

Isolation and Identification of *Saccharomyces cerevisiae var boulardii* and its Uses as a Probiotic (*in vitro*)

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ABSTRACT

The study included isolation and identification of *Saccharomyces cerevisiae var. boulardii* from fresh and dried fruits such as (fig, grape, apricot, lychees, mangosteen) which were available in local markets in Erbil city. Fifty isolates of the yeast were obtained depending on morphological, physiological and biochemical tests. The antibacterial activity of the isolated yeasts against pathogenic bacteria *Salmonella enteric sub spp. enterica* (Typhimurium) *in vitro* was determined by Well's Diffusion method. Most of the isolated yeasts showed antibacterial activity against tested bacterium, the highest diameter of inhibition zone was (16 mm) for isolates (S.b32). The ability to tolerate different concentrations of bile salt and gastric acids were also studied. Ten strains of the isolated yeast had the highest inhibitory activity and were used to study physiological properties (bile salt concentration and gastric acidity) which were (S.b6, S.c1, S.b2, S.c36, S.b13, S.b28, S.b32, S.b22, S.b48, and S.b5). The result showed that the isolate (S.b32) was more resistant than other strains when exposed to bile salt and gastric acids.

Keywords: *Saccharomyces cerevisiae*, probiotic.

Saccharomyces cerevisiae var. boulardii

(*in vitro*)

Saccharomyces cerevisiae var. boulardii

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Saccharomyces cerevisiae var. boulardii

50

Salmonella enteric sub spp. enterica (Typhimurium)

.(Well Diffusion)

(S.b32) S.Typhimurium (16)

(S.b6, S.c1, S.b2, S.c36, S.b13, S.b28, S.b32, S.b22, S.b48, and S.b5)

(S.b32)

.*Saccharomyces cerevisiae*, probiotic :

INTRODUCTION

Sacch. cerevisiae var boulardii possesses many properties that make it a potential probiotic survives transit through the GI tract, its temperature optimum is 37°C, both *in vitro* and *in vivo*, it inhibits the growth of a number of microbial pathogens. However, *Sacch. cerevisiae var boulardii* belongs to the group of simple eukaryotic cells and, it thus differs from bacterial probiotics that are prokaryotes. *Sacch. cerevisiae var boulardii*; was effective in the treatment of acute diarrhea in children (Cetina and Siemo, 1999; Zeng *et al.*, 2003). And critically ill tube fed patients. *Sacch. cerevisiae var. boulardii* release polyamines which help in repairing mucous membranes. These polyamines increase the activity of short chain fatty acids (SCFA) and disaccharide enzymes (lactase, maltase, sucrase). Polyamines stimulate the repair of intestinal cells and the growth of colonic mucosa (Buts *et al.*, 1994; Edwards-Ingram *et al.*, 2007). The beneficial properties of *Saccharomyces spp.* strains are well documented (Rodrigues *et al.*, 1996; Martins *et al.*, 2005). In addition to their nutritive value (e.g. provision of vitamins of the B group), probiotic yeasts are generally resistant to gastrointestinal passage and are resistant to most antibiotics. Yeast preparations have also been successfully applied, in combination with antibiotics, to treat *Clostridium difficile* -related diarrhea commonly known as antibiotic associated diarrhea. Probiotic *Saccharomyces spp.* may also help to re-establish a normal gut function, after long term antibiotic therapy (McFarland *et al.*, 1994; Katarzyna and Alina 2010). The recent study aimed to study the isolation and identification of local isolates the yeast *Sacch. cerevisiae* from different dried and fresh fruits sources in Erbil city; it also studies the ability of using these isolated yeasts as a probiotics (Edwards-Ingram *et al.*, 2007; DeVrese and Schrezenmeir, 2008).

MATERIALS AND METHODS

Isolation of the yeasts

One hundred and twenty Samples of fresh and dried fruits were collected from different sources that were available in local markets in Erbil city such as lychee, grape, mangosteen, apricot and fig. One gram of fruit sample, blended and mixed with sterilized nine milliliters peptone water then a serial of dilution was made until 10^{-5} , one ml of the last dilution was cultured by pour plate method using sabouraud dextrose agar (Oxide) pH 5.5 (Deak and Beuchat, 1987). Morphological appearance and microscopic examination of the colonies were done after staining them with simple stain (methylene blue).

Identification of isolated yeast:

As mentioned by (Lodder, 1974; Barnett *et al.*, 1985; Barnett *et al.*, 1990), biochemical tests were done such as; Carbon source fermentation, Nitrogen source utilization, acid production from fermented sugars, Ester production, Urea hydrolysis were tested to isolate yeasts.

Cyclohexamide(Actidione):

Two solutions were prepared (Frazier, 1985)

Solution No.1: Prepared by dissolving 10mg actidione in 90ml distilled water and then sterilized by filtration (Millipore filter 0.22 μ m).

Solution No.2: Prepared by dissolving 6.7gm of Yeast Nitrogen Base (YNB) and 1gm glucose in 100 ml distilled water then sterilized by filtration (Millipore filter 0.22 μ m), 4.5 ml of solution No.1 was mixed with 0.5 ml of solution No.2 in sterilized test tubes, the tubes were inoculated with yeast isolates, then incubated at 28 °C, the tubes were monitored weekly. The tubes don't show any growth after three weeks are considered to be very sensitive to the antibiotic.

Antibacterial activity against tested pathogenic bacteria:

The isolated yeasts were inoculated in Yeast Extract Glucose peptone (YEGP) and incubated at 32°C for 24 hr, 50ml of the same medium was inoculated by 5ml of yeast suspension and incubated at 32°C for 24 hr. The pathogenic bacteria (from Media Center/Erbil) was cultured in nutrient broth at 37°C for 3 hr, Plates were inoculated by 0.1 ml of the pathogenic bacteria, left to dry for 15 minutes at 37°C, 4 wells in each plate (with diameter 5mm) were done using Cork Borer and filled with 0.1ml of yeast suspension after centrifugation at 3000 rpm for 5 minutes the plates were incubated for 24 hr at 37°C then the inhibition zones were measured (Izguet *al.*, 1997).

Study the tolerance of *Saccharomyces cerevisiae var. boulardii* for some gastrointestinal conditions (*in vitro*)

The isolated yeasts were used to examine tolerance to bile salt and gastric acidity which had the best activity against *S. Typhimurium*, the isolated yeasts were (S.b6, S.c1, S.b2, S.c36, S.b13, S.b28, S.b32, S.b22, S.b48, and S.b5).

Bile salt tolerance

Yeast cultures were activated by two transfers in liquid Yeast Peptone Dextrose (YPD) and inoculated at 32°C for 48hr and centrifuged at 3000 rpm for 10 minutes, then sterile phosphate buffered saline was added to the cells and mixed by vortex mixer, then the tubes contain YPD medium supplemented with different concentrations of bile salt (0.2, 0.4, 0.6, 0.8, and 1%), and inoculated with (10^6 CFU/ml) of yeast cells. The growth of the yeast cells was estimated after 1, 8, 16 and 24 hr of incubation at 32°C by measuring absorbance at 660 nm (Katarzyna and Alina, 2010; Bhukya *et al.*, 2010).

Tolerance to acidity

Yeast cultures were activated in YPD and incubated at 32°C for 24 hr., then centrifugation at 3000 rpm for 10 minutes. Sterile phosphate buffered saline PBS was added to the cells and mixed by vortex mixer. Tubes containing artificial gastric juice were inoculated with (10^6 CFU/ml) of yeast cells in which the pH was adjusted 2.5 (the pH of stomach). The tubes incubated at 32°C and the number of viable cells was determined by plate count method using (colony counter) after 0, 1, 2, 3, 24 hr of incubation (Katarzyna

and Alina, 2010) and the result expressed as percentage log survival was calculated according to (Williamson and Johnson,1981).

RESULTS AND DISCUSSION

Fifty isolated yeasts were obtained from different sources such as fresh and dried fruits and all of them were identified by morphological, biochemical and physiological tests, as shown in Table (1). Some taxonomic studies have indicated that *Saccharomyces boulardii* should be rather considered as *Saccharomyces cerevisiae*. DNA-DNA reassociation studies indicated that this yeast was distinct from *Saccharomyces cerevisiae* (Cardinali and Martini,1994). DNA analysis showed the presence of similarity in the genome of these two yeasts is more than 95 % (Baleiras *et al.*, 1995; Edwards-Ingram *et al.*, 2004; Dixit and Gandhi, 2006). The yeast *Sacch. Cerevisiae* should be referred to as *Saccharomyces cerevisiae var. boulardii* (Czerucka *et al.*, 2007; Edwards-Ingram *et al.*, 2007; DeVrese and Schrezenmeir, 2008). Nevertheless, *Sacch. cerevisiae var boulardii* are reported in the scientific reports for their biotherapeutic activities in the prevention of several types of diarrhoea and colitis in humans (Surawicz *et al.*, 1989; McFarland and Bernasconi, 1993).

Antibacterial activity of isolated yeasts against *S. Typhimurium*

The inhibitory activity of isolated yeast against *S. Typhimurium*, was shown in Table (2) *Saccharomyces cerevisiae var boulardii* have an obvious effect against bacterial growth by producing inhibitory substances that prevent the growth of pathogenic bacteria. In using the well diffusion method, isolated yeasts (S.b2, S.b5, S.c36, S.b48, S.b32, S.b13) had larger diameter of inhibition zone which was 15 and 16 mm and the smallest diameter of inhibition zone was 10 mm for the isolates (S.c15, S.c19, S.b18, S.c 33). A study proved that *Sacch. cerevisiae var boulardii* has ability to inhibit the diarrhea causative bacteria *S. Typhimurium* has shown that they strongly adhere to mannose on the surface of *Sacch. cerevisiae var boulardii* via lectin receptors, once the invading microbe is bound to *Sacch. cerevisiae var boulardii*, its prevented from attaching to the intestinal cell wall (Gedek,1999). Also it was proved that the yeast could be used for the treatment of diarrhea (Zouch *et al.*, 2000).

Another study showed the antagonistic activity of the yeast against *Salmonella*, *Shigella* and *E. coli* (Zeng *et al.*, 2003). The outer membrane of *Sacch cerevisiae var boulardii* is rich in mannose, allowing pathogens such as *Salmonella* and others which contain in their structure type1 pili (mannose-binding fibers) this enables pathogens to bind to this mannose rich membrane and this binding action prevents *E. coli* and other harmful bacteria from adhering to intestinal cells. (Rodrigues *et al.*, 1996).

Tolerance of *Saccharomyces cerevesiae var boulardii* for some gastrointestinal conditions (*in vitro*)

The promising activities of yeasts as well as their ability to survive during the passage through the human gastro-intestinal tract, tolerating exposures to low pH and to bile salts, have drawn attention to their possible use as probiotics (Lourens-Hattingh and Viljoen, 2001), even if their use for humans is currently restricted (Psomas *et al.*, 2001). Furthermore, few studies have specifically focused on selecting or studying new probiotic yeast strains (Agarwal *et al.*, 2000; Psomas *et al.*, 2001; Martins *et al.*, 2005).

Bile salt tolerance:

As shown in (Fig. 1) the isolated (S.b32) had shown the best ability to tolerance bile salts more than the other isolated yeasts and had good growth rate even in 1% bile salt, next to it (S.b2) had also a good ability to tolerance and the other strains showed different ability for exposure to different concentrations of bile salts. Generally all the isolated yeasts showed different abilities of tolerance to bile salts. Microorganisms that survive in the acidic condition of the stomach also have to survive in intestinal secretion and the bile salts in the duodenum (Erkkila and Petaja, 2000). Bile is yellow green aqueous solution whose major constituent includes bile acids, cholesterol phospholipids and pigment biliverdin (Carey and Duane, 1994; Hofmann,1994). It's synthesized in the pericentral hepatocytes of the liver, stored and concentrated in the gall bladder interdigestively, concentration used for the screening of a resistant probiotic strain is 0.3% w/ v (Gilliland *et al.*, 1984). Bile resistance is an important criterion in the selection of culture as a dietary adjunct (Gilliland and Walker, 1990; Walker and Gilliland, 1993), because it could allow the growth of the ingested probiotic microorganism in the intestinal tract (Gilliland *et al.*,1984; Suskovic *et al.*, 1997). and the bile salts in the duodenum (Erkkila and Petaja, 2000). Bile is yellow green aqueous solution whose major constituent include bile acids, cholesterol phospholipids and pigment biliverdin (Carey and Duane, 1994; Hofmann,1994). It's synthesized in the pericentral hepatocytes of the liver, stored and concentrated in the gall bladder interdigestively, concentration used for the screening of a resistant probiotic strain is 0.3% w/ v (Gilliland *et al.*, 1984). Bile resistance is an important criterion in the selection of culture as a dietary adjunct (Gilliland and Walker, 1990; Walker and Gilliland, 1993), because it could allow the growth of the ingested probiotic microorganism in the intestinal tract (Gilliland *et al.*,1984; Suskovic *et al.*, 1997).

Table 1: The Results of diagnostic tests used for identification *Sacchromyces cerevisiea* var.*boulardii*

Tests		Results
Utilization of carbon	Glucose	+
	Fructose	+
	Sucrose	+
	Galactose	+/-
	Raffinose	+
	Lactose	-
	Starch	+
Utilization of nitrogen	Peptone	+
	Asparagine	+
	Ammonium sulphate	+
	Nitrate	-
Acid production		+
Urea hydrolysis		-
Ester production		+
Cyclohexamide Resistance		-

Table 2: Antibacterial activity of isolated yeasts against *S.Typhimurium*

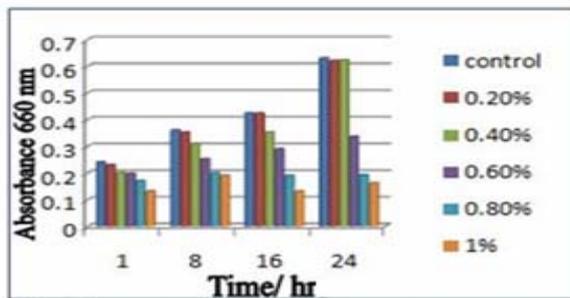
Yeast Isolates*	Inhibition zone (mm)
S.c 1	14
S.b 2	15
S.b 3	13
S.c 4	11
S.b 5	15
S.b 6	14
S.c 8	12
S.b 9	3
S.b 11	13
S.c 12	12
S.b 13	15
S.c 15	10
S.b 16	12
S.b 17	13
S.b 18	10
S.c 19	10
S.b 20	11
S.b 22	14
S.c 23	11
S.b 24	13
S.b 25	12
S.c 26	13
S.c 27	13
S.b 28	14
S.c 29	12
S.c 31	13
S.b 32	16
S.c 33	10
S.b 34	13
S.c 36	16
S.c 38	11
S.b 40	12
S.c 41	11
S.b 42	13
S.b 43	12
S.c 45	11
S.c 47	11
S.b 48	15
S.b 49	12

* s.b.30, s.b.37, s.c.50 had no effect against tested bacteria

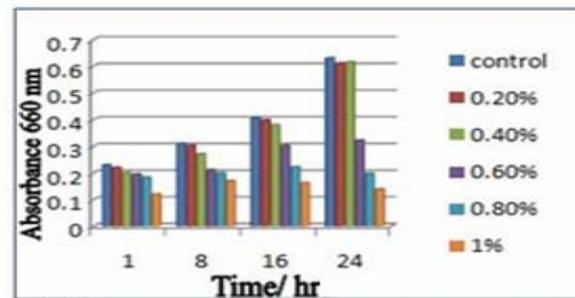
Tolerance to acidity:

Before reaching the intestinal tract, probiotic yeast must survive transit through the stomach and exposure to gastric acid constituents, which is a primary defense mechanism

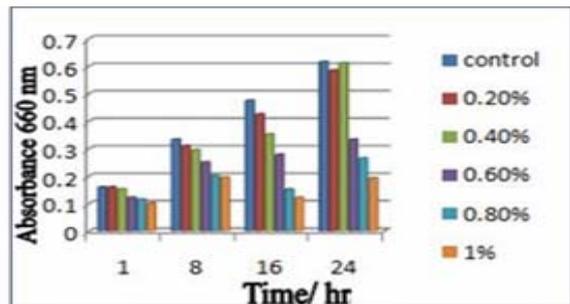
against most ingested microorganisms. (Marteau *et al.*, 1993). It's shown in (Fig. 2) that the isolated yeast strains have the ability to resist acidity, and the strains have different ability to resist low pH. The isolated yeast (S.b32) had highest percentage of survival and the others (S.c36, S.b13, S.b28, S.c1, S.b2, S.b6) also tolerated acidity but compared to strain (S.b32) was lower. The first main barrier that microorganisms after ingestion is exposed to gastric juice, in which the inhibitory effect is strictly related to the pH and the concentration of hydrochloric acid HCl (Psomas *et al.*, 2001). The pH of excreted HCl in the stomach is 0.9, but the presence of food raises the pH to 3.0 (Erkkila and Petaja, 2000). Moreover, the presence of food or other food components could buffer the probiotics ingested, conferring some protective effect on the microbial cells in the stomach. (Conway *et al.*, 1987 ; Prasad *et al.*, 1998).



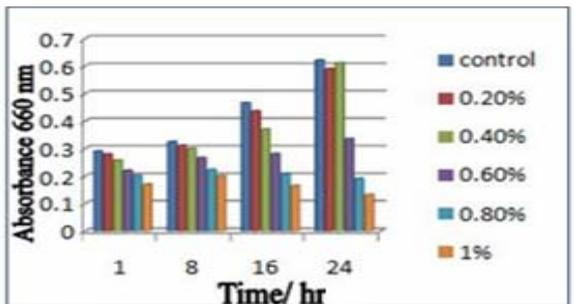
S.b48



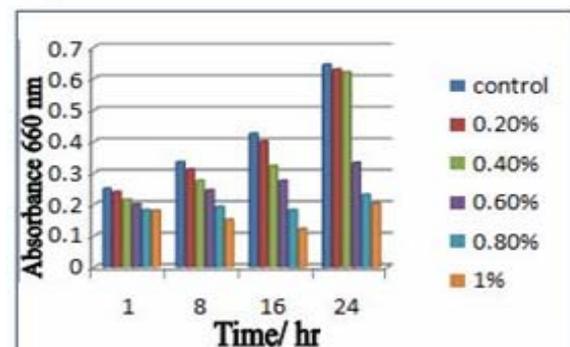
S.b5



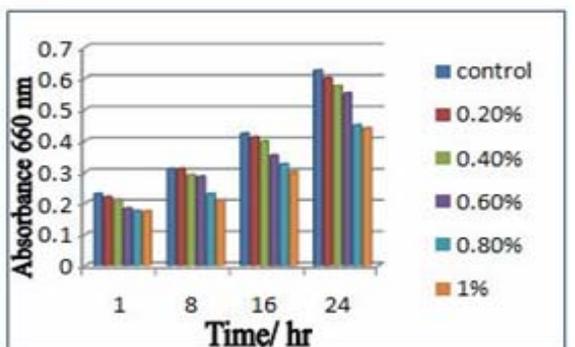
S.b22



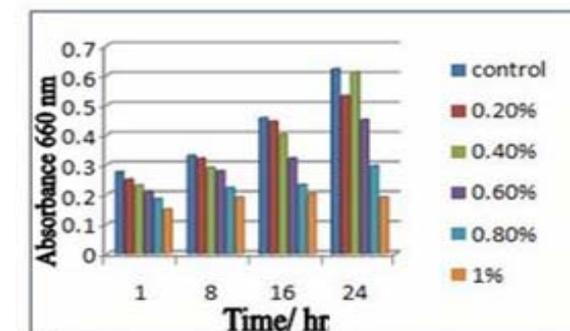
S.b13



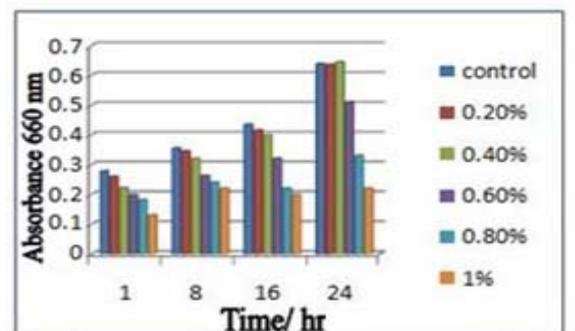
S.b28



S.b32



S.b6



S.c1

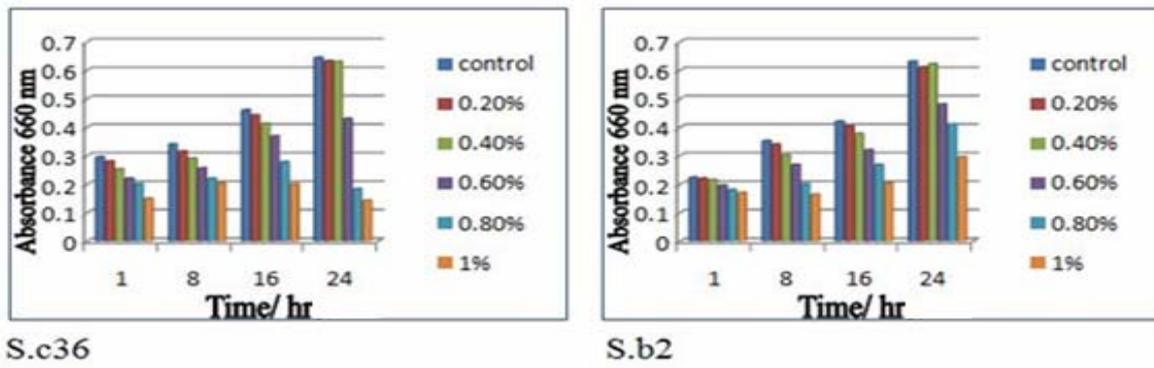


Fig. 1: Ability of isolated yeasts to tolerate different concentrations of bile salts

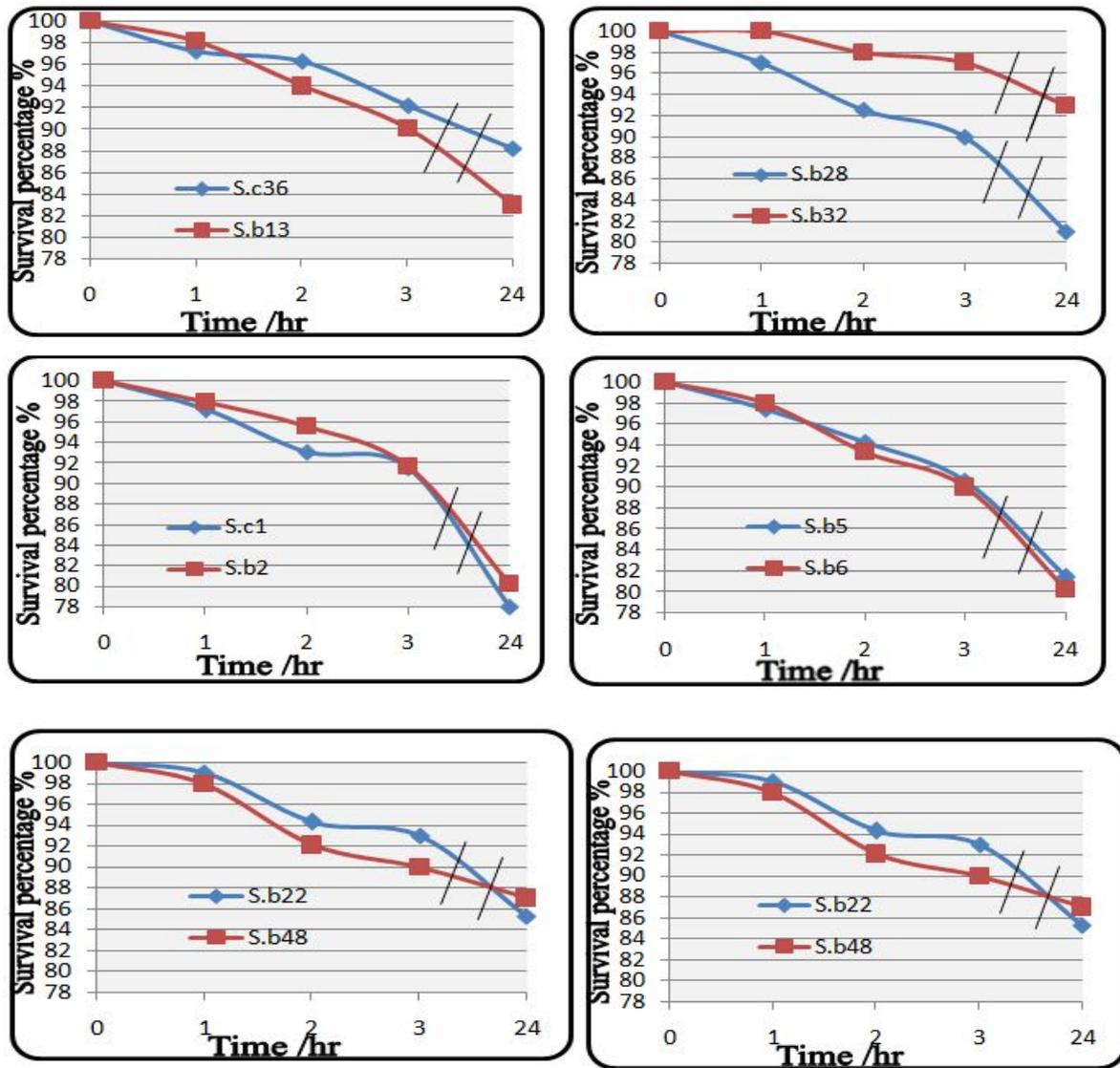


Fig. 2: Percentages of survival of *Saccharomyces cerevesiae var boulardii* in artificial gastric juice (pH 2.5).

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