A recombinant *Saccharomyces cerevisiae* strain for efficient conversion of lactose in salted and unsalted cheese whey into ethanol

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For utilization of lactose in salted and unsalted cheese whey, intergeneric protoplast fusion between lactose nonfermenting, salt-tolerant *Saccharomyces cerevisiae* ATCC4126 and lactose fermenting *Kluyveromyces lactis* CBS683 was carried out. The fusion process gave rise to new hybrid yeast strains that revealed higher significant DNA contents than parental strains. The recombinants showed growth on either lactose or sucrose. The ethanol yields by some recombinants were 5.55% from sweet whey and 4.66% from salted whey containing up to 6% sodium chloride compared to 4.15 and 2.86% for parental *K. lactis* CBS683, respectively.

1 Introduction

Manufacturing of cheese from either whole or skim milk produces a greenish fluid known as whey. Disposal of cheese whey is a major problem for the dairy industry. World output of cheese whey is increasing in line with the growth of cheese industry which has shown a sustained increase in output of about 2% *per annum* [1]. Also, long-term land disposal of whey can cause environmental pollution problems as reported by several authors [2, 3]. The nitrogen in cheese whey is water-soluble and may be subject to leaching into ground water, thus becoming a threat to human and animal health. However, aerobic yeast fermentation of cheese whey can be used to reduce its pollution potential as well as to produce single-cell protein (SCP) [4]. Ben-Hassan and Ghaly [5] reported that aerobic fermentation of cheese whey using the yeast *Kluyveromyces fragilis* was successful in reducing the total chemical oxygen demand (COD) by 42%. The major problem to use lactose for alcohol production is the inability of *Saccharomyces cerevisiae* to ferment lactose since it lacks both lactose permease and β-galactosidase activity [6], but can produce ethanol. The relatively small number of other microorganisms which are capable of fermenting lactose usually give a low yield of ethanol, *e.g., Kluyveromyces* sp., are able to carry on sustained alcoholic fermentation [7, 8].

In 1974, 32.5 billion pounds of whey were produced, over half of which was disposed as waste [9]. This represents a pool of 1.6 billion pounds of lactose, which if converted into usable food products, could be of sizable monetary value to the dairy industry. Since lactose is the major component of whey solids [10], it must be taken into consideration in any effective utilization process. Utilization of unsalted whey as a fermentation substrate to produce biomass and organic acids was described earlier by Rogosa *et al.* [11]. In Egypt, most of produced milk is converted to cheese after being salted with sodium chloride of up to 6%. Thus, the major problem of using salted whey as a fermentation medium has been the fact that relatively few organisms are able to ferment the high energy content (lactose) in the presence of sodium chloride.

Several processes have been proposed for whey utilization, largely based on fermentation by microorganisms, *K. fragilis* [12], *Candida* sp., *Lactobacillus* sp., *etc.* [8, 13]. Also, the yeast *Candida pseudotropicalis* was used for ethanol production from cheese whey [14, 15]. Lactose is one of the several least expensive carbon sources for ethanol production. Although attempts were made to use lactose for alcohol production since 1940 [11], the major problem is the inability of *S. cerevisiae* to ferment lactose. The relatively small number of other microorganisms which are capable of fermenting lactose usually give a low yield of ethanol, *e. g., K. fragilis* [16]. However, it has been shown that only a fraction of the available lactose was converted to alcohol, possibly due to an alcohol inhibition [17].

Protoplast fusion technology allows an alternative solution of the problem; both lactose permease and β-galactosidase of *Kluyveromyces* sp. have been fused and expressed in *S. cerevisiae* [18]. Farahnak *et al.* [19] used the protoplast fusion technique to construct hybrids between the auxotrophic strain of *S. cerevisiae* having high ethanol tolerance and the auxotrophic strain of lactose-fermenting *K. fragilis* isolated by ethylmethane sulfonate mutagenesis. The fusants obtained were prototrophic and capable of assimilating lactose and producing ethanol in excess of 13% v/v. In order to construct a yeast strain having high ethanol tolerance together with good lactose fermentation ability, the protoplast fusion using *S. cerevisiae* STV8a and *K. fragilis* CBS397 was tried [20]. Spencer *et al.* [21] used the protoplast fusion technique to prepare yeast hybrids with improved properties. Using polyethylene glycol, it was possible to obtain a *S. cerevisiae* strain that ferments lactose which has been produced by transferring β-galactosidase and lactose permease in successive fusions from *K. lactis*. By using a fusant strain prepared from *S. cerevisiae* and *K. fragilis*, the ethanol fermentation capacity and ethanol tolerance was increased up to 8.0% compared with the parental *K. fragilis* [22].

The present work was carried out aiming to prepare a new hybrid yeast strain from crossing of *S. cerevisiae* ATCC4126 and *K. lactis* CBS683 of high efficiency to convert lactose in salted cheese whey into ethanol.

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Abbreviations: MIC, minimum inhibitory concentration; **MM**, minimal media; **OMM**, osmotically stabilized minimal medium; **ONPG**, *O*-nitrophenyl-β-D-galactopyranoside; **YEPD**, yeast extract peptone dextrose medium

Keywords: Cheese whey / *Kluyveromyces lactis* / Protoplast fusion / Recombinants / *Saccharomyces cerevisiae*

2 Materials and methods

2.1 Microorganisms

The yeast strains used were *S. cerevisiae* ATCC4126 obtained from the American Type Culture Collection (Maryland, USA); *K. lactis* CBS683 was provided by Centraalbureau voor Schimmel Cultures, The Netherlands. The yeasts were propagated regularly by subculturing on yeast extract peptone dextrose medium (YEPD) and incubation at 28 °C.

2.2 Chemicals

2.2.1 Systemic fungicides

Sodium phenyl phenate was obtained from BDH Chemicals (Poole, England); acriflavin, 5-fluorouracil and pimiracin are products of Sigma Chemicals (St. Louis, MO, USA). Cycloheximide is a product of Aldrich Chemical Company (Milwaukee, WI, USA). Benlate (Benomyl) was obtained from Dupont (Wilmington, DE, USA). *p*-Chloronitrobenzene, virdazan, carboxin and imazilil were gifts from the Department of Botany, University of Nottingham, Nottingham, NG72RD, UK.

2.2.2 Lytic enzymes

Zymolase-20T was a gift from Kirin Brewery Pharmaceutical Laboratory (Takasaki Lab., Gunma, Japan). Cellulase CPD30 was obtained from Rhone-Poulenc ABM Brewing & Food Group (Cheshire, England). β-Glucuronidase (EC 3.21.31) type H-5 from *Helix pomatia* and Novozym 234 (lysing enzymes) from *Trichoderma harzianum* were obtained from Sigma Chemical. Acid dye fuchsin was obtained from BDH Chemicals and *O*-nitrophenyl-β-D-galactopyranoside (ONPG) was provided by Aldrich-Chemie (Deisenhofen, Germany). All other chemicals used were obtained from Sigma Chemical.

2.3 Media

Botham and Ratledge medium (CM) was prepared according to Botham and Ratledge [23]. Yeast extract peptone dextrose (YEPD) was prepared as recommended by Ohta *et al.* [24]. Sporulation medium is a Fowell's medium [25] with a little modification. Regeneration minimal media (MM): Peberdy and Ferenczy's minimal was used for regeneration of protoplasts according to [26]. Peberdy and Ferenczy's regeneration osmotically stabilized minimal medium (OMM) is the previous minimal medium except it was stabilized by the addition of 0.6 M KCl. Wickerham's regeneration minimal medium was prepared according to [27]. Fermentation medium (FM) was prepared according to Ballesterose *et al.* Cheese whey medium (CWM) was prepared according to Mahoney *et al.* [29].

2.4 Methods

2.4.1 Introduction of genetic markers into yeast genome using systemic fungicides

Acriflavine (a mixture of 2,8-diamino-10-methyl acridinium chloride and 2,8-diamino acridine), cycloheximide (3-[2,3,5-dimethyl-2-oxo-cyclohexyl]-2-hydroxyethylglutarimide), imazilil (1-β-[alkyloxy]-2,4-chlorophenetylimidazole phosphate) and pimiracin were used for labeling different yeasts. Acriflavine was dissolved in distilled water, while the other inhibitors were dissolved in methanol to give a final concentration of various fungicides from 1–100 µg/mL of YEPD medium. This was carried out to determine the minimum inhibitory concentration (MIC) of each inhibitor. The resistant mutants of the investigated yeast strains towards different fungicides were obtained by subculturing the yeasts at two to five times the MIC each systemic fungicide.

2.4.2 Isolation of resistant mutant spores

Sporulation was performed by subculturing the yeast strains under consideration at a density of 10^6 on slants of 9 mL at pH 10.6. The slants were incubated at 25 °C until the appearance of tetrads followed by dissection of asci to obtain single spores.

2.4.3 Protoplast isolation and fusion

Protoplast isolation was achieved first by growing resistant mutant spores of each strain until early log phase. A 10^6 of each spore was inoculated in YEPD liquid medium separately at 28 °C for 20 h under shaking at 200 rpm. Protoplast fusion was carried out according to Peberdy and Ferenczy [26]. The germinated spores were collected by centrifugation at 5000 rpm for 10 min. The pellet was suspended in 0.6 M KCl containing 0.1% of 2-mercaptoethanol, then centrifugation was repeated. The digestion of yeast cell walls was performed by the addition of snail enzyme solution which consisted of 5 mg/mL of lyophilized snail enzyme in 0.6 M KCl containing 0.1% of 2-mercaptoethanol. The process was completed by mild shaking at 75 rpm and 30 °C for 90 min. The protoplasts were collected by centrifugation at 1500–2000 rpm for 10 min, washed at least three times with 0.6 M KCl. The supernatant was removed and 5 mL of 0.3 M $CaCl_2$ were added and the protoplasts were resuspended in 0.6 M KCl. The fusion was carried out by mixing equal numbers of protoplasts (10^6 of each strain). The process was completed by the addition of 2 mL of 35% polyethylene glycol (PEG) 6000 and 0.1 M $CaCl_2$. The mixture was incubated at room temperature for 20 min. The fused cells (aggregated protoplasts) were washed with 0.6 M KCl followed by centrifugation at 2000 rpm. The pellet obtained was washed with 0.3 M $CaCl_2$, followed by centrifugation at 2000 rpm. The resultant pellet was diluted with 0.6 M KCl. Aliquots of the fusion product were subcultured embedded in a regeneration OMM, supplemented with vitamins and 0.6 M KCl, followed by laying another layer of OMM containing the systemic fungicides. The protoplasts were incubated at 28–30 °C and the fusion products were transferred from OMM regeneration medium to Wickerham's MM after 14 days. Complementary colonies that appeared on OMM fusion plates were subjected to successive passages on MM before further analysis.

2.4.4 Measurement of DNA

Nucleic acid extraction was achieved according to Juni [30], whereas, the DNA content was determined using the diphenyl amine reagent method according to Burton [31].

2.4.5 Nuclear staining and measurement of nuclear diameter

Parental strains and diploids were fixed and stained according to Robinow [32]; the nuclear diameter was measured according to the method of Clutterbuck and Roper [33].

2.4.6 Alcohol fermentation and measurement

Fifty mL of the fermentation medium or cheese whey medium used for ethanol production were transferred into 100 mL fermentation bottles with a rubber stopper equipped with hypodermic syringe. The bottles were inoculated with a cell suspension of 10^6 cells/mL and incubated by shaking at 150 rpm at 30 °C for 96 h. Ethanol concentration in culture filtrates was measured using a Shimadzu GC-8A (Shimadzu, Japan) gas chromatograph equipped with a flame ionization detector (FID). A column (2 m by 2 mm) packed with chromosorb W/AW (60–80 mesh) impregnated with 15% carbowax 20 M was used. The temperature of the detector and the injector was maintained at 220 °C and the column oven was operated isothermally at 130 °C. A sensitivity of 10^2 and a range of 32 resulted in a stable baseline.

2.4.7 Measurement of β-galactosidase activity

The cell mass was suspended in phosphate buffer, pH 7, and 2% toluene was added for cell autolysis, according to Mahoney *et al.* [29]. Cell-free extract was subjected to the measurement of β-galactosidase activity and the absorbance of the nitrophenolate was measured at 420 nm [12]. One ONPG unit of the enzyme activity is defined as the amount of enzyme which liberates 1 μM *O*-nitrophenol per min under the conditions described. The statistical analysis was carried out using the randomized complete analysis.

3 Results and discussion

3.1 Introduction of genetic markers into yeasts

For subsequent studies concerning selection of fusants on OMM plates, genetic markers were introduced into parental yeast strains including *S. cerevisiae* ATCC 4126 and *K. lactis* CBS683. For that purpose, each of the two parental strains was grown separately in the presence of each antifungal inhibitors at different concentrations. This was carried out aiming to determine the MIC of each fungicide. The use of antifungal inhibitors as genetic markers was recommended earlier [34], as the use of auxotrophic markers was criticized because they may result in a strain of inferior activity than parental strains [35]. The results obtained indicated that *S. cerevisiae* ATCC4126 was more sensitive towards cycloheximide as its MIC was detected at 5 μg/mL, which is lower than the MIC of other antifungal inhibitors tried. Table 1 indicates also that *K. lactis* CBS683 was more sensitive to imazilil than others and the MIC of this inhibitor towards that yeast strain was detected at 10 μg/mL. To isolate resistant mutants of above yeasts towards any inhibitor, the yeasts were grown at two- to fivefold the concentration of the MIC, however, the use of fivefold concentration is not recommended as it might cause chromosomal aberration [36]. Therefore, resistant yeast mutants were obtained by growing the yeasts at twofold the MIC of the specified inhibitors.

Table 1. MIC[a] of some systemic fungicides that effectively inhibited the growth of *S. cerevisiae* ATCC4126 and *K. lactis* CBS683

Inhibitor (μg/mL)	*S. cerevisiae* ATCC4126	*K. lactis* CBS683
Cycloheximide	5	70
Carboxine	>100	>100
Imazilil	>100	10
Acriflavin	62.5	50
Benomyl	75	–
Pimiracin	5.5	–
Mycostatin	35	–

a) Average of three replicates.

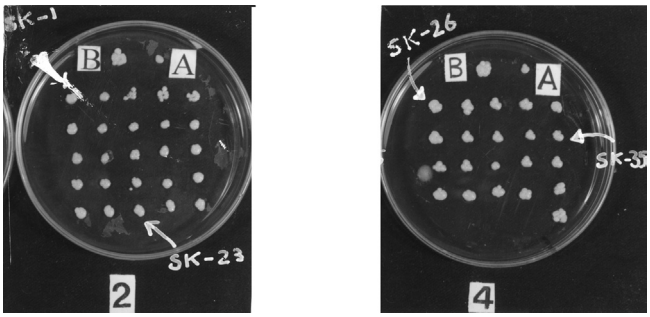

Figure 1. Growth pattern of parental *S. cerevisiae* ATCC4126, *K. lactis* CBS683 and some selected fusants from SK-1 to SK-45 on YEP containing 10% lactose. A = ATCC4126; B = CBS683; 2 = SK-1 to SK-25 on YEP containing 10% lactose; 4 = SK-25 to SK-45 on YEP containing 10% lactose.

The incidence of intergeneric protoplast fusion between *S. cerevisiae* ATCC4126 and lactose fermenting *K. lactis* CBS683 was confirmed first by examining the colonies grown on OMM plates containing 10 μg/mL cycloheximide and 20 μg/mL imazilil. Accordingly, a number of 57 colonies out of hundreds were chosen on the basis of their vigorous growth in the presence of the two inhibitors. For subsequent investigations the above colonies were subjected to different measurements including nuclear diameter, DNA content of hybrid per parent, the growth on lactose or sucrose as sole carbon source and the β-galactosidase activity as proofs for the incidence of the fusion event. Further proofs in that respect included the capacity of the new hybrid strains to produce ethanol from lactose and the growth at different concentrations of sodium chloride. Subculturing of selected colonies on YEP medium containing 10% lactose as sole carbon source at 30 °C revealed faster and dense growth of the fusant strains than *S. cerevisiae* ATCC4126 (Fig. 1). Growing the selected colonies on YEP medium containing 10% sucrose indicated the inability of some fusants such as SK-48 and SK-49 to grow on sucrose. Consequently, later fusants are proposed to be the result of fusion between two nuclei of *K. lactis*, while the others are *S. cerevisiae* with transformed lactose system from *K. lactis*.

3.2 Identification of fusion event between *S. cerevisiae* ATCC4126 and *K. lactis* CBS683

Among the 57 fusants that were selected from the fusion plates, 18 showed a high DNA content of hybrid per parent and a high nuclear diameter compared to each of the two parental strains. The use of above criteria as proofs for the incidence of protoplast fusion could be substantiated by previous results [37, 38]. As shown in Table 2, the DNA content of hybrid per parent *K. lactis* CBS683 varied between 0.85 and 1.87. Whereas, such values were found to vary between 1.29 and 2.23 calculated per parental *S. cerevisiae* ATCC4126. Previous studies [19, 39–41] have indicated that a DNA content of 2–4 times that of parent is a fixed proof for the incidence of

Table 2. Some properties of some selected hybrids as compared with parental *S. cerevisiae* ATCC4126 and *K. lactis* CBS683 grown on 10% lactose at 30 °C for 96 h

Nuclei	DNA content µg/10^6 protoplasts of hybrid per parent		Nuclear diameter (mm)
	CBS683	ATCC4126	
CBS683			0.00068 c
ATCC4126			0.00079 c
SK-1	1.65 b	1.75 c	0.00140 a
SK-23	1.63 b	1.53 e	0.00120 b
SK-26	1.47 c	1.66 d	0.00130 ab
SK-35	1.87 a	1.81 b	0.00130 ab
SK-48	0.85 f	1.29 f	0.00120 b
SK-49	1.25 d	1.84 b	0.00120 b
SK-53	1.52 c	2.23 a	0.00120 b

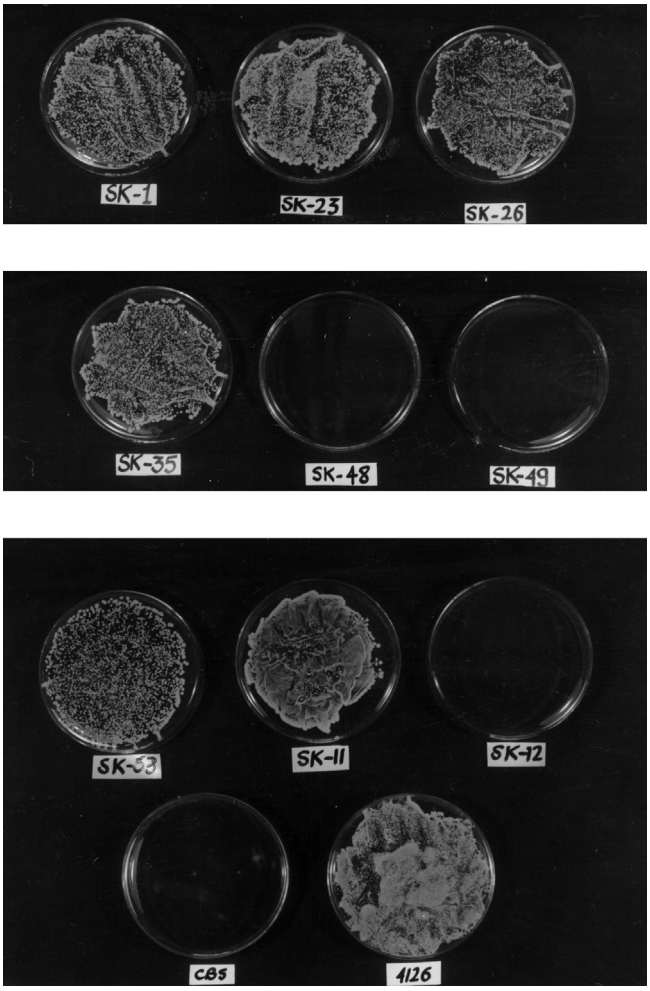

Figure 2. Growth pattern of parental *S. cerevisiae* ATCC4126, *K. lactis* CBS683 and some selected fusants on YEP containing 10% sucrose at 30 °C for 48 h.

nuclear fusion. Whereas, it was reported [42] that the incidence of intergeneric fusion between *S. cerevisiae* and *Candida* sp. is realized on exceeding a ratio of 1.6 the DNA content of hybrid per parent.

Analysis of data of Table 2 by the randomized complete design indicated that new hybrid yeast strains had significantly higher DNA content than parental *K. lactis* CBS683. However, the strain SK-35 showed the highest significant DNA content of hybrid per parent (1.87) than the DNA content of other hybrids. Similarly, hybrid SK-53 revealed the highest significant DNA content (2.23) compared with *S. cerevisiae* ATCC4126 and other hybrids compared with above parent. The nuclear diameter of different hybrids obtained from crossing *S. cerevisiae* and *K. lactis* varied between 0.0012 mm and 0.0014 mm calculated on the basis of hybrid per each of the two parental strains. Statistical data indicated that all hybrid strains investigated had higher significant nuclear diameter than each of the two parental strains. Whereas, strain SK-1 showed the highest significant increase in nuclear diameter that reached 0.0014 mm. From above findings and discussion, it could be concluded that the new hybrid yeast strains described are prognated from the fusion event between *S. cerevisiae* ATCC4126 and *K. lactis* CBS683.

Table 3. β-Galactosidase activity, lactose consumption and the properties of some selected fusants with elevated ethanol yields grown at 30 °C in fermentation medium containing 10% lactose for 96 h as compared to parental strains CBS683 and ATCC4126

Nuclei	β-Galactosidase activity total units/106 protoplasts	Lactose consumption % w/v	Ethanol % v/v
CBS683	763.00 i	76.80 c	4.56 bcd
ATCC4126	30.48 i	2.31 d	0.23 c
SK-1	569.00 f	86.40 a	6.17 cd
SK-23	505.00 g	81.70 b	5.97 cd
SK-26	773.00 c	76.10 c	7.42 ab
SK-35	789.40 a	77.00 c	8.06 a
SK-48	782.00 b	77.30 c	6.97 abc
SK-49	385.00 h	77.00 c	5.66 d
SK-53	750.00 e	82.25 b	6.28 cd

The relationship between β-galactosidase activity, lactose consumption and ethanol yield by some fusant strains is shown in Table 3. The results denote that *S. cerevisiae* ATCC4126 exhibited low β-galactosidase activity and consequently low ethanol yield from lactose. Contrary to that, parental *K. lactis* CBS683 gave an ethanol yield of 4.56%. The β-galactosidase activity of different hybrids varied between 385 and 789 U/10^6 protoplasts as compared with the mean value of the enzyme activity of the two parents CBS683 and ATCC4126 that reached 396.74 U/10^6 protoplasts. The statistical analysis shown in the above table indicates that hybrid strain SK-35 revealed the highest significant increase in β-galactosidase activity and ethanol yields compared with the mean value of the two parental strains and different hybrids investigated. However, lactose consumption was not consistent with the β-galactosidase activity as hybrid strain SK-1 indicated the highest significant increase in that respect than the mean value of the two parental strains and other hybrids.

The intracellular lactase system including β-galactosidase, galactoside permease and galactoside transacylase was used earlier by several authors as confirmatory tools for the incidence of the transfer of lactase gene by protoplast fusion between donor lactase microorganism *K. fragilis* and recipient strain *S. cerevisiae*. Spencer *et al*. [21] reported that transfer of β-galactosidase and lactose permease in successive fusion from *K. lactis* to *S. cerevisiae* was achieved. Similarly, Woo *et al.* [20] used the β-galactosidase activity as a proof for the

Table 4. % v/v Ethanol by *K. lactis* CBS683 and some selected hybrids grown in cheese whey medium containing different concentrations of sodium chloride grown at 30 °C for 96 h

Nuclei	Sodium chloride concentration (%)						
	0 (sweet whey)	2	4	6	8	10	Mean
	% v/v Ethanol						
CBS683	4.15 gh	3.85 i	3.83 i	3.55 jkl	2.86 n	2.16 p	3.40 D
SK-1	5.29 b	4.69 d	4.66 d	4.66 d	2.57 o	2.60 o	4.08 A
SK-23	4.65 d	4.48 e	4.13 h	4.14 h	2.59 o	2.17 p	3.69 C
SK-26	5.28 b	4.66 d	4.10 h	3.60 jk	3.20 m	2.60 o	3.91 B
SK-35	5.55 a	3.53 kl	3.45 kl	1.77 q	1.65 q	1.71 q	2.94 G
SK-48	4.90 c	4.05 h	3.20 m	2.62 o	2.62 o	2.14 p	3.26 E
SK-49	4.15 gh	4.31 f	4.09 h	3.70 ij	3.46 kl	3.40 l	3.85 B
SK-53	4.30 fg	3.43 l	3.50 kl	2.58 o	2.66 o	2.13 p	3.10 F

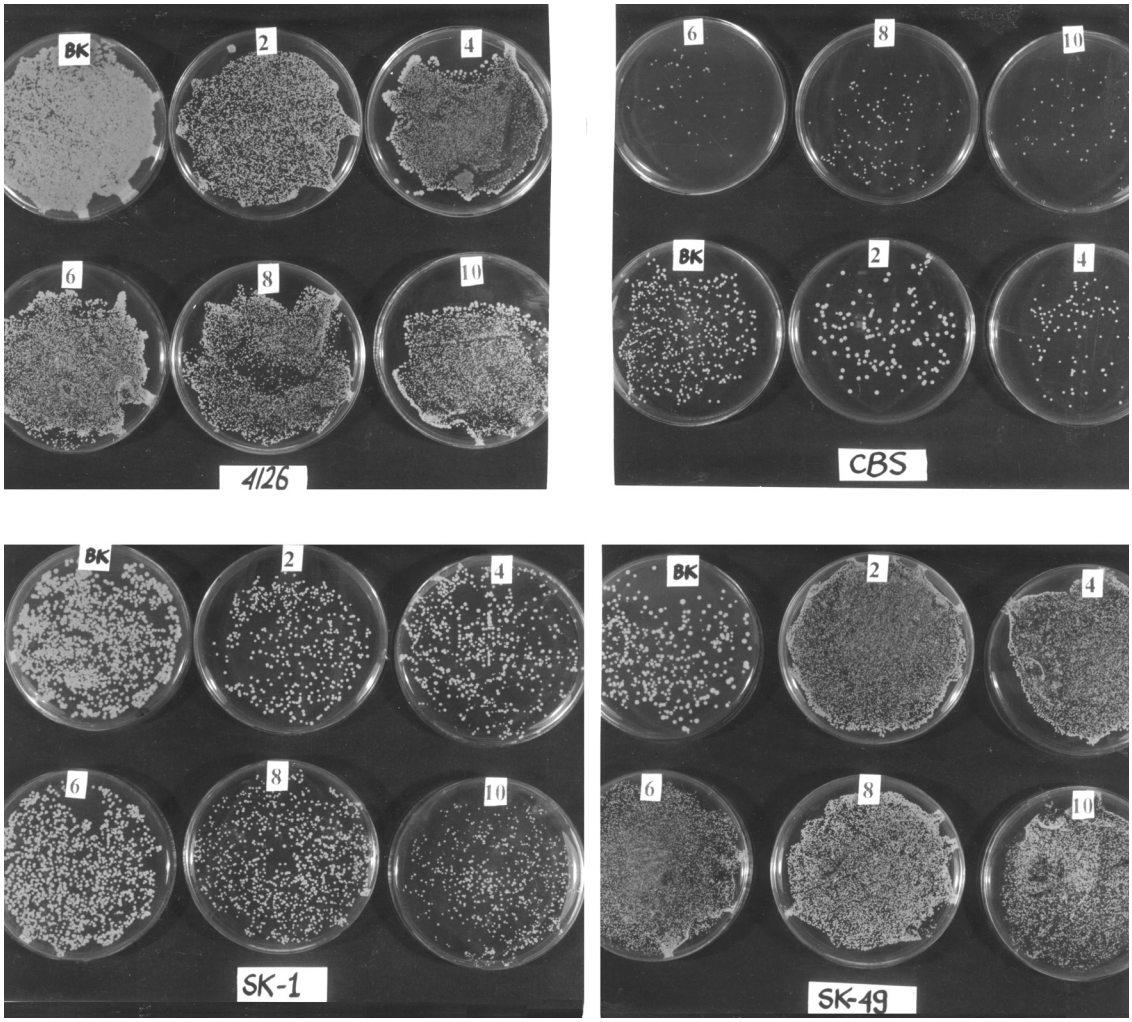

Figure 3. Growth pattern of parental *S. cerevisiae* ATCC4126, *K. lactis* CBS683 and fusants SK-1 on YEP containing different concentrations of sodium chloride at 30 °C for 48 h. BK = control. 2, 4, 6, 8 and 10% NaCl.

incidence of recombination between *S. cerevisiae* and lactose fermenting *K. fragilis*.

3.3 Conversion of lactose in sweet and salted cheese whey into ethanol by recombinant *S. cerevisiae*

The major objective of the present study is to assess the feasibility of using the fusant yeast strains prepared by nuclear fusion of parental *S. cerevisiae* ATCC4126 and *K. lactis* CBS683 to convert lactose in salted whey into ethanol. Thus, the influence of sodium chloride on the growth of the fusants and parental strains was investigated on solid medium containing 0–10% sodium chloride. Figure 3 indicates that parental *S. cerevisiae* revealed more and dense growth in the presence of up to 10% sodium chloride than parental *K. lactis*. While, fusants SK-1

and SK-49 revealed growth similar to parental *S. cerevisiae* which is also greater than that of other fusants. Table 4 illustrates ethanol yields from salted and unsalted cheese whey produced by some selected hybrid strains and parental *K. lactis* CBS683. Using sweet cheese whey, all hybrid strains except SK-49 showed significantly higher ethanol yields than the parent strain, whereas hybrid SK-35 revealed the significantly highest yield compared to other hybrids. In salted cheese whey, hybrid strain SK-1 gave the significantly highest ethanol yields that reached 4.69, 4.66 and 4.66% in the presence of 2, 4 and 6% sodium chloride as compared with *K. lactis* that gave 3.85, 3.83 and 3.55% ethanol, respectively. At high sodium chloride concentrations (8 and 10%), hybrid yeast strain SK-49 produced the significantly highest ethanol yield (3.46 and 3.4%) than other hybrids and parental strain *K. lactis* that was found to be 2.86 and 2.16% at 8 and 10% sodium chloride, respectively. Generally, the average of ethanol yield by hybrid SK-1 at all sodium chloride concentrations used was greater than other hybrids and parental yeast strain. Moreover, hybrid SK-49 was more tolerant to high sodium chloride concentrations 8 and 10% than other hybrids. Above findings clearly indicate that new yeast hybrids are more efficient in converting lactose in sweet and salted cheese whey into ethanol than other recombinant strains prepared earlier [22] by nuclear fusion of *S. cerevisiae* and *K. fragilis*.

4 Concluding remarks

1. Protoplast fusion between lactose nonfermenting, sodium chloride tolerant *S. cerevisiae* ATCC4126 and lactose fermenting *K. lactis* CBS683 gave rise to a large number of fusant strains.

2. The new hybrid yeast strains exhibited higher DNA content and higher nuclear diameter than parental yeast strains. They revealed also growth on either lactose or sucrose indicating the incidence of nuclear fusion between the two parents.

3. Hybrids prognated from nuclear fusion of *S. cerevisiae* ATCC4126 and *K. lactis* CBS683 revealed significantly high ethanol yields from lactose in synthetic medium as well as from sweet and salted cheese whey of up to 10% NaCl.

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