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Identification of strain isolated from dates (*Phœnix dactylifera* L.) for enhancing very high gravity ethanol production

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Abstract Ethanol production from by-products of dates in very high gravity was conducted in batch fermentation using two yeasts, *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii*, as well as a native strain: an osmophilic strain of bacteria which was isolated for the first time from the juice of dates (*Phoenix dactylifera* L.). The phylogenetic analysis based on the 16S ribosomal RNA and *gyr*B sequence and physiological analysis indicated that the strain identified belongs to the genus of *Bacillus*, *B. amyloliquefaciens*. The ethanol yields produced from the syrup of dates (175 g L^{-1} and 360 g L^{-1} of total sugar) were 40.6% and 29.5%, respectively. By comparing the ethanol production by the isolated bacteria to that obtained using *Z. rouxii* and *S. cerevisiae*, it can be concluded that *B. amyloliquefaciens* was suitable for ethanol production from the syrup of dates and can consume the three types of sugar (glucose, fructose, and sucrose). Using *Z. rouxii*, fructose was preferentially consumed, while glucose was consumed only after fructose depletion. From this, *B. amyloliquefaciens* was promising for the bioethanol industry. In addition, this latter showed a good tolerance for high sugar concentration (36%), allowing ethanol production in batch fermentation at pH 5.0 and 28 °C in date syrup medium. Promising ethanol yield produced to sugar consumed were observed for the two osmotolerant microorganisms, *Z. rouxii* and *B. amyloliquefaciens*, nearly 32–33%, which were further improved when they were cocultivated, leading to an ethanol to glucose yield of 42–43%.

Keywords *Bacillus amyloliquefaciens* · *Saccharomyces cerevisiae* · *Zygosaccharomyces rouxii* · By-products of dates · Ethanol · VHG fermentation

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Introduction

Biomass, is currently considered as one of the most promising alternative for the use of conventional fossil fuels, due to the foreseeable low cost and abundant resource. The most important advantage of bioenergy is to reduce dependence on nonrenewable fossil fuel sources. It can also provide good opportunities to convert renewable organic waste materials into energy. Moreover, biomass with high sugar content allows to work in fermentation using very high gravity (VHG) conditions. The efficiency of transformation processes are energetically optimized (Laopaiboon et al. 2009; Pereira et al. 2010; Larnaudie et al. 2016).

The production of dates is accompanied by a substantial increase of loss during harvest, storage, commercialization, and conditioning process. Date by-products are not consumed because of their low quality (Abbes et al. 2011). Currently, very little use of these by-products is made; they are discarded

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or used in limited cases for animal feed. Research into date by-products has not been a true reflection of the importance and potential of this crop, since dates are a rich source of certain nutrients and sugars (70–80%) in the form of glucose, fructose, and sucrose (Al-Farsi 2011).

Recently, there has been increased attention in the field of bioenergy as world energy consumption has increased. Ethanol is a renewable energy with high efficiency and low environmental impact. Ethanol production via yeast fermentation may provide an economically competitive source of energy. Among the crucial factors affecting alcoholic fermentation are substrate selection and preparation, microbial selection and adaptation, optimization of fermentation conditions, and improvement of fermentation technology (Kasavi et al. 2012).

Saccharomyces cerevisiae is traditionally used for ethanol production. Alfenor et al. (2002) observed that *S. cerevisiae* increased ethanol production in the presence of vitamins. A maximum productivity of 0.5 g L^{-1} h^{-1} was reached in the best fermentation. The aeration strategy is another parameter very determinant for ethanol production with an increase of 25% of ethanol productivity compared with microaeration, by *S. cerevisiae* (Alfenor et al. 2004). Djelal et al. (2006) studied the effect of aeration of ethanol and glycerol production under salt conditions by *Hansenula anomala*. They well described the effect of oxygen concentration on the bioproduction of both products. More recently, Bideaux et al. (2016) showed that the lower the oxygen to xylose ratio, the higher the ethanol production yield. It should be noted that the performance of *S. cerevisiae* during fermentation was compromised by the impact of variable environmental factors such as hyperosmolarity due to the large amount of sugar (Belloch et al. 2008; Djelal et al. 2012).

Three yeasts *(S. cerevisiae, Candida pelliculosa*, and *Zygosaccharomyces rouxii*) were tested for ethanol production from date syrup. However, performances during fermentation (72 h) were compromised by the impact of the hyperosmolarity due to the large amount of sugars. Only *Z. rouxii* can grow under this extreme environmental stress, but it cannot consume glucose and sucrose (Chniti et al. 2014).

It was observed that the strains isolated from a natural substrate gave much better results than those obtained with pure strains. Thus, Abd-Alla et al. (2015) observed that some native strains isolated from agricultural soils cultivated with different plants have the high ability to produce biobutanol. A similar study was conducted by Sarris et al. (2009) with a newly isolated *S. cerevisiae* strain for the production of ethanol. The authors observed that this strain is performed to convert raw materials into ethanol in high concentration.

This investigation was designed to use an isolated (indigenous) microorganism from dates, by-products of the *Deglet Nour* variety, and to evaluate its efficiency for ethanol production in date syrup medium under high initial sugar concentration. In the first part, a comparative study was performed with *S. cerevisiae* 522D and the osmotolerant yeast *Z. rouxii* after 150 h of batch fermentation. In the second part, a study of the kinetics of sugar consumption, ethanol, and glycerol production by *Z. rouxii* and *B. amyliloquifaciens* was described. Finally, to improve the ethanol production, a coculture was conducted with *Z. rouxii* and the isolated strain.

Materials and methods

Raw material treatment

Date by-products, "Deglet Nour," was procured from the Tunisian conditional unit of dates "ALKHALIJ." The date juice was prepared according to Acourene et al. (2011) and Chniti et al. (2014).

Yeast strains and inoculum preparation

The fermentative yeasts *S. cerevisiae* 522D and *Z. rouxii* (IP 2021.92) were obtained from the culture collection of the Pasteur Institute (Paris, France). The yeast strains were maintained in a synthetic medium whose compositions (in grams per liter) were glucose 20; yeast extract 10; peptone 10; and agar 10. In all cases, cultures were maintained at 28 °C for 24 h and then stored at 4 °C. A given number of drops of a yeast suspension in KCl 150 mmol L^{-1} were grown in 25 mL of synthetic medium (in grams per liter): glucose, 20; peptone, 10; and yeast extract, 10; in a 0.25-L bottle on a rotating shaker (New Brunswick, INNOVA 40, NJ, USA) at 180 rpm, 28 °C for 18 h. After centrifugation (3000 rpm, 4 °C, and 5 min), cells were harvested, resuspended in 25 mL KCl 150 mmol L^{-1}, and centrifuged again in similar conditions. The suspension obtained after harvesting cells and resuspending in 10 mL KCl 150 mmol L^{-1} was used to inoculate culture media (Djelal et al. 2005).

Strain isolation from by-products of dates

A date juice sample was put in fermentation at room temperature for some days until the appearance of ethanol odor. The composition of the tubes of the enrichment medium was (in grams per liter) yeast extract 4; peptone 5; glucose 25; KH_2PO_4 0.55; KCl 0.42; $CaCl_2$ 0.12; $FeCl_3$ 0.0025; $MnSO_4$ 0.0025; and date juice, 50 mL L^{-1}. The tubes were seeded by positive culture then brought to the incubation at 25 °C. Strains were purified by subsequent streaking onto the surface of the Sabouraud chloramphenicol agar (HiMedia Laboratories). The typical colonies were transferred from the solid medium to the broth medium (enrichment medium), then incubated at 25 °C for 72 h. From the positive tubes (alcoholic fermentation), streaks were made onto the surface of the

Wallerstein Laboratory Nutrient Medium (WLNM; Merck) and the observations were made after 72 h of incubation at 25 °C (Pereira-Dias et al. 2000). Only characteristic colonies (green, smooth, curved) were transferred from the WLNM agar to tubes containing the broth medium. The purification was performed by streaking onto the surface of the Sabouraud chloramphenicol agar.

Identification of the isolated strain

Identification of the isolated strain was done in EQUASA (Technopole Brest-IROISE, Plouzane, France). 16S rDNA was amplified and sequenced and then a portion of the gene encoding gyrase (*gyr*B). The sequences were verified using the software BioNumerics and compared to NCBI databases. The phylogenetic tree was realized with the Figtree software.

Biochemical characteristics

The isolated strain was identified according to their biochemical profiles using the API50 CHB test kit (Biomérieux, Marcy L'Etoile, France).

Date syrup medium preparation for fermentation

Date syrup at 175 g L^{-1} (medium A) and 360 g L^{-1} (medium B) of total sugar were supplemented with mineral medium which was well described in Chniti et al. (2014). The total sugar content was expressed in equivalents of glucose (glucose + fructose + 1.05 × sucrose) (Guigou et al. 2011). The pH was adjusted to 6.0 using KOH 1 mol L^{-1}. The medium was transferred into a 500-mL bottle with a final working volume of 300 mL and was autoclaved at 120 °C for 20 min before adding NH_4Cl sterilized by filtration on a 0.2-μm membrane (Sartorius, Goettingen, Germany). The medium was inoculated with 200 μl of yeast or bacterial suspension. Batch fermentation was carried out on an incubator shaker (New Brunswick, INNOVA 40, NJ, USA) at 20 rad sec^{-1}, 28 °C for 150 h. All fermentations were performed at least in duplicate.

Date syrup at an initial concentration close to 200 g L^{-1} (medium A) and 360 g L^{-1} (medium B) of total sugar were supplemented with mineral medium which was well described in Chniti et al. (2014). The total sugar content was expressed in equivalents of glucose (glucose + fructose + 1.05 × sucrose) (Guigou et al. 2011). The pH was adjusted to 6.0 using KOH 1 mol L^{-1}. The medium was transferred into a 500-mL bottle with a final working volume of 300 mL and was autoclaved at 120 °C for 20 min before adding NH_4Cl sterilized by filtration on a 0.2-μm membrane (Sartorius, Goettingen, Germany). The medium was inoculated with 200 μl of yeast or bacterial suspension. Batch fermentation was carried out on an incubator shaker (New Brunswick, INNOVA 40, NJ, USA) at 20 rad sec^{-1}, 28 °C for 150 h. All fermentations were performed at least in duplicate.

Analytical methods

During the fermentation period, a 5.0-mL sample was taken at several times and centrifuged at 3000 rpm, 4 °C for 5 min. Absorbance at 600 nm was then measured. The supernatant was used to analyze ethanol, glycerol, and residual sugar concentrations with HPLC (Djelal et al. 2005).

The yield of ethanol production ($Y_{P/S}$) was calculated as the actual ethanol produced and expressed as grams of ethanol per gram of total sugar utilized (grams per gram). The volumetric ethanol productivity (Q, grams per liter per hour) was determined from the concentration produced (P, grams per liter) divided by the fermentation time (t, h) giving the highest ethanol concentration.

Statistical analysis

Each analysis was done in duplicate and the results were expressed as mean and standard deviation (SD). The Duncan test was used to compare all mean pairs in conjunction with analysis of variance (ANOVA) using XLSTAT software, version 2013.3.01 (Addinsoft). Differences between means were considered significant when $p < 0.05$.

Results and discussion

Phylogenetic analysis

The quality of the sequencing allowed the use of only one of the two sequences obtained. At this stage, it was not possible to confirm the identification of the strain (more than 98% sequence similarities); it can be either *Bacillus subtilis* or *Bacillus amyloliquefaciens*. According to Larsen et al. (2014), a portion of the gene encoding a part of gyrase seems more specific to differentiate these two species. To refine the identification of the strain, a phylogenetic tree based on the analysis of a portion of the gene encoding a part of gyrase (*gyr*B gene), with strains of *B. subtilis* and *B. amyloliquefaciens*, was constructed (Fig. 1).

The results of analysis of the sequences suggest that the strain isolated from dates belonged to *B. amyloliquefaciens* species, possibly subspecies *plantarum*. The percentage of identification was higher than 98%. The biochemical test confirmed the identification of the isolated strain as *B. amyloliquefaciens* species.

Alvindi and Natsuaki (2009) have isolated *B. amyloliquefaciens* from banana fruit surface, namely able to grow at low water activity, as it is the case in the date syrup. *B. amyloliquefaciens* strain was also isolated from salt spring in

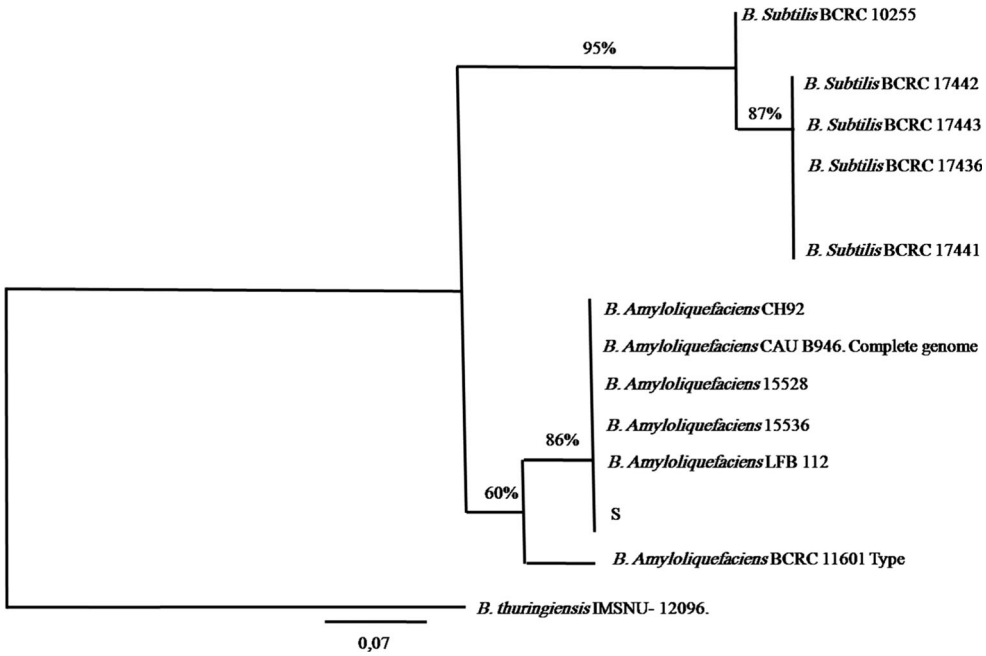

Fig. 1 Phylogenetic tree of members of the genus *Bacillus*, based on *gyr*B gene sequences

Ovca located in the Republic of Serbia, indicating the osmotolerant character of the strain which would explain its adaptation on dry medium such as this of dates (Loncar et al. 2014). Manhar et al. (2015) isolated *B. amyloliquefaciens* from traditional fermented soybean (Churpi) and observed the high ability of this strain to degrade cellulose. It is well known that *Bacillus* species have emerged as a robust organism that can grow in extreme environmental conditions and grow easily to very high densities (Kumar et al. 2013).

Comparison of ethanol fermentation by *B. amyloliquefaciens*, *S. cerevisia*, and *Z. rouxii* in two culture mediums

The production of ethanol was observed for the two yeasts and the isolated bacteria in a culture medium with 175 g L^{-1} (medium A) of sugar content (Table 1); while with a total sugar concentration of 360 g L^{-1} (medium B), the ethanol production was observed only with *B. amyloliquefaciens* and *Z. rouxii* and no noticeable amount of ethanol was produced by *S. cerevisiae* (Table 1). With *B. amyloliquefaciens* as inoculum strain for the medium A, the maximum ethanol concentration reached 89.8 g L^{-1} after 150 h and it was 73.6 g L^{-1} and 62.6 g L^{-1} for *S. cerevisiae* and *Z. rouxii*, respectively (Table 1). Among the three tested species, *B. amyloliquefaciens* was therefore the most efficient species for ethanol production with near complete sugar consumption (313.56 g L^{-1} total sugar). The yield of ethanol produced per total sugar consumed by the tested strains from date juice is close to those found in the scientific literature, for example: from molasses and olive mill wastewater (Sarris et al. 2014), from food waste hydrolysate (Thongdumyu et al. 2014), and from enriched culture medium with vitamins (Alfenore et al. 2012). Given the promising early results, we will study the kinetics of consumption of sugar and ethanol production.

Kinetics of sugar consumption by *B. amyloliquefaciens* and *Z. rouxii*

Sugar consumption during culture showed different trends regarding on the one hand the considered sugar and on the other hand the considered yeast. For 200 g L^{-1} of total sugar, a high consumption of glucose and fructose was observed for *B. amyloliquefaciens* after a 3-day culture (Fig. 2a). Contrarily, the fructose consumption was high for *Z. rouxii* if compared to its consumption of glucose (Fig. 2b); while the consumption of sucrose was observed only for *B. amyloliquefaciens*.

As shown in Fig. 2a and c, all glucose, fructose, and sucrose were consumed simultaneously and completely by *B. amyloliquefaciens*. Sucrose may be hydrolyzed in two ways: in the first and predominant mechanism, sucrose is hydrolyzed by an extracellular invertase. Hydrolysis yields glucose and fructose, which enter into the cell by facilitated diffusion via hexose transporters. In the second mechanism, sucrose can be actively transported in the cells by a proton-symport mechanism and hydrolyzed intracellularly (Stambuk et al. 2000; Batista et al. 2004). More recently, some authors well described why *Z. rouxii* uses firstly fructose in the presence of glucose (Dalkia et al. 2014).

Concerning consumption of sugar by *Z. rouxii* at a high sugar concentration (360 g L^{-1}), it should be observed that

Table 1 Comparison of ethanol and glycerol l production from date syrup at 150 h of culture

	Consumed sugar (g L^{-1})		Ethanol (g L^{-1})		Glycerol (g L^{-1})		$Y_{EtOH/S}$ (%)		Q_{EtOH} (g L^{-1} h^{-1})	
Culture medium	A	B	A	B	A	B	A	B	A	B
B. amyloliquefaciens	173.60[a]	313.56[a]	89.80 ± 0.5[a]	92.40 ± 0.5[a]	10.0 ± 0.10[a]	8.20 ± 0.2[a]	40.6[a]	29.5[a]	0.59[a]	0.61[a]
S. cerevisiae	158.37[b]	3.96[b]	73.60 ± 0.6[b]	<LD	8.20 ± 0.3[b]	<LD	47.2[a]	<LD	0.49[b]	<LD[b]
Z. rouxii	155.05[b]	213.84[c]	62.60 ± 4.5[c]	78.80 ± 3[b]	8.40 ± 0.3[b]	10.30 ± 0.1[b]	36.4[a]	36.7[b]	0.42[c]	0.52[c]

Medium A at 175 g L^{-1} total sugars; medium B at 360 g L^{-1} total sugars

<LD <Limit of detection

[a, b, c] Means followed by the same letter within the same column are not significantly different using Duncan's multiple range tests at the level of 0.05

the assimilation of fructose was observed from the beginning of the culture, while glucose was only used after total consumption of fructose as an energy source for cell maintenance (Fig. 2d).

These results indicate that at high concentrations of reducing sugars, *Z. rouxii* consumed fructose faster than glucose and sucrose, in agreement with its fructophilic character (Leandro et al. 2011). At a high concentration (360 g L^{-1}), fructose significantly inactivated the glucose transporter, preventing the uptake of this sugar. Fructose was able to utilize the glucose transporter, by competing with glucose.

Contrarily and even at very high gravity fermentation, *B. amyloliquefaciens* was able to ferment glucose, fructose, and sucrose simultaneously, even if after 7 days of culture, sucrose

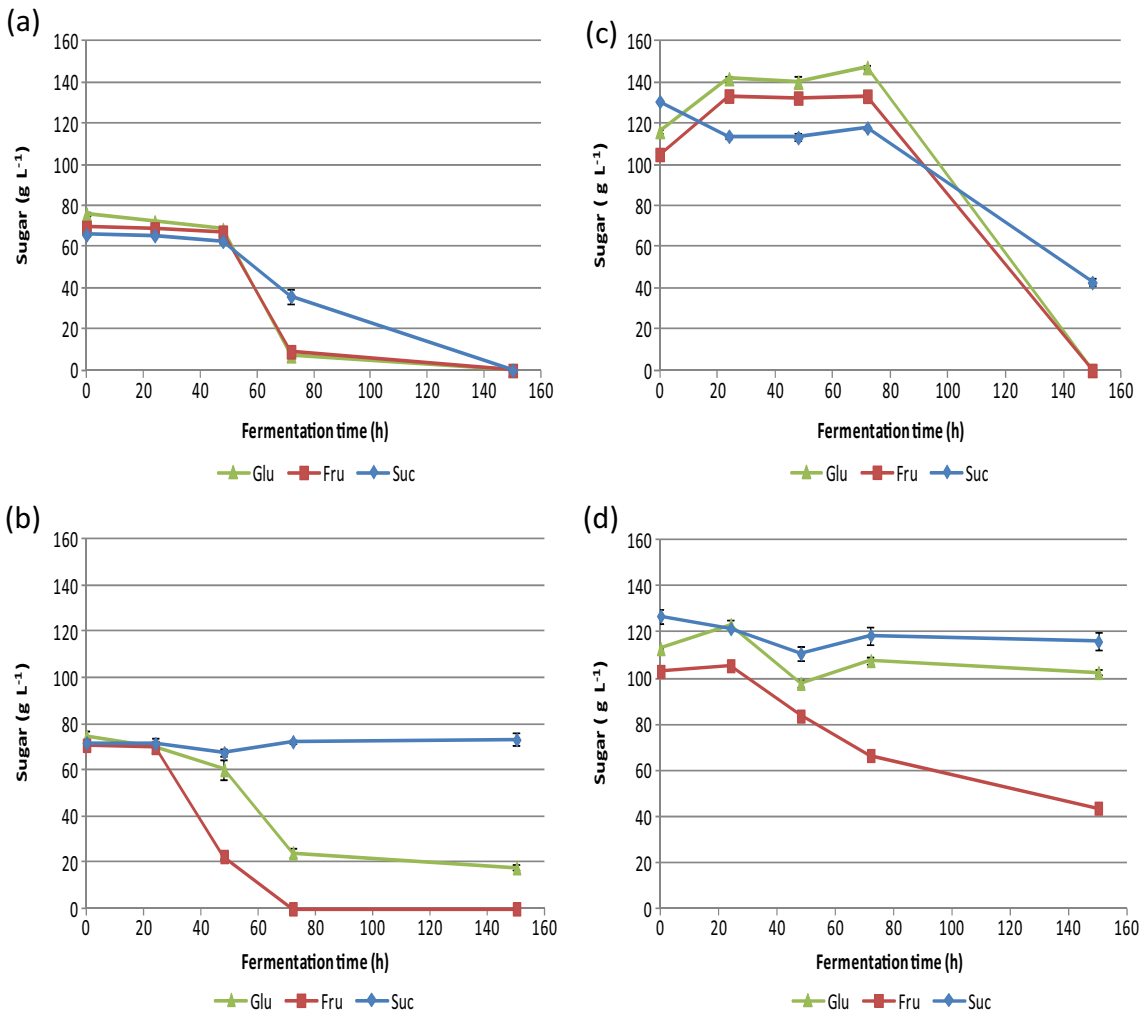

Fig. 2 Sugar consumption by *B. amyloliquefaciens* at 175 g L^{-1} (**a**) and 360 g L^{-1} (**c**) and *Z. rouxii* at 175 g L^{-1} (**b**) and 360 g L^{-1} (d)

was not completely consumed (Fig. 2c). The isolated strain, which showed the ability to metabolize all sugars present in the culture medium, appears therefore more interesting than the osmophilic yeast *Z. rouxii*.

Kinetics of ethanol production by *B. amyloliquefaciens* and *Z. rouxii*

Both ethanol and glycerol productions were observed for *Z. rouxii* and *B. amyloliquefaciens* in the culture media (A and B) (Fig. 3). The highest ethanol production was observed for *B. amyloliquefaciens*, 90 and 92 g L^{-1} for culture media A and B, respectively (Fig. 3a, b). It is noteworthy that nearly similar amounts of ethanol were produced during culture in medium A; while and in close connection with sugar consumption (Fig. 2c, d), ethanol production by *B. amyloliquefaciens* was significantly higher than the amount produced by *Z. rouxii* during VHG fermentation (medium B), 92 and 62 g L^{-1}, respectively (Fig. 3a, b). Regarding the osmoprotective metabolite (glycerol), roughly similar amounts were produced by the two species and in both media (A and B) (Fig. 3c, d).

The isolated strain was able to produce ethanol from concentrated date syrup and can consume the three sugars contained in the medium (glucose, fructose, and sucrose). Attfield (1997) and Jiménez-Marti et al. (2011) indicated that, under a particular environment, yeasts have to cope with osmotic stress caused by high sugar concentrations; a part of the assimilated sugar is used for cell maintenance to produce glycerol as osmolyte.

The comparison of the ethanol production obtained in this study, using palm date, to those of the literature performed in similar conditions show that the ethanol productivity by the isolated strain gave similar results to those obtained with other strains cultivated on several sources (Table 2). Sarris et al. (2009) observed the possibility of utilizing an industrial grape must enriched with commercial sugars for the high production of ethanol with the newly isolated *S. cerevisiae* strain. It could be noted that in addition to ethanol production, some studies show the coproduction of added-value compounds as glycerol (Djelal et al. 2006) and volatile compounds (Sarri et al. 2009) or to mix bioproduction of ethanol with treatment of olive mill wastewater-based media (Sarris et al. 2013).

According to our results, the strain *B. amyloliquefaciens* isolated from dates was promising for the production of ethanol. These results are in agreement with those of Di Pasqua et al. (2014) who showed a high enzymatic activity on lignocellulosic biomass by *B. amyloliquefaciens* isolated from compost. A microbial mixing gave better ethanol productivity than the pure culture of a given strain (Kalyani et al. 2013). Furthermore, the isolation and characterization of novel cellulose hydrolyzing enzymes from bacteria are still an active research area, because bacteria have a higher growth rate than fungi, leading to a greater production of enzyme (Maki et al. 2009).

Batch fermentation with cocultures of *B. amyloliquefaciens* and *Z. rouxii*

To check whether the coculture of indigenous strain (*B. amyliloquifaciens*) and exogenous strain (*Z. rouxii*) enhance the production of ethanol from date syrup at 400 g L^{-1} of total sugars, an assay was made in the same conditions as previously described in this study. In view of comparison, pure cultures of each microorganism, *B. amyliloquifaciens* and *Z. rouxii*, were also made. In Fig. 4, the growth of the strains was represented. Before 50 h of culture, no growth was observed for the indigenous strain, while after this time, the cells went up significantly and then leveled off. The growth of the exogenous strain did not follow that of *B. amyliloquifaciens*. Its adaptation required more time than for *Z. rouxii*. On the other side, the coculture in the ratio S1/Z.r:1/1 and S1/Z.r:1/3 did not affect the growth of *Z. rouxii*. At 150 h of fermentation, the yield of ethanol production compared to sugar consumed ($Y_{EtOH/S}$) is quite similar for the two pure cultures. But this yield increased when strains were mixed by 25% (Fig. 5). The data indicated that the use of *B. amyliloquifaciens* in coculture improved the productivity of ethanol by 20% (Fig. 5). The strain ratio did not affect the results of productivity of ethanol. Of the same, De Bari et al. (2013) observed that the cocultures of *Scheffersomyces stipitis* with *S. cerevisiae* ensured faster processes with higher productivity of ethanol than single cultures. For instance, Thongdumyu et al. (2014) showed that the fermentation of food waste hydrolysate by cocultures of *Zymomonas mobilis* and *Candida shehatae* showed an increase of ethanol production by 30% comparative with the single culture (Table 2).

The implantation of industrial ethanol production from by-products of dates could be necessary in hot climates and high-temperature seasonal deviations. The use of strain acclimated in these conditions will be a definite plus. Developing a nonsterile continuous bioprocess is indisputably necessary for a viable business model. For instance, Kopsahelis et al. (2009, 2012) studied an integrated cost-effective system for continuous fermentation of nonsterilized molasses during 32 days at 40 °C. They observed no contamination and similar production of ethanol compared to sterilized molasses. Sarris et al. (2014) observed that bioethanol production by *S. cerevisiae* under nonaseptic conditions from molasses and olive mill wastewater blends had the same effect as with aseptic conditions. Thongdumyu et al. (2014) conducted a study of ethanol production from food waste under a nonsterile condition and concluded that this raw material is an interesting biomass resource for ethanol production. But it is also important to consider the cost of the preparation of the raw material and to take account of

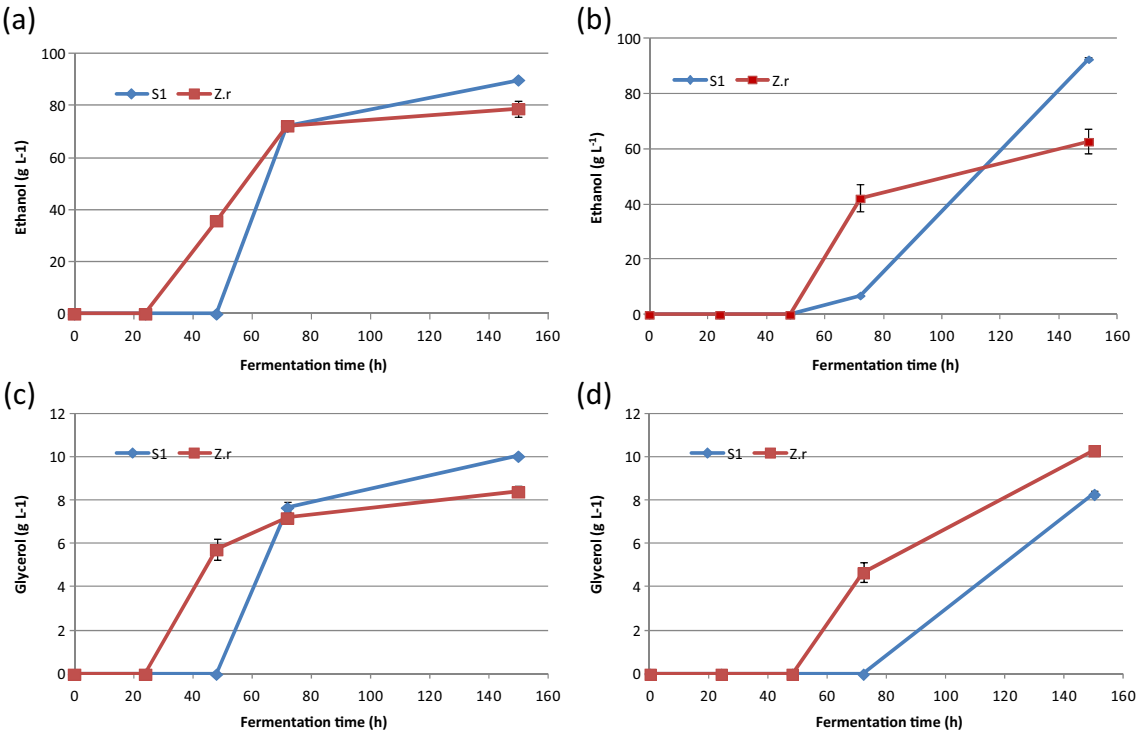

Fig. 3 Ethanol (**a**) and glycerol (**c**) productions at 175 g L^{-1}. Ethanol (**b**) and glycerol (**d**) productions at 360 g L^{-1} by *B. amyloliquefaciens* (S1) and *Z. rouxii* (Z.r)

Table 2 Ethanol productivity by some strains cultivated on various sources

Strain	Sources	Ethanol productivity (g L^{-1} h^{-1})	References
S. cerevisiae	Coconut fiber mature	0.30	Goncalvez et al. 2016
P. stipitis		0.23	
Z. mobilis		0.30	
S. cerevisiae	Date syrup	0.90	Chniti et al. 2014
C. pelliculosa		0.60	
Z. mobilis	Soybean molasses	1.53	Letti et al. 2012
S. cerevisiae	Sweet sorghum juice	2.01	Laopaiboon et al. 2009
S. cerevisiae	Synthetic medium in presence of vitamin	9.50	Alfenore et al. 2002
S. cerevisiae	Alkali-pretreated *P. densiflora*	0.55	Kalyani et al. 2013
P. stipitis		0.44	
S. cerevisiae + *P. stipitis*		0.72	
S. cerevisiae + *S. stipitis*	Mixed syrup of glucose and xylose	0.72	De Bari et al. 2013
Z. mobilis	Food waste hydrolysate	0.75	Thongdumyu et al. 2014
C. shehatae		0.66	
Z. mobilis + *C. shehatae*		1.07	
B. amyloliquefaciens	Date syrup	0.35	In this study
Z. rouxii		0.40	
B. amyloliquefaciens + *Z. rouxii*		0.50	

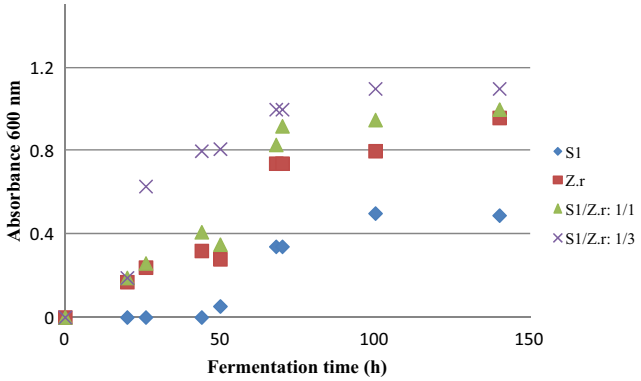

Fig. 4 Growth of *B. amyliloquifaciens* (S1), *Z. rouxii* (Z.r), and their coculture in the ratio S1/Z.r:1/1 and S1/Z.r:1/3 from date syrup with an initial concentration of 400 g L^{-1} of total sugars

different sugars obtained after hydrolysis (Limayen and Ricke 2012). The cost of enzymatic hydrolysis increased the global cost of the production of ethanol from natural raw material or by-product of the food industry. Justly, the coculture with fungi such as *Aspergillus niger* and yeast such as *S. cerevisiae* appeared to be an opportunity for the production of enzyme simultaneous with the production of ethanol (Izmirlioglu and Demirci 2016). It would be, therefore, more interesting to operate with a microbial consortium for enhancing the ethanol bioproduction from low-cost biomass.

Conclusion

The present study was undertaken to assess the ethanol production from by-products of dates by VHG fermentation process using *S. cerevisiae*, *Z. rouxii*, and an isolated osmotolerant bacterial strain, which was identified and belonged to "*B. amyloliquefaciens*." This strain was able to grow in concentrated date juice which presented a very high osmotic pressure, higher than that supported by the conventional yeast *S. cerevisiae*. Under the conditions considered in the present work, *B. amyloliquefaciens* can consume the three types of sugars (glucose, fructose, and sucrose). These results show the prospect for the use of new isolated bacteria for ethanol production from the by-products of dates or other substrates used for ethanol production. Moreover, *B. amyloliquefaciens* led to high ethanol productivity, 32% ethanol produced to sugar consumed yield, which was further improved when growing in mixed culture with the osmotolerant yeast *Z. rouxii*, resulting in 42% Y_{EtOH} yield.

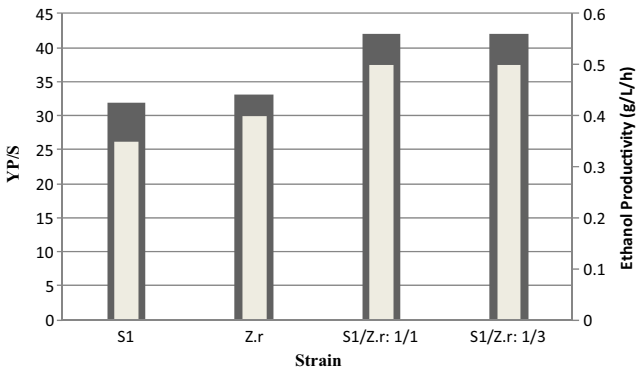

Fig. 5 Comparison of ethanol production yield (*gray bars*) and ethanol productivity (*white bars*) during single cultures of *B. amyliloquifaciens* (S1), *Z. rouxii* (Z.r), and their coculture in the ratio S1/Z.r:1/1 and S1/Z.r:1/3 from date syrup with an initial concentration of 400 g L^{-1} of total sugars at 150 h of culture

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