355	1.13	film yeast
356	1.12	
357	1.35	
358	1.13	
359	1.23	film yeast
360	0.10	"
361	0	
362	0.29	
363	0.43	
364	0.84	
365	0.27	
366	0.54	
367	0.53	
368	0.11	
369	0.92	
370	0.52	
371	1.36	
372	0.14	
373	1.43	
374	0.51	
375	0.62	
376	0.51	

Table 3 (continue)

Strain No.	Ethanol at 48h % W/V	Remark
377	0.55	
378	0.48	
379	0.42	
380	0.38	
381	0.16	
382	0.17	
383	0.75	
384	0.91	
385	1.18	
386	0.84	
387	0.93	
388	0.84	
389	0.78	
390	0.82	
391	0.97	
392	0.87	
393	0.88	
394	1.04	
395	0.81	
396	0.22	
397	0.13	film yeast
398	0.11	"
399	2.24	"
400	0.10	"
401	0.02	"
402	3.42	
403	1.21	film yeast
404	0.59	"
405	0.76	"
406	0.26	

Table 3 (continue)

Strain No.	Ethanol at 48 h % W/V	Remark
407	0.60	film yeast
408	1.33	
409	1.21	
410	0.62	film yeast
411	0.62	"
412	0.74	"
413	0.67	"
s001	1.28	
s002	2.62	
s003	2.31	
s004	0.74	
s005	2.46	
s006	2.86	
s007	1.22	film yeast
s008	1.80	
s009	2.31	
s010	2.20	
s011	1.57	
s012	1.79	
s013	3.25	
s014	2.21	
s015	2.23	
s016	1.39	
s017	1.08	
s018	1.91	
s019	2.76	
s020	2.64	
s021	2.34	
s022	2.67	
s023	NT	
s024	0.43	
s025	1.51	
s026	1.82	
s027	1.35	
s028	0.58	
s029	0.76	
s030	0.41	Film yeast
s031	1.44	
s032	1.15	

Table 7(continue)

Strain No.	Ethanol at 48 h % W/V	Remark
s033	1.21	film yeast
s034	0.58	"
s035	1.57	
s036	2.06	
s037	2.09	
s038	0.54	
s039	1.75	film yeast
s040	0.27	
s041	1.04	film yeast
s042	0.29	"
s043	0.32	"
s044	0.32	"
s045	0.29	"
s046	0.34	"
s047	0.34	"
s048	0.26	"
s049	0.31	"
s050	1.45	"
s051	0.38	"
s052	0.61	"
s053	2.34	
s054	0.96	
s055	1.86	
s056	1.58	film yeast
s057	0.94	
s058	0.06	
s059	0.83	
s060	0.70	
s061	0.63	
s062	0.93	
s063	0.93	
s064	1.69	film yeast

Table 3 (continue)

Strain No.	Ethanol at 48 h % W/V	Remark
s065	2.57	
s066	NT	
s067	NT	
s068	0.38	
s069	1.16	
s070	1.24	
s071	NT	
s072	NT	
s073	NT	
s074	NT	
s075	2.93	
s076	2.45	
s077	3.64	
s078	2.14	
s079	2.60	
s080	1.81	
s081	2.46	
s082	2.65	
s083	2.46	
s084	3.09	
s085	2.23	
s086	2.47	
s087	1.51	
s088	1.45	
s089	2.36	
s090	[illegible].20	
s091	0.52	film yeast
s092	0.60	"
s093	0.53	"
s094	0.71	"
s095	2.17	"

Table 3 (continue)

Strain No.	Ethanol at 48 h % W/V	Remark
s096	0.49	film yeast
s097	0.51	"
s098	0.51	"
s099	0.44	"
s100	0.46	"
s101	2.09	
s102	2.30	film yeast
s103	NT	
s104	1.33	film yeast
s105	1.91	
s106	1.79	
s107	2.24	
s108	2.33	
s109	1.08	film yeast
s110	0.81	
s111	1.11	
s112	1.10	
s113	1.05	
s114	1.92	
s115	1.65	
s116	1.87	
s117	1.53	
s118	0.72	film yeast
s119	NT	
s120	1.10	film yeast
s121	1.03	"
s122	NT	
s123	0.87	
s124	0.96	
s125	1.47	
s126	0.50	
s127	0.52	

Table3(continue)

Strain No.	Ethanol at 24 h % W/V	Remark
s128	1.77	film yeast
s129	0.82	"
s130	0.78	"
s131	1.14	"
s132	1.42	film yeast
s133	1.49	"
s134	1.76	"
s135	1.52	"
s136	1.66	"
s137	0.75	"
s138	1.33	"
s139	0.84	"
s140	0.82	"
s141	1.35	"
s142	1.30	"
s143	1.23	"
s144	2.32	"
s145	1.44	"
s146	1.75	
s147	1.85	film yeast
s148	0.87	"
s149	0.92	"
s150	1.88	"
s151	1.80	"
s152	1.43	"
s153	0.97	"
s154	1.21	"
s155	0.73	"
s156	1.99	"
s157	1.00	"
s158	2.00	"
s159	1.31	"

Table 3(continue)

Strain No.	Ethanol at 48 h % W/V	Remark
s160	0.90	film yeast
s161	1.01	"
s162	1.18	"
s163	1.02	"
s164	1.57	"
s165	1.71	"
s166	2.17	"
s167	0.91	
s168	2.12	film yeast
s169	1.44	"
s170	1.51	"
s171	1.06	
s172	1.26	
s173	NT	
s174	1.23	
s175	NT	
s176	0.11	
s177	0.27	film yeast
s178	0.18	"
s179	0.40	
s180	0.23	
s181	NT	
s182	NT	
s183	NT	
s184	0.39	
s185	0.02	
s186	0.28	
s187	0.04	film yeast
s188	0.26	
s189	NT	
s190	0.30	film yeast
s191	1.10	

Table 3 (continue)

Strain No.	Ethanol at 48 h % W/V	Remark
s192	0.53	film yeast
s193	1.08	
s194	1.20	
s195	1.32	
s196	0.78	film yeast
s197	0.60	"
s198	0	
s199	0.04	
s200	NT	
s201	0.11	
s202	0.10	
s203	0.14	
s204	0	film yeast
s205	0.15	
s206	0.23	
s207	0	
s208	0.10	
s209	0.13	
s210	0.67	
s211	0.88	
s212	NT	
s213	0.04	
s214	0.43	film yeast
s215	0.32	"

Table3(continue)

Strain No.	Ethanol at 48 h % W/V	Remark
s216	0.46	film yeast
s217	1.81	
s218	0.28	
s219	0.35	film yeast
s220	0.38	"
s221	NT	
s222	NT	
s223	0.22	film yeast
s224	0	
s225	0.20	film yeast
s226	0.28	"
s227	NT	
s228	0.22	film yeast
s229	0.40	
s230	0.11	film yeast
s231	0.20	"
s232	0.18	"
s233	0.21	"
s234	0	
s235	0.26	
s236	NT	
s237	0	
s238	0.13	
s239	0.20	

Table 3 (continue)

Strain No.	Ethanol at 48 h % W/V	Remark
s240	0.29	
s241	0.19	
s242	0.12	
s243	0	
s244	0.29	
s245	0.10	
s246	0.41	
s247	NT	
s248	0	film yeast
s249	0.13	
s250	NT	
s251	0.32	film yeast
s252	NT	
s253	NT	
s254	0.27	film yeast
s255	0.16	
s256	0.19	
s257	0.85	
s258	0.51	
s259	NT	
s260	0.01	
s261	0.01	

Table 4 Growth of halotolerant yeasts in molasses medium contained 17% of sodium chloride, at 40°c.

Strain No.	Growth of 96 h (OD 660)
001	0.76
002	1.11
003	0.80
004	0.76
005	1.12
006	1.12
007	0.68
008	1.55
009	1.11
010	0.82
011	0.91
012	2.01
013	1.81
014	1.66
015	1.44
016	1.04
017	0.88
018	1.10
019	0.94
020	0.93
021	1.62
022	1.74
023	1.18
024	1.03
025	NT
026	1.15
027	NT
028	1.40
029	1.64
030	0.79
031	2.79
032	1.14
033	1.70

NT = non test

Table 4 (continue)

Strain NO.	Growth of 96 h (OD 660)
034	1.74
035	1.49
036	1.51
037	NT
038	1.78
039	NT
040	1.28
041	1.34
042	1.30
043	NT
044	1.30
045	1.79
046	1.51
047	0.93
048	1.06
049	1.00
050	2.12
051	1.18
052	1.17
053	2.02
054	1.46
055	1.32
056	1.08
057	NT
058	NT
059	NT
060	1.53
061	1.14
062	3.82
063	1.81
064	NT
065	2.19

Table 4 (continue)

Strain NO.	Growth at 96 h (OD 660)
066	2.16
067	1.13
068	1.16
069	NT
070	1.03
071	0.72
072	1.58
073	1.31
074	1.66
075	NT
076	0.80
077	1.46
078	2.29
079	1.83
080	0.76
081	1.30
082	0.88
083	NT
084	2.00
085	1.32
086	4.41
087	NT
088	NT
089	NT
090	5.18
091	NT
092	2.51
093	1.41
094	NT
095	1.16
096	0.75
097	1.42
098	1.43

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
099	1.62
100	1.00
101	1.92
102	2.44
103	0.52
104	2.25
105	1.11
106	1.15
107	0.54
108	1.63
109	1.16
110	1.35
111	1.97
112	NT
113	1.25
114	1.88
115	0.52
116	2.59
117	1.43
118	1.52
119	1.84
120	2.29
121	1.68
122	11.52
123	2.06
124	2.19
125	1.19
126	3.12
127	1.26
128	1.22
129	1.68
130	3.09
131	3.26

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
132	0.20
133	0.61
134	0.78
135	1.59
136	NT
137	0.27
138	1.35
139	1.69
140	NT
141	2.06
142	1.08
143	1.03
144	1.46
145	NT
146	1.07
147	1.31
148	0.92
149	2.55
150	NT
151	0.31
152	2.28
153	1.51
154	2.24
155	2.34
156	NT
157	0.83
158	NT
159	NT
160	0.52
161	0.47
162	0.18
163	1.55

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
164	NT
165	1.40
166	NT
167	2.80
168	0.97
169	NT
170	NT
171	NT
172	NT
173	1.62
174	2.59
175	NT
176	1.25
177	2.40
178	2.16
179	1.97
180	2.11
181	1.48
182	2.27
183	2.11
184	1.60
185	NT
186	1.79
187	NT
188	1.52
189	NT
190	1.30
191	2.59
192	2.73
193	1.58
194	0.41
195	6.52

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
196	6.49
197	2.11
198	0.66
199	0.79
200	0.47
201	0.78
202	0.51
203	NT
204	0.69
205	0.55
206	0.73
207	NT
208	1.80
209	0.77
210	2.01
211	0.97
212	1.07
213	0.72
214	2.05
215	NT
216	1.91
217	1.18
218	0.59
219	0.81
220	0.75
221	1.03
222	0.97
223	1.61
224	0.67
225	0.46
226	0.66
227	1.68
228	1.82

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
229	3.54
230	2.08
231	2.22
232	1.25
233	2.23
234	2.15
235	1.32
236	0.70
237	0.96
238	0.46
239	0.67
240	NT
241	2.15
242	0.92
243	3.60
244	2.06
245	0.43
246	2.59
247	NT
248	1.81
249	0.78
250	0.83
251	1.93
252	2.65
253	0.86
254	2.74
255	2.27
256	NT
257	2.82
258	1.13
259	1.95
260	1.55

Table 4 (continue)

Strain NO.	Growth at 96h (OD660)
261	
262	0.82
263	1.61
264	1.45
265	1.66
266	1.94
267	1.31
268	NT
269	2.01
270	2.47
271	NT
272	1.21
273	1.22
274	1.57
275	1.87
276	1.72
277	1.22
278	2.06
279	2.29
280	1.47
281	1.99
282	0.94
283	1.34
284	1.41
285	2.01
286	2.22
287	2.37
288	2.64
289	2.23
290	2.45
291	2.42
292	1.56
293	1.84

Table 4 (continue)

Strain NO.	Growth at 96h (OD 660)
294	2.63
295	2.56
296	2.10
297	1.83
298	1.97
299	2.06
300	1.65
301	1.63
302	2.37
303	1.60
304	1.68
308	1.31
309	2.15
310	1.92
311	1.68
312	1.76
313	1.25
314	1.85
315	1.65
316	1.55
317	2.26
318	2.20
319	2.00
320	1.56
321	1.25
322	0.49
323	2.34
324	1.37
325	1.62
326	2.52
327	0.47
328	0.99
329	1.68

Table 4 (continue)

Strain NO.	Growth at96 h (OD660)
330	1.54
331	2.29
332	1.76
333	1.89
334	5.74
335	5.87
336	5.56
337	5.90
338	2.16
339	1.29
340	1.16
341	5.17
342	5.90
343	7.24
344	3.02
345	3.81
346	3.85
347	1.73
348	1.99
349	6.81
350	2.01
351	1.99
352	2.93
353	1.37
354	3.96
355	1.64
356	3.08
357	2.87
358	2.35
359	2.42
360	1.31
361	2.08
362	1.90

Tabl (continue)

Strain NO.	Growth at 96 h (OD660)
363	2.14
364	4.75
365	2.54
366	4.62
367	1.78
368	1.73
369	0.50
370	2.84
371	1.09
372	0.89
373	1.14
374	0.60
375	1.70
376	1.38
377	0.97
378	0.60
379	0.46
380	0.41
381	0.53
382	1.00
383	6.95
384	7.57
385	3.48
386	6.80
387	2.47
388	6.24
389	1.52
390	8 96
391	5.40
392	8.50
393	1.73

Table 4 (continue)

Strain NO.	Growth at 96h (OD660)
394	2.69
395	7.74
396	3.23
397	2.27
398	2.15
399	2.77
400	2.23
401	2.27
402	2.74
403	2.61
404	2.17
405	2.76
406	2.61
407	2.52
408	2.84
409	2.71
410	2.04
411	2.21
412	1.76
413	2.12
s001	1.60
s002	1.68
s003	1.49
s004	1.87
s005	1.59
s006	1.82
s007	1.29
s008	2.03
s009	1.65
s010	3.12
s011	3.46

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
s012	1.76
s013	1.56
s014	1.91
s015	2.31
s016	1.23
s017	0.93
s018	2.10
s019	0.97
s020	0.34
s021	0.87
s022	1.47
s023	1.33
s024	1.63
s025	1.68
s026	1.55
s027	1.65
s028	1.21
s029	1.64
s030	1.24
s031	1.17
s032	1.55
s033	1.66
s034	0.90
s035	1.68
s036	1.60
s037	1.74
s038	1.56
s039	0.96
s040	1.11
s041	NT
s042	0.65
s043	1.06

Table 4 (continue)

Strain NO.	Growth at96 h (OD660)
s044	1.94
s045	0.66
s046	0.84
s047	0.78
s048	0.46
s049	0.97
s050	1.39
s051	1.57
s052	1.45
s053	NT
s054	1.76
s055	0.93
s056	NT
s057	NT
s058	NT
s059	NT
s060	1.48
s061	NT
s062	NT
s063	NT
s064	1.29
s065	1.70
s066	1.14
s067	1.40
s068	2.74
s069	2.45
s070	2.22
s071	1.61
s072	1.46
s073	1.18
s074	1.16
s075	1.73
s076	1.78

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
s077	1.84
s078	1.73
s079	2.10
s080	1.77
s081	2.05
s082	1.32
s083	1.35
s084	1.67
s085	1.50
s086	1.77
s087	2.27
s088	1.69
s089	1.89
s090	2.00
s091	1.20
s092	0.90
s093	1.10
s094	1.17
s095	1.35
s096	0.90
s097	1.07
s098	0.90
s099	0.93
s100	1.46
s101	0.68
s102	0.87
s103	1.02
s104	0.71
s105	0.79
s106	1.33
s107	2.16
s108	1.87

Table4 (continue)

Strain NO.	Growth at 96h (OD660)
s109	0.32
s110	0.33
s111	1.79
s112	0.63
s113	0.80
s114	1.10
s115	1.57
s116	1.64
s117	1.66
s118	1.67
s119	1.69
s120	1.10
s121	NT
s122	1.26
s123	1.39
s124	1.39
s125	1.44
s126	1.24
s127	1.51
s128	1.40
s129	1.31
s130	1.81
s131	1.53
s132	0.87
s133	1.10
s134	1.75
s135	1.64
s136	0.69
s137	0.86
s138	0.58
s139	0.81
s140	1.27

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
s141	0.46
s142	1.96
s143	NT
s144	1.89
s145	0.98
s146	0.80
s147	0.79
s148	1.43
s149	1.14
s150	1.61
s151	1.64
s152	1.27
s153	1.87
s154	1.32
s155	2.24
s156	1.24
s157	1.31
s158	1.88
s159	0.49
s160	1.53
s161	1.67
s162	1.38
s163	1.08
s164	0.67
s165	1.34
s166	0.90
s167	1.35
s168	0.76
s169	0.79
s170	1.04
s171	1.50
s172	0.99
s173	1.86

Table 4 (continue)

Strain NO.	Growth at 96h (OD660)
s174	1.04
s175	1.58
s176	1.04
s177	1.58
s178	1.93
s179	2.27
s180	1.86
s181	2.00
s182	NT
s183	0.98
s184	1.79
s185	0.95
s186	2.11
s187	0.82
s188	1.16
s189	1.70
s190	2.04
s191	1.30
s192	2.21
s193	1.19
s194	2.08
s195	1.85
s196	1.73
s197	1.32
s198	1.76
s199	1.03
s200	0.70
s201	1.11
s202	1.13
s203	1.78

Table 4 (continue)

Strain NO.	Growth at 96 h (OD660)
s204	1.29
s205	3.54
s206	1.32
s207	0.86
s208	0.75
s209	1.55
s210	1.53
s211	1.27
s212	1.19
s213	1.54
s214	1.59
s215	1.40
s216	2.02
s217	1.54
s218	1.47
s219	1.34
s220	1.27
s221	NT
s222	1.93
s223	0.69
s224	1.55
s225	1.22
s226	1.81
s227	NT
s228	0.88
s229	1.12
s230	1.01
s231	1.40
s232	1.36
s233	3.02
s234	2.25
s235	1.06

Table 4 (continue)

Strain NO.	Growth at 96h (OD660)
s236	1.31
s237	0.94
s238	2.36
s239	2.36
s240	1.38
s241	1.60
s242	1.69
s243	0.87
s244	2.60
s245	1.58
s246	0.84
s247	1.53
s248	1.24
s249	NT
s250	1.38
s251	1.43
s252	NT
s253	1.00
s254	1.94
s255	NT
s256	0.89
s257	1.45
s258	2.42
s259	0.54
s260	0.98
s261	1.22

Table 5 Growth and ethanol production by halotolerant yeasts in molasses medium contained 17% of sodium chloride, at 40°C.

Strain No.	Cell concentration(OD660)			Ethanol concentration		
	0 h	72 h	72-0 h	0 h	72 h	72-0 h
031	0.54	0.73	0.19	0.50	0.40	NEP
062	0.49	6.31	5.82	2.0	5.89	3.89
065	0.85	4.22	3.37	0.3	5.97	5.67
086	1.18	3.06	1.88	1.90	1.50	NEP
090	1.66	5.75	4.09	0.50	5.80	5.30
092	0.69	6.24	5.55	0.30	6.60	6.30
116	0.98	3.49	2.51	0.15	4.20	4.05
126	2.83	9.04	6.22	0.10	16.99	16.89
130	1.16	4.43	3.27	0.99	11.58	10.59
149	0.60	2.05	1.45	1.29	1.70	1.15
167	1.49	3.02	1.53	1.51	1.26	NEP
174	0.62	0.48	NG	0.40	0.15	NEP
192	2.07	2.16	0.09	1.06	2.51	NEP
229	1.38	4.59	3.21	1.26	6.29	5.03
246	0.70	4.16	3.46	0.50	6.60	6.10
252	1.29	0.95	NG	0.80	0.20	NEP
254	1.30	1.37	0.07	1.81	1.35	NEP
257	0.26	1.36	1.10	0.69	3.75	3.06
270	1.21	0.72	NG	0.20	0.04	NEP
288	1.84	1.11	NG	0.10	0.20	0.10
290	0.62	0.92	0.34	0.40	0.20	NEP
294	0.56	0.55	NG	0.99	0.76	NEP
295	0.65	0.80	0.15	0.20	0.20	NEP
326	1.06	3.09	2.03	0.10	4.00	3.90
344	1.06	1.96	0.09	0.63	2.09	1.46
346	1.15	1.76	0.61	0.51	17.75	17.24
352	0.83	0.73	NG	0.30	0	NEP
356	1.86	6.90	5.04	1.51	17.75	16.24
357	0.72	1.93	1.21	1.26	1.11	NEP
365	0.61	1.32	0.71	0.30	6.80	6.50
366	1.03	5.27	4.24	0.80	9.02	8.22

Table 5 (continue)

Strain No.	Cell concentration(OD660)			Ethanol concentration		
	0 h	72 h	72-0 h	0 h	72 h	72-0 h
370	0.64	3.67	3.03	1.0	12.32	11.32
394	0.29	4.65	4.36	0	7.40	7.40
396	0.54	0.60	0.06	0.40	0.20	NG
399	0.92	0.69	NG	0.50	0.10	NG
402	0.99	0.84	NG	0.50	0.30	NG
403	0.66	1.00	0.34	0.30	0.10	NG
405	0.66	0.92	0.26	0.50	0.50	NG
406	1.11	0.84	0.27	0.40	0.10	NG
407	0.62	0.73	NG	0.40	0.20	NG
408	0.66	1.66	1.00	0.40	2.00	1.60
409	1.08	0.99	NG	0.80	1.11	0.31
s010	0.68	1.76	1.08	1.22	2.79	1.57
s011	1.40	1.85	0.45	1.05	1.85	0.80
s068	1.18	2.28	1.10	1.70	1.19	0.51
s069	0.71	0.72	0.01	0.86	0.80	NEP
s205	0.61	0.26	NG	0.20	0	NEP
s233	0.98	1.10	0.12	0.20	0	NEP
s244	0.65	0.35	NG	0.20	0.20	NEP

NG = No growth

NEP = No ethanol production

Table 6 Ethanol production and growth of *S. cerevisiae* M30 in molasses medium contained 5 % of sodium chloride , at 40°c.

Time (h)	Cell concentration (OD 660)	Ethanol concentration (g/l)
0	0.92	2.51
6	1.10	2.68
12	1.17	3.49
18	1.54	5.15
24	0.95	NT
30	1.85	NT
36	1.47	9.98
42	1.49	10.04
48	1.73	NT
54	1.21	13.81
60	1.07	16.40
66	1.35	15.53
72	0.74	NT

* Intitial sugar concentration was equivalent to 23.6 % of sucrose

Table 7 Ethanol production and growth of *S. cerevisiae* AM12 in molasses medium contained 5 % of sodium chloride , at 40°c.

Time (h)	Cell concentration (OD 660)	Ethanol concentration (g/l)
0	0.97	2.96
6	0.38	3.28
12	0.60	3.56
18	1.21	3.62
24	1.49	3.39
30	0.61	4.16
36	NT	4.22
42	1.00	4.20
48	0.75	5.34
54	0.70	5.86
60	1.18	6.07
66	1.12	6.11
72	1.87	7.16

* Initial sugar concentration was equivalent to 23.4 % of sucrose

Table 8 Ethanol production and growth of *S. cerevisiae* Sc90 in molasses medium contained 5 % of sodium chloride, at 40°c.

Time (h)	Cell concentration (OD 660)	Ethanol concentration (g/l)
0	0.70	2.23
4	1.00	2.65
8	0.80	2.80
12	0.85	2.28
16	0.64	2.76
20	1.17	2.77
24	0.51	2.74
30	0.81	2.66
36	0.37	2.95
42	0.58	2,85
48	0.52	3.29
54	0.40	2.87
60	0.43	2.97
66	0.59	3.17
72	0.29	NT

* Initial sugar concentration was equivalent to 22 % of sucrose

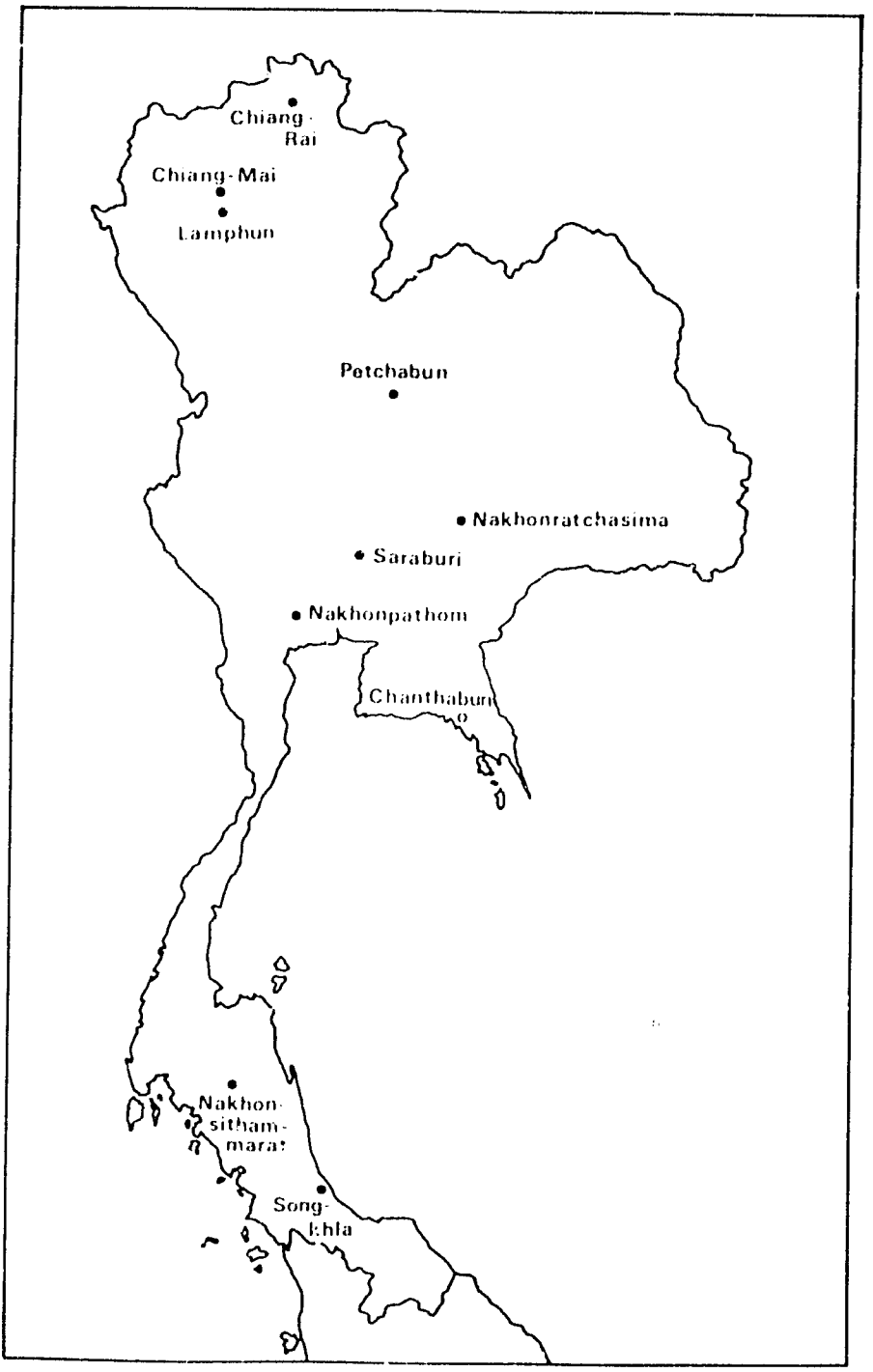

FIG. 1 MAP OF THAILAND SHOWING ADDITIONAL SITES OF SAMPLE COLLECTION

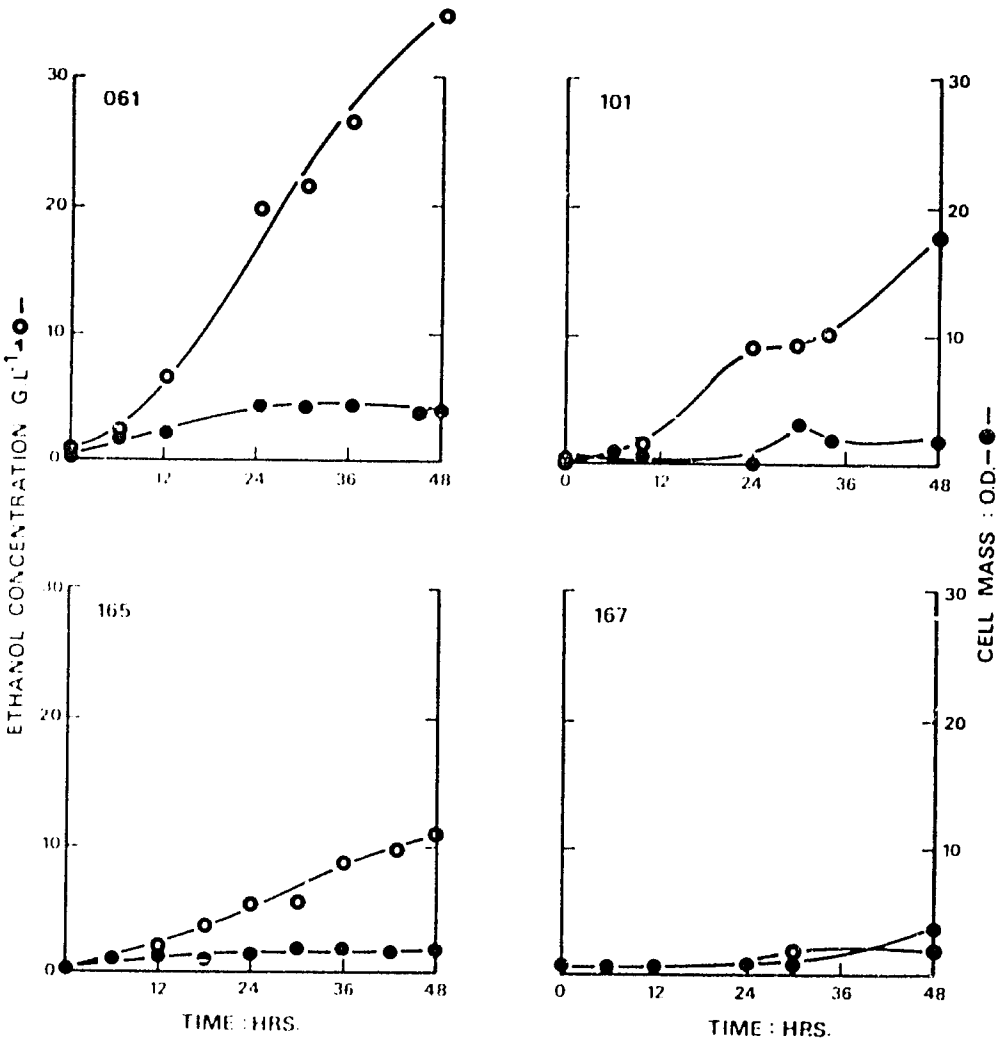

FIG. 2 TIME COURSE OF ETHANOL PRODUCTION AND GROWTH BY HALOTOLERANT YEASTS IN MOLASSES MEDIUM CONTAINING 5% NaCl AT 40°C.

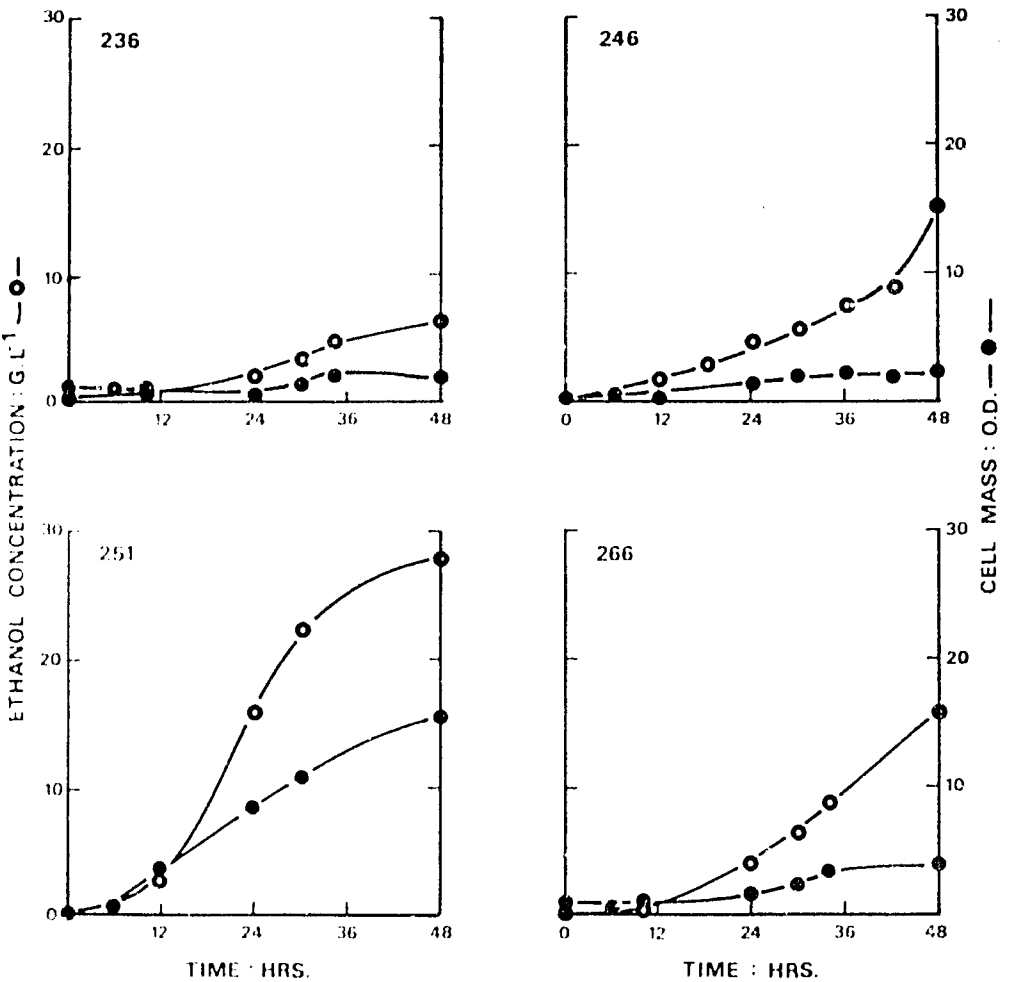

FIG. 2 CONTINUED.

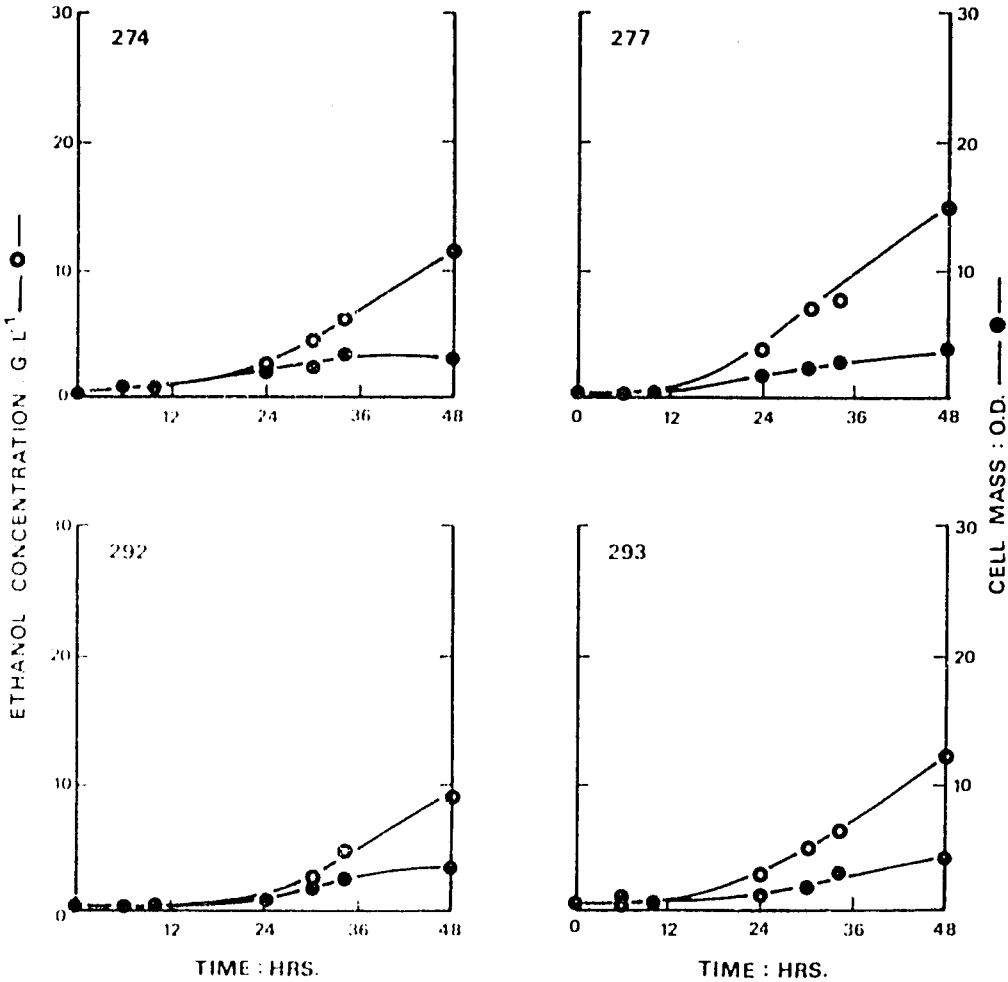

FIG. 2 CONTINUED.

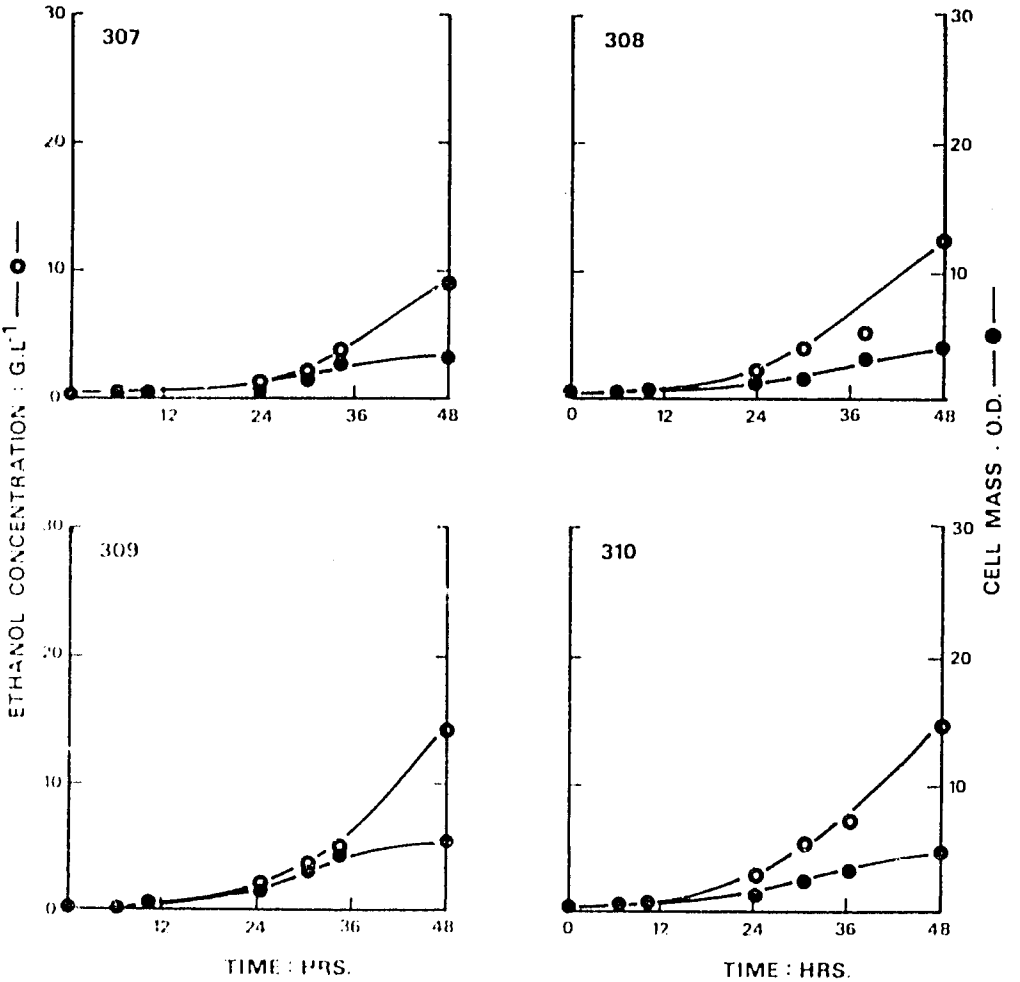

FIG. 2 CONTINUED.

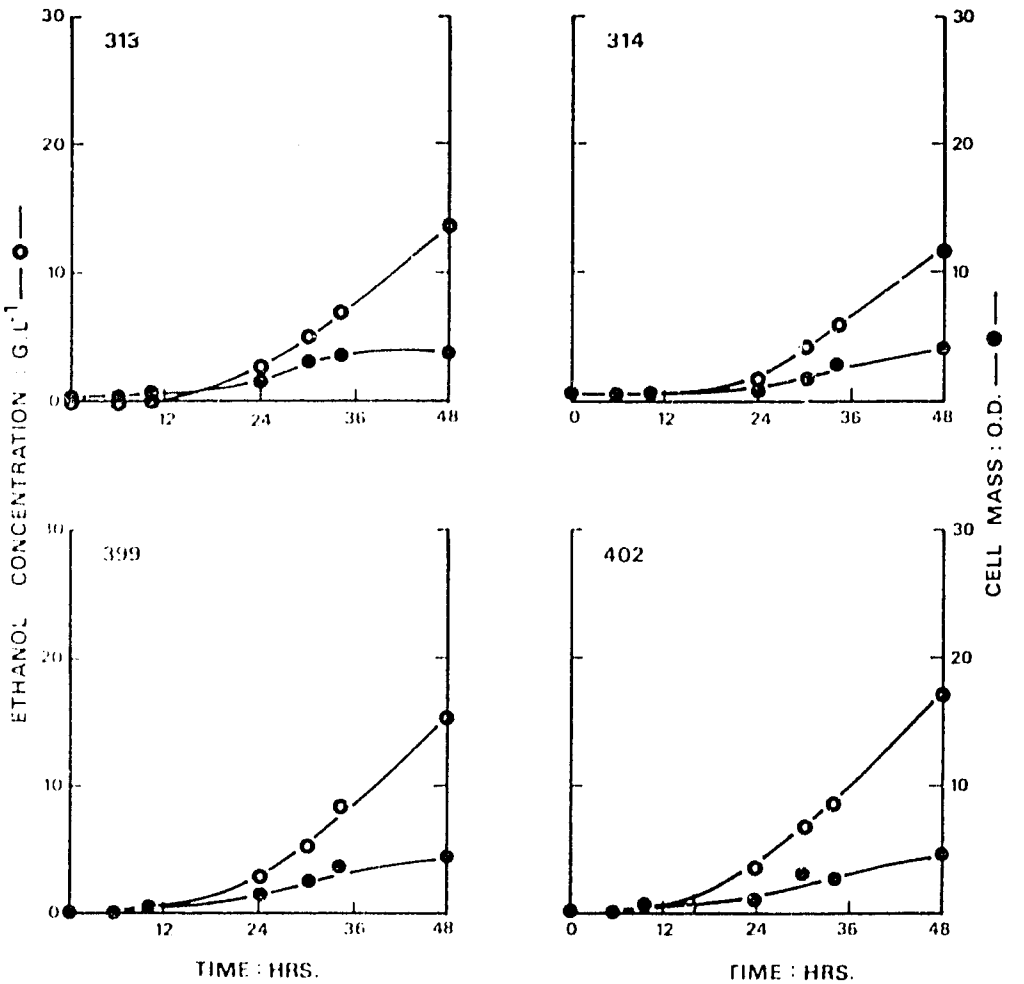

FIG. 2 CONTINUED.

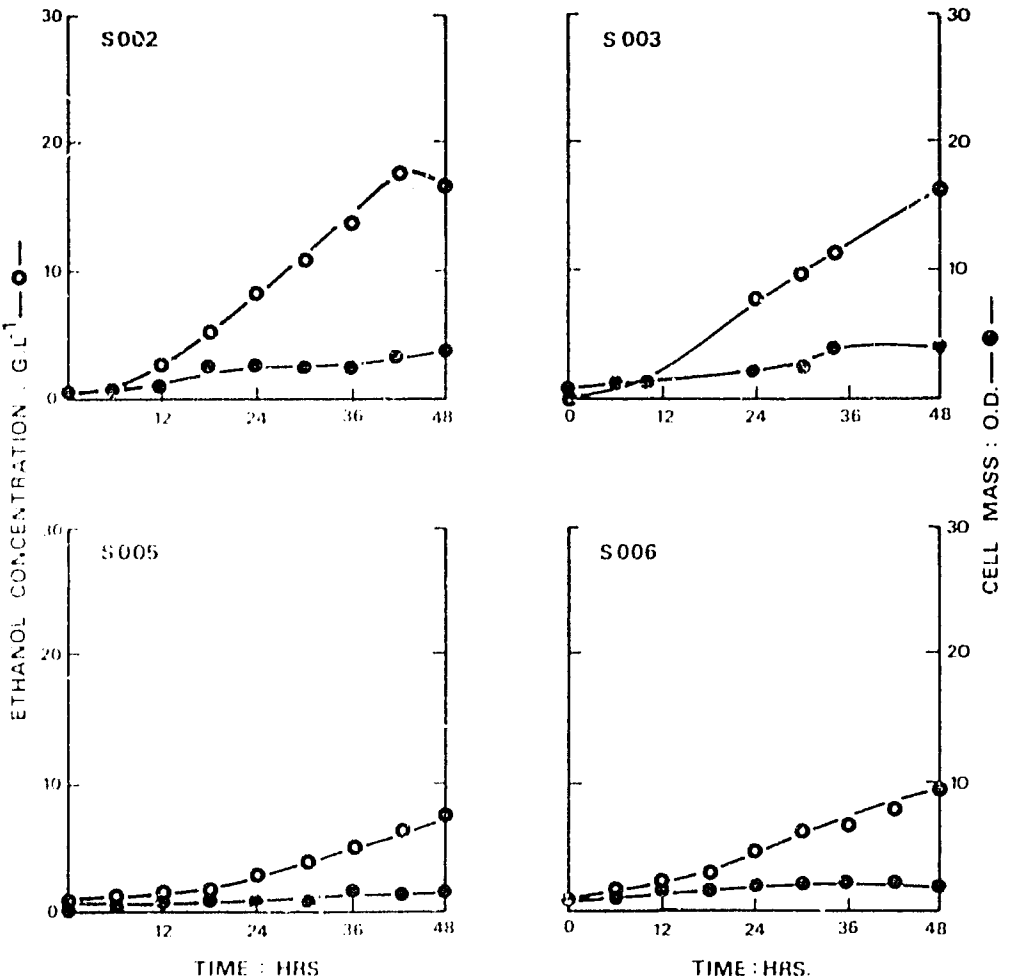

FIG. 2 CONTINUED.

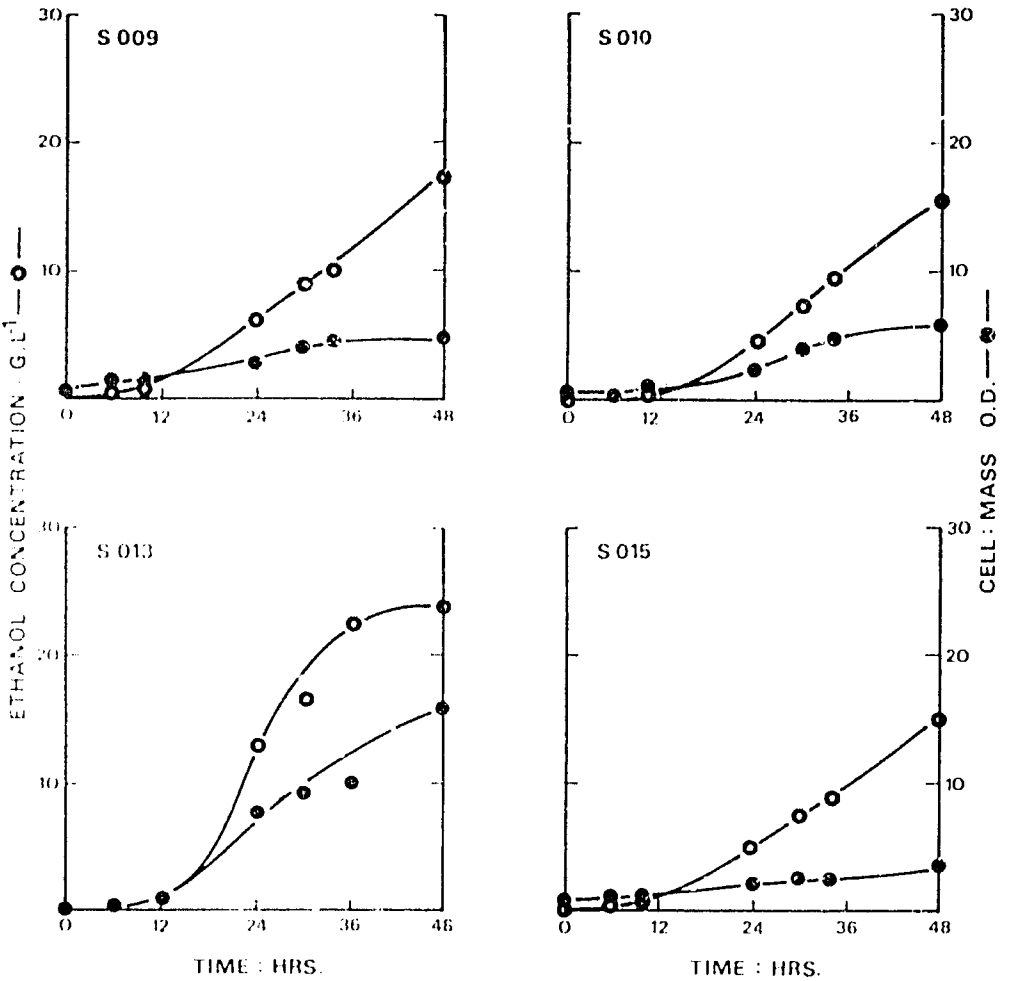

FIG. 2 CONTINUED.

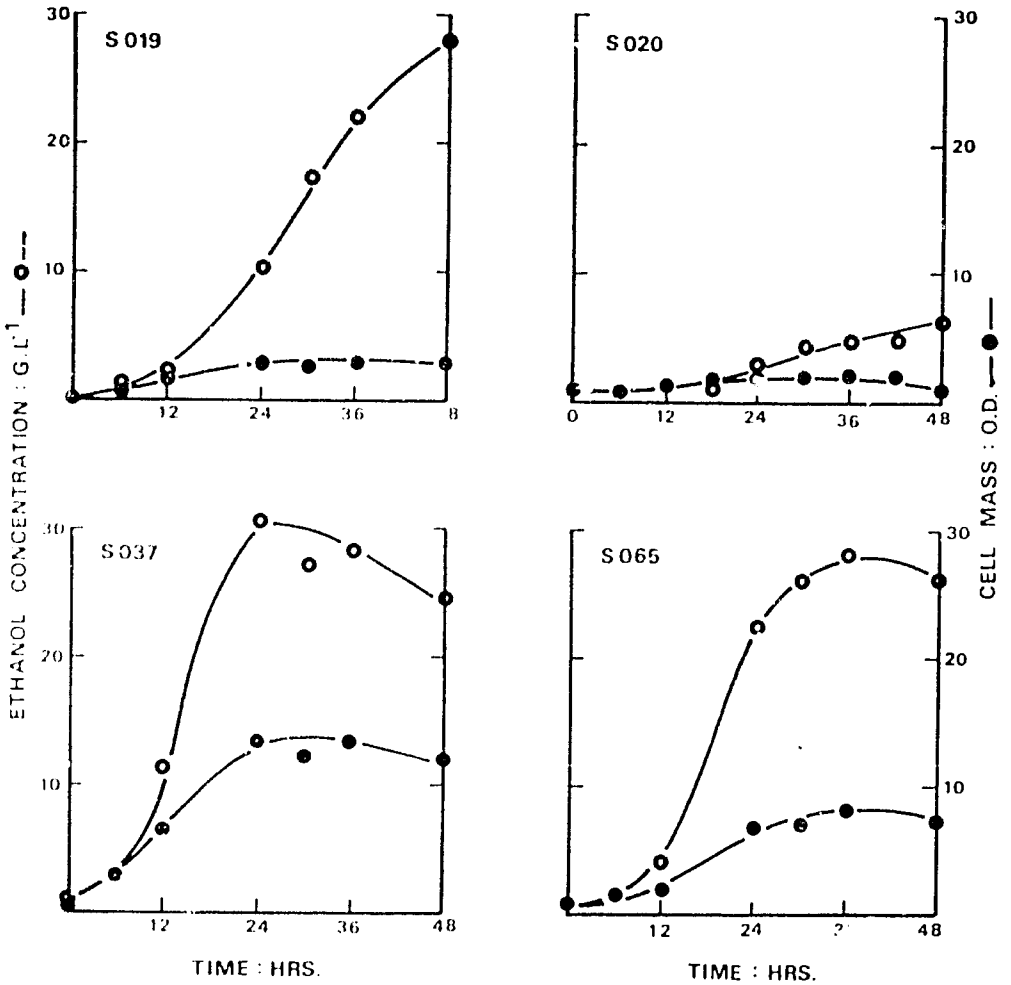

FIG.2 CONTINUED.

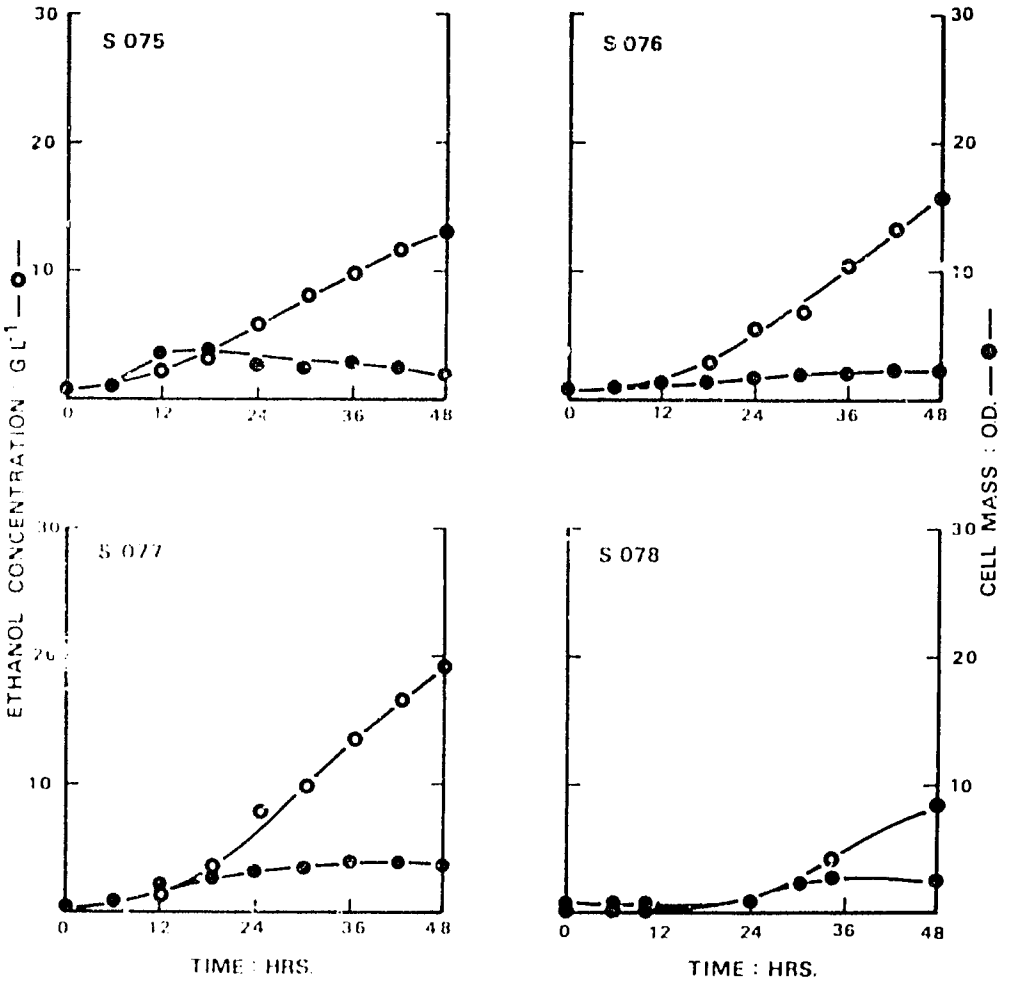

FIG. 2 CONTINUED.

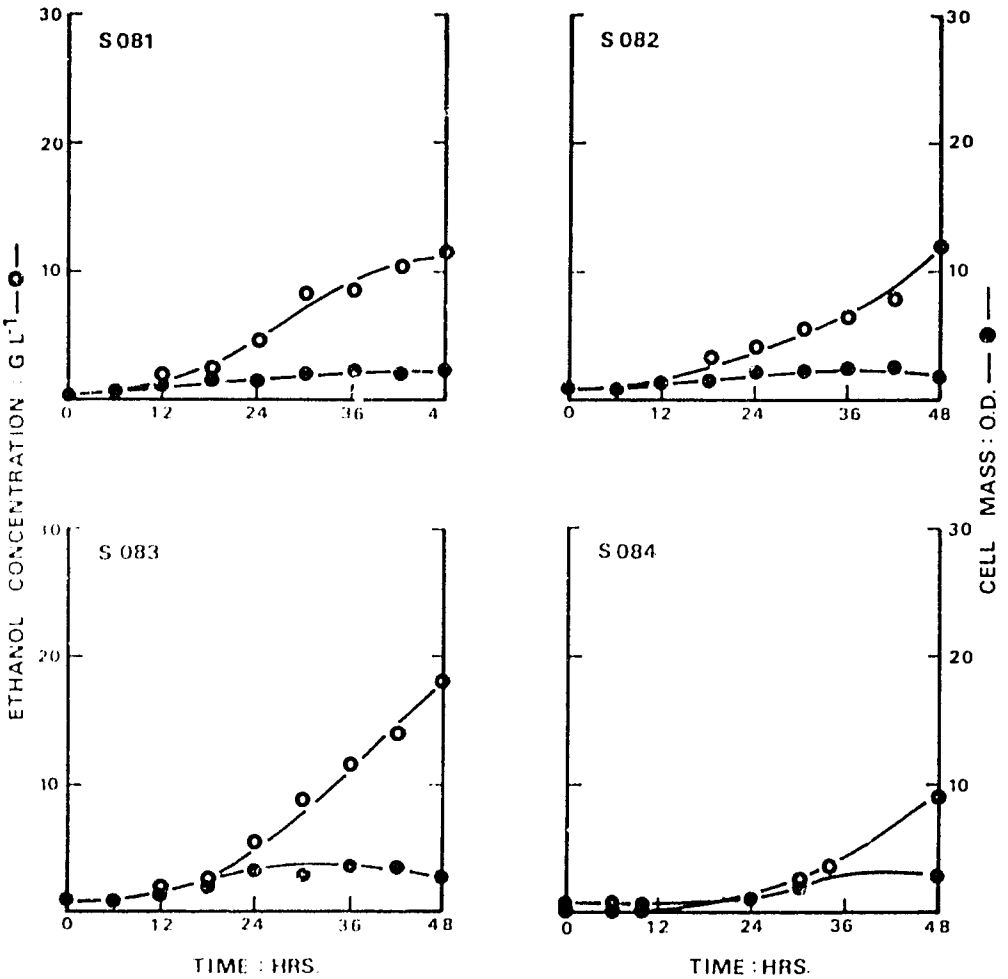

FIG. 2 CONTINUED.

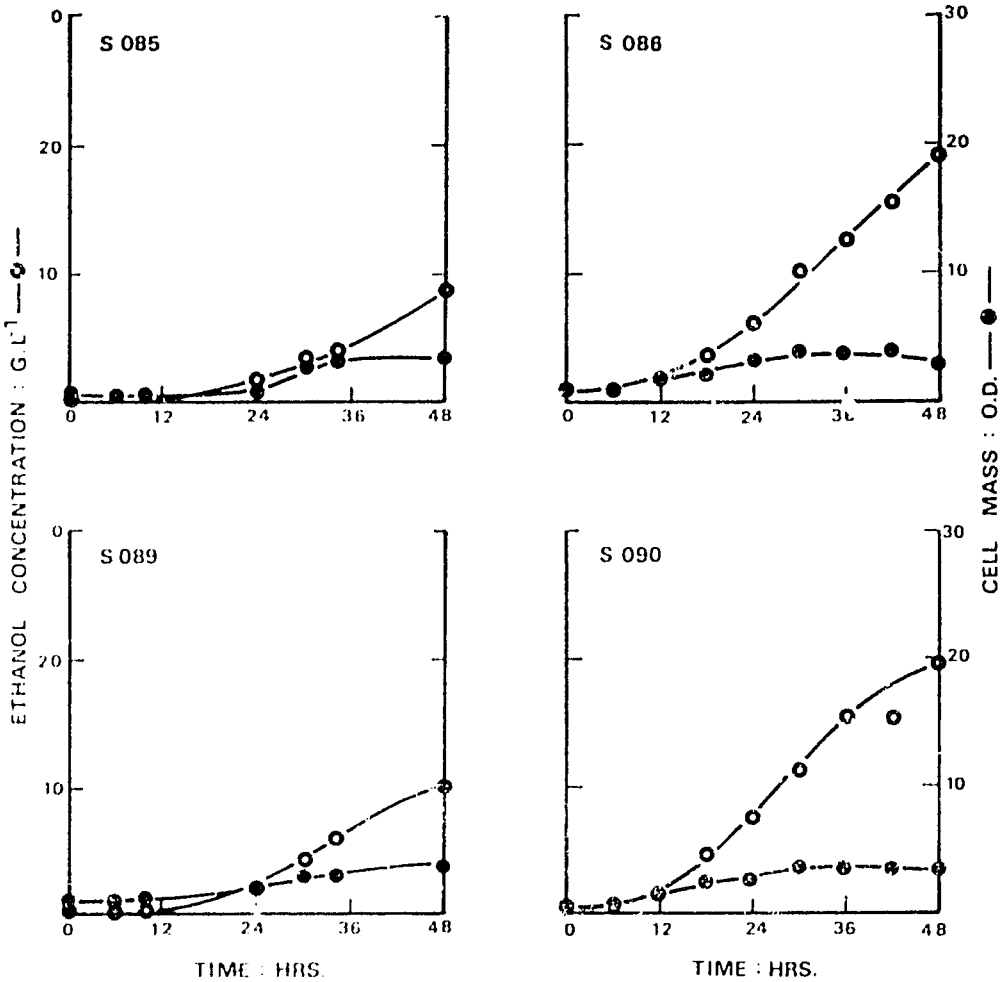

FIG. 2 CONTINUED.

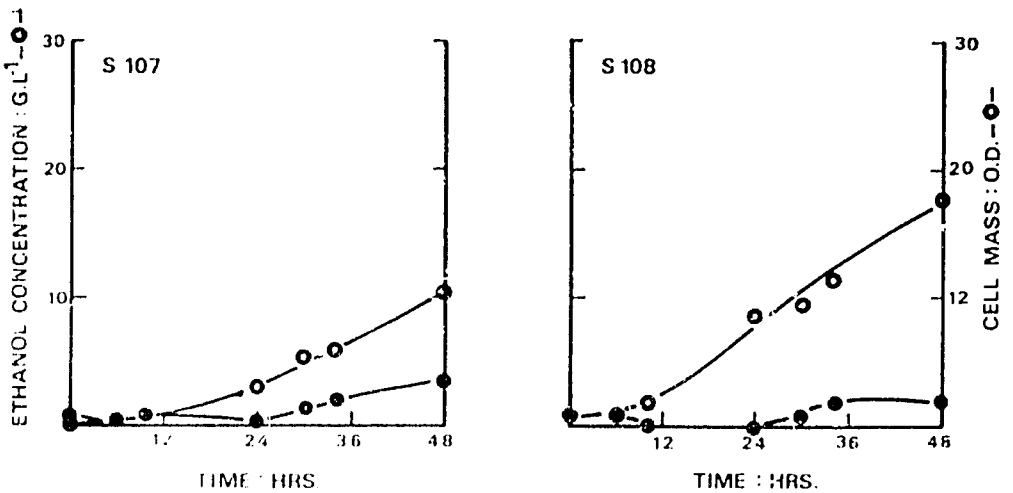

FIG. 2 CONTINUED.

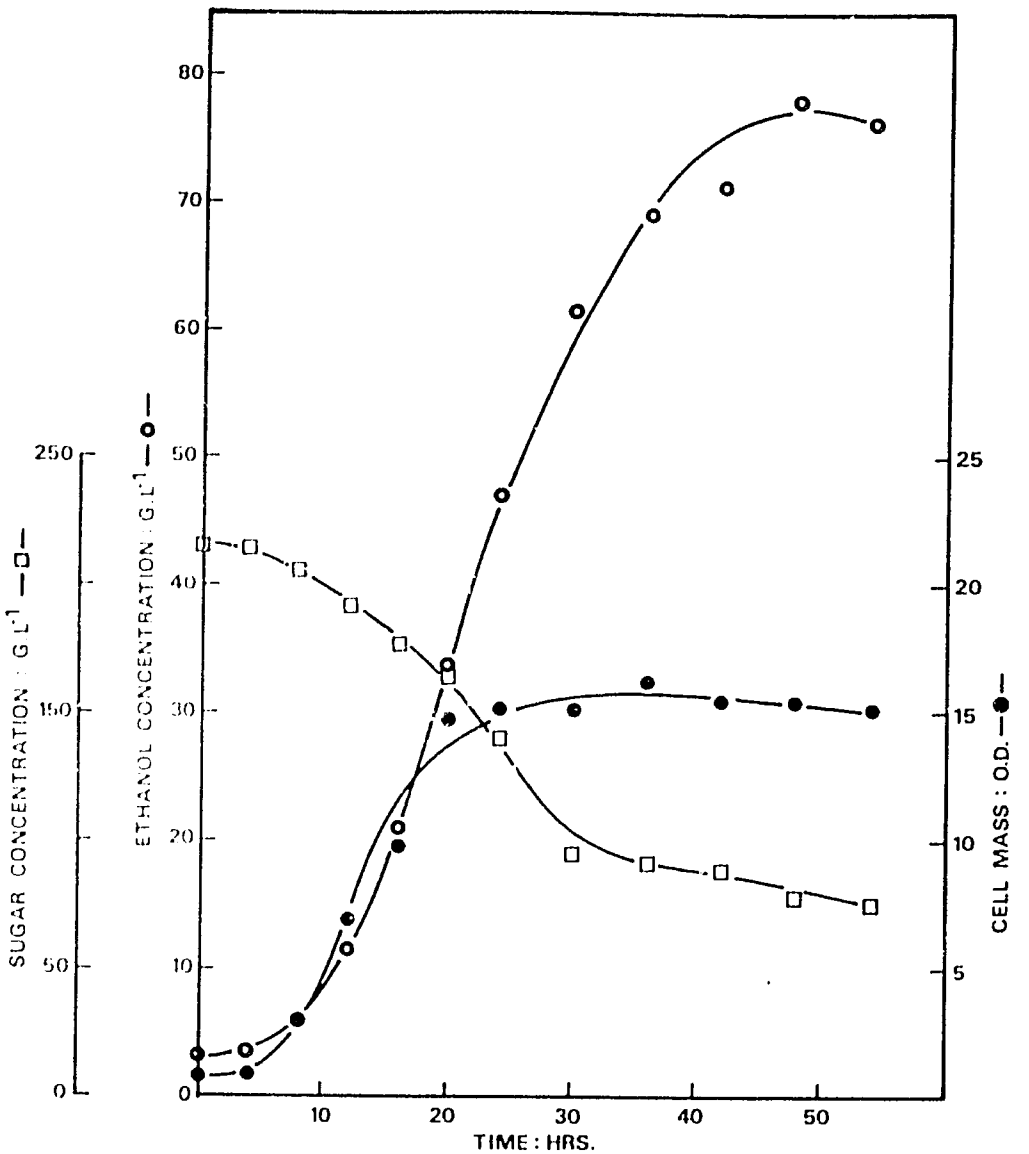

FIG. 3 TIME COURSE OF ETHANOL PRODUCTION AND GROWTH BY INDUSTRIAL YEAST STRAIN, M 30, IN 5 LITER FERMENTER CONTAINING MOLASSES MEDIUM AT 40°C.

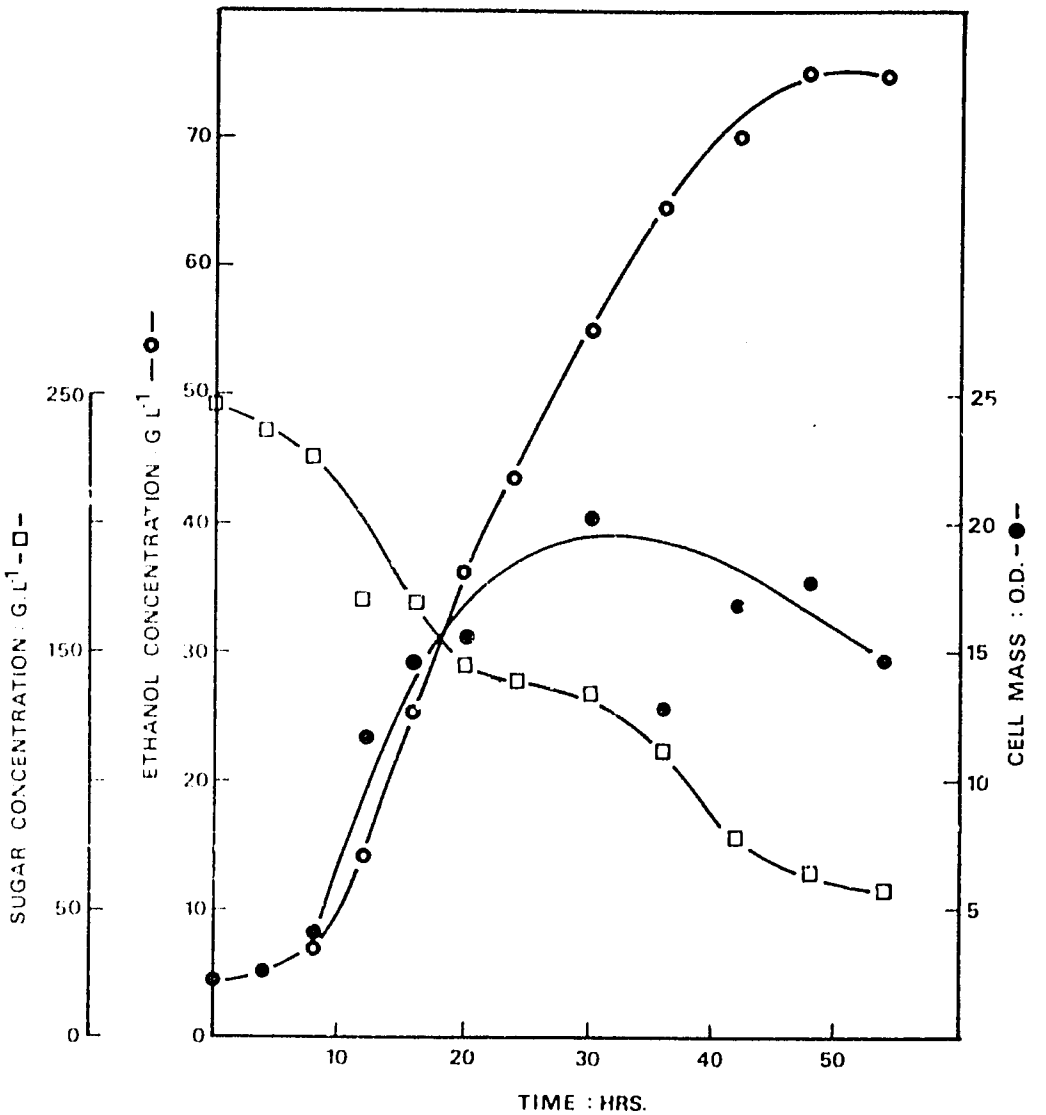

FIG.4 TIME COURSE OF ETHANOL PRODUCTION AND GROWTH BY INDUSTRIAL YEAST STRAIN, AM 12, IN 5 LITER FENTER CONTAINING MOLASSES MEDIUM AT 40°C.

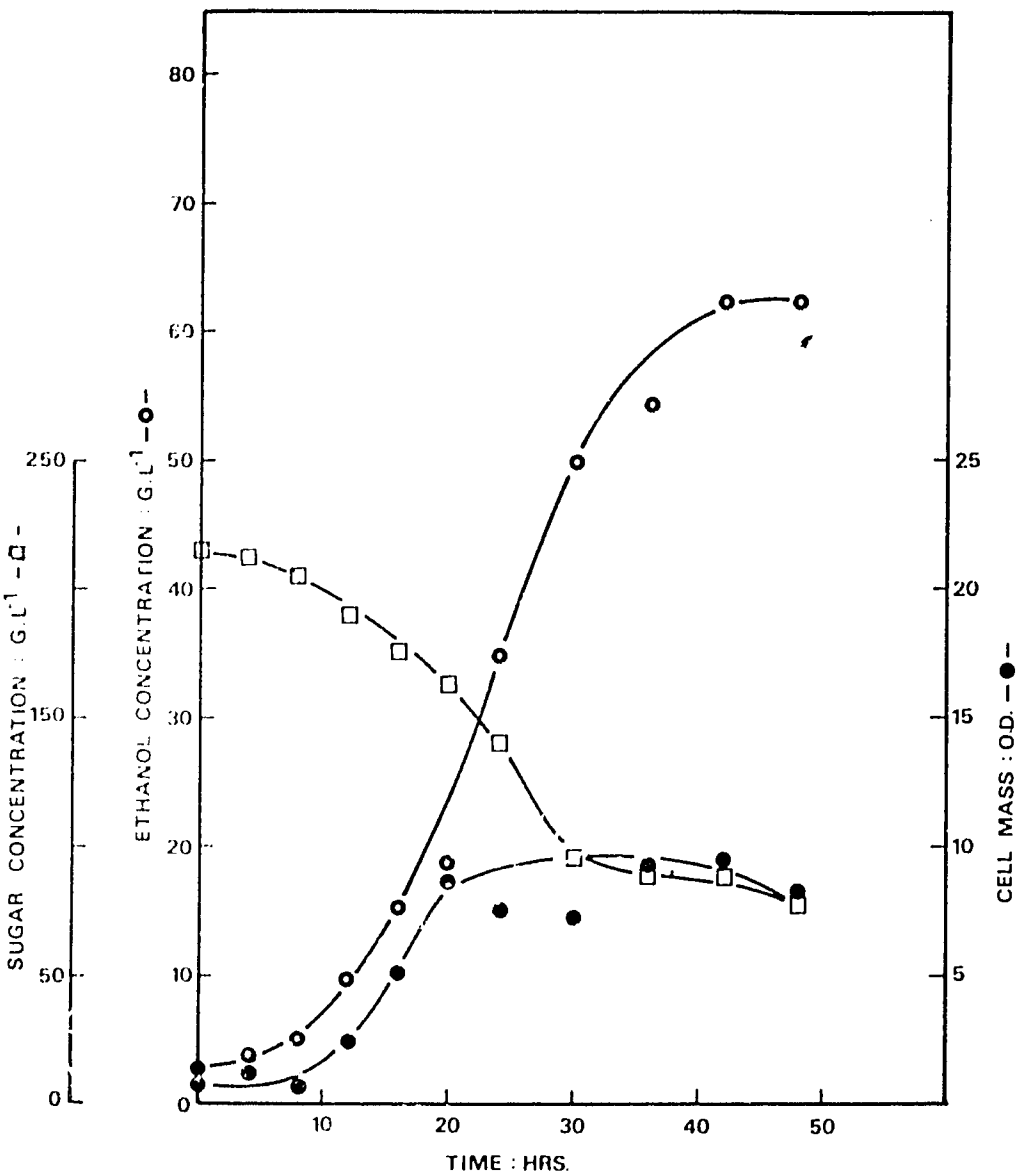

FIG. 5 TIME COURSE OF ETHANOL PRODUCTION AND GROWTH BY INDUSTRIAL YEAST STRAIN, Sc90, IN 5 LITER FERMENTER CONTAINING MOLASSES MEDIUM AT 40°C.

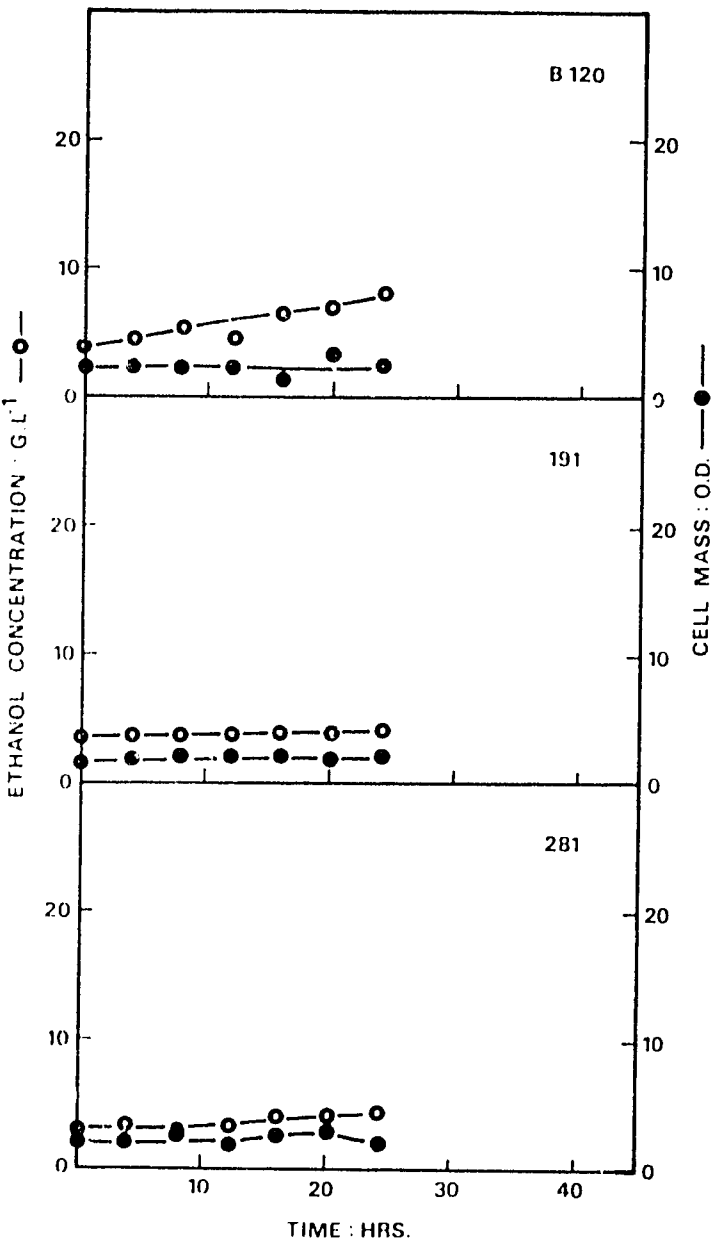

FIG. 6 TIME COURSE OF ETHANOL PRODUCTION AND GROWTH BY INDUSTRIAL YEAST STRAINS IN 5 LITER FERMENTER CONTAINING 5% NaCl MOLASSES MEDIUM AT 40°C.