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Fermentative Strength of Yeasts Strain, Naturally Isolated Using Common Date in South-West of Algeria

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Abstract : The bioconversion of by-products coming from the date palm can constitute future projects for the recovery and development of arid and semi-arid regions of North Africa, Middle East and South Asian countries. The treatment of common dates of low commercial value using biochemical transformations provides new efficient products with added-value easy to commercialize at high price. Among these derivative products we notice the syrups, the alcohols, the yeasts and others substances. The conversion involves complex biochemical processes based on various types of microorganisms; each one has its specificity and role among the biotechnological processes. The yeast strain plays a major role in an alcoholic transformation because it consumes simple sugars of dates and converts them into ethanol. The composition of the substrate and the control of the fermentation process are also important.
In the present paper, we are studied one variety of common dates, having low commercial value originated from Adrar province in south Algeria called (*Tinaceur*) as a substrate to isolate new efficient yeast strains for using them in the alcoholic fermentation to produce a bioethanol compared to the use of commercial well-known yeast strain *Saccharomyces cerevisiae*. The main objective of the present work includes the isolation of new yeast strains from the mash of dates during the fermentation process and determination the fermentative strength of this strain on the substrate of the variety of dates studied by physical and chemical analysis of (pH, juice density, alcohol concentration, total sugar and Brix degree).
Keywords. South-West of Algeria, common date (Tinaceur), yeast strain (*Saccharomyces cerevisiae)*, alcohol fermentation, bioethanol.

Introduction

Algerian country imports a huge quantity of yeast strain (*Saccharomyces cerevisiae)* every year for making bakery yeast, in order to satisfy the increasingly request of the population in bread, and consequently in yeast for divers uses. In all times the Saharan peoples made their own locally traditional vinegar from existing yeast strains of naturally microorganisms based on dates[1]. The phoenic culture is the central axis of the Saharian agriculture in Algeria where the date palm predominates with about 22% of the total area of plantations. The number of date palm reaches 18 million, of which 11 million are productive according to the[2] and produces about 492,000 tons of dates[3]. A quantity of 200,000 to 250,000 tons is not appreciated, and it is commercialized with great difficultly in the local markets[4,5].

The bioconversion of the by-products coming from date palms can constituting a future project for the development of Saharan agriculture[1], by finding new profitable applications for these derived products such as syrups, alcohols, yeasts and others[6].These conversion processes involve complex biochemical phenomena and several types of specific microorganisms. Among them, the alcoholic fermentation, who transforms sugar of the juice of dates in presence of yeast into a bioethanol[7].

The yeast has been used by humans for several thousands of years, without known it, in particular in the production of the alcoholic beverages and bread. The importance role of the yeast in the fermentation process is highlighted after the celebrate Professor L. Pasteur (1899-1876) by the works[8,9].

Lots of yeasts used in biotechnology are commonly publicly obtained by natural habitats that are developed an adaptation faculty of several ecological niches due to their physicochemical proprieties. In addition, the evaluation of dates by biotechnological processes allows the production of biomass and different metabolites. This biomass is the basis for many industrial activities: the production of wines, the dietary yeast and mostly unicellular derived proteins. The metabolism of yeast and fermentation activity depends on the presence or absence of the oxygen in the fermentation medium.

The ethanol is an important industrial chemical product provided several purposes in chemical industry, in pharmaceutical, in cosmetic etc. Nowadays, the ethanol acquires new potential as a clean biofuel. Bioethanol thanks to its physic-chemical properties compatible with gasoline represents a promising alternative to fossil fuels, expensive and polluting. Bioethanol is produced from sugar by an alcoholic fermentation from sweet biomass (sugar cane, sugar beet, and others), starch product (corn, potato, cassava etc…), or from lignocellulosic biomass such as grass, agro-forestry residues, wood, and others [10].The objective of the present work consists in the purification and isolation of yeast strains from biological sampling of date juice in the south-West of Algeria (Adrar region). The capacity, productivity and fermentation strength of yeast strain are also discussed.

Materials and Methods

Sampling

The sampling substrate is based on common date "Tinaceur"is coming from Adrar region in South-West of Algeria after the harvest season during October-November period.

Fermentation and yeast strainseparation

For each sampling, 2kg of dates are collected aseptically into a sterilized bottle and immediately stored at 4°C, then transported to the laboratory into an isothermal closed container for the purpose of analysis. The dates' juice is fermented at 30°C into a small bottle with a volume of 500ml and it is subjected to a mechanical stirring at 20 rpm/min. The fermentation process is controlled every day by determination of the mass loss of the date mash. Once the mass rate observed reached 70gr/L which is corresponding to the consumption of an approximately 2/3 of their sugar content, the samples were diluted to 10^4 and 10^5 [11]. Each dilution is spread on the surface of Sabouraud agar medium. In addition, we added of quantity of gentamicin 0.04gr/L to inhibit the growth of $Gram^+$ and $Gram^-$ bacteria. The inoculation is carried out on this surface by spreading 0.1mL of the culture on[12,13]. The Petri dishes prepared are incubated at 30°C for 24h to 72h[14].

Yeast strain purification

Strains of different morphologies are well separated then transplanted on an Agar Sabouraud medium. The purification of the yeast strains is carried out by streaks method on a solid Sabouraud medium. These stepsare repeated several times until obtained pure homogeneous colonies, which are retained for the present study.

Yeast strain conservation

The conservation of yeast strainsis the easiest and the most used technique to apply. It consists to transplant the strains in tube on slant Potato Dextrose AgarPDA or Malt Agar MA. The culture is incubated during 3 to 5 days to ensure maximum growth, and then it is stored at 4°C for one month to promote its viability

and to limit the possibility of variations[15].

Fermentation strength of isolated yeast strains in dates' juice

- **Mash preparation and alcoholic fermentation**

The raw material retained in the present study consists in common date called (Tinaceur) coming from the Adrar region, due to their abundance, richness in sugars, and low commercialized values (see Figure 1).

Figure 1 : Common dates

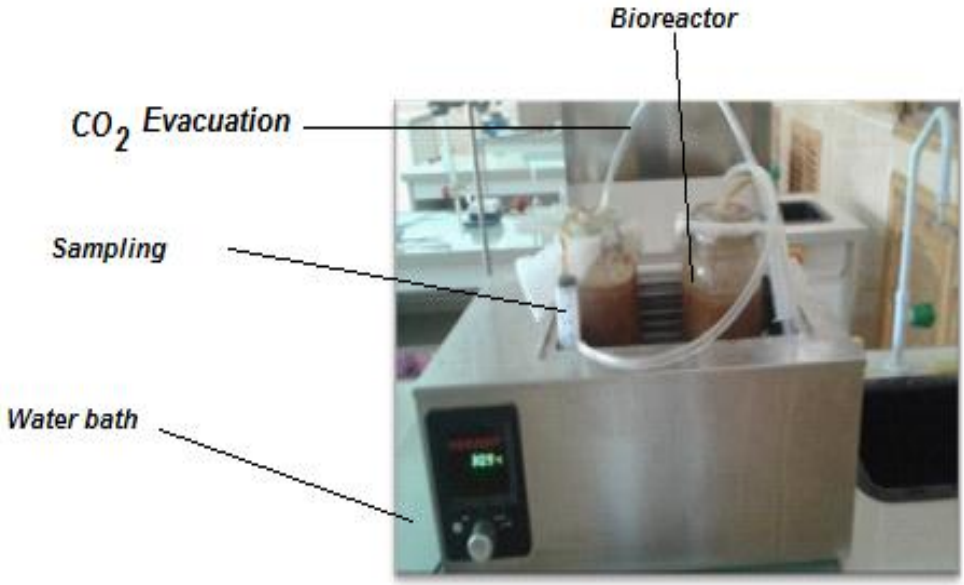

Figure 2: Alcoholic fermentation system

The fermentation process is performed according to the following steps:

Step 1: The yeast strain is introduced into a rich medium in sugar Oxytetracycline Glucose Agar OGA medium without Agar, the test tube is maintained during 24h into a stoveat 25°C.

Step 2 : Consists to :

- Preparation of mash of dates of the common dates variety "Tinaceur"studied;
- Sterilization of the mash of dates in an autoclave at 110°C during 20min;
- Adding 9mL of yeast strains in the bioreactor;
- Putting the bioreactor into a water bath heated at 30°C with agitation.

- **Analyzesconducted**

- pH determination of the wine of dates according to the NF ISO 1842-1992 of fruit and vegetable of pH, based on potential gradient method.
- Dosage of the total sugar based on Dubois method (1956).
- Density measurement of the mash of dates by means a digital density meter, graduated at 2g/mL at 20°C, model Fisher.
- Total soluble solids rate TSS is controlled according to the ISO 2173 (2003), standard method, who is reviewed and confirmed in (2016), by using refractometric method[16].
- Determination of alcoholic concentration.

Results and Discussions

The dates sampling allowed the separation of three yeast strains (S1, S2 and S3). The testing results showed that all the sampling ofmashof dates collected from different zones of Adrar region, has relatively uniform yeast strain communities. The communities color is ranging from white to cream and their shapes are spherical or oval. The ambient temperature of southern Algerian remains a crucial factor for the yeast strain growth. Generally the most yeast strains are active between 15 and 35°C. Some strains requires higher temperatures between 40 and 45°C and other strains requires very low temperatures of 0°C to growth[9]. Furthermore, some yeast strains resists well in this hot and dry region. Yeast strains coming from Adrar province growth perfectly on a Sabouraud medium (Figure 3, P1, P1 and P3) and provide three types of community yeast strain purified distinguished showed in (Figure 3, S1, S2 and S3).

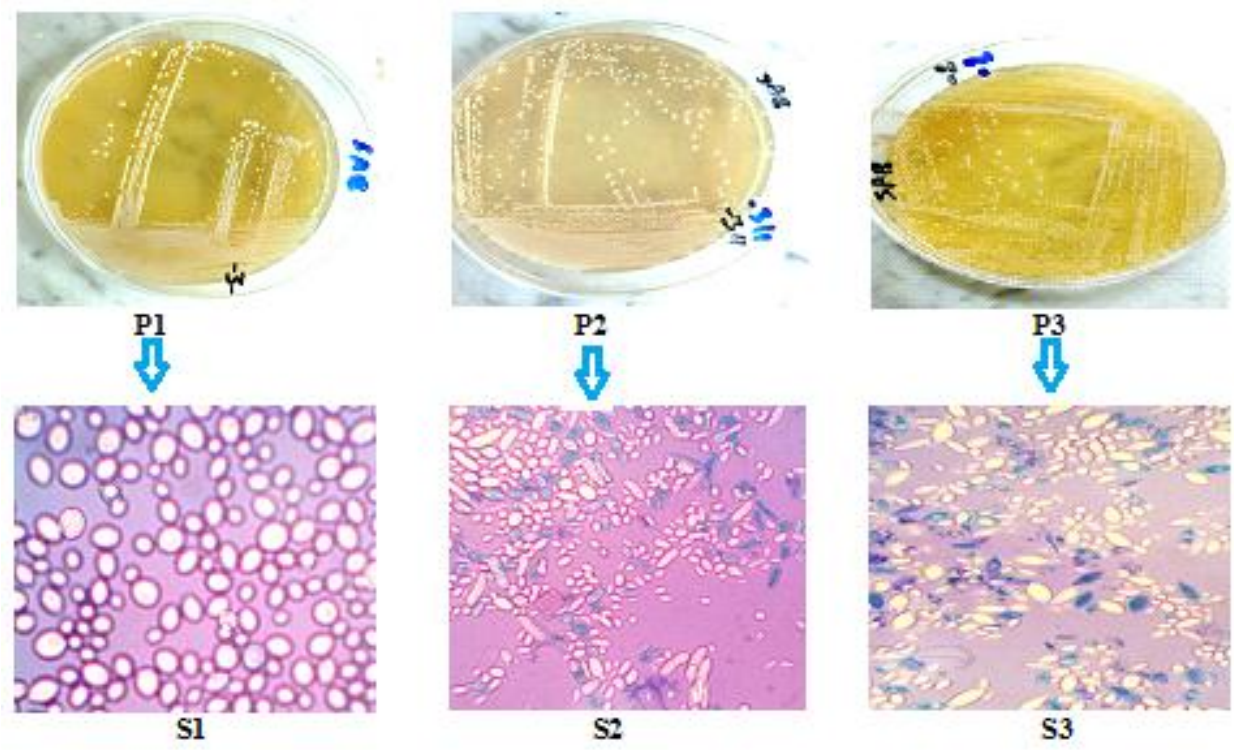

Figure 3: Yeast strains separated (S1, S2 and S3)under microscopic observations(x100).

The pH

The pH is kept constant, the cytoplasmic pH is essential for the survival of the yeast strains. The limits of the pH reported in the literature range from 2.4 to 8.6 with an optimal range between 4.4 and 6.5[17]. The nature of the acidic composition (dissociated form or not) is also important. The Figure 4 showed a slight decrease in pH for the strains studied, due to the formation of the alcohol in fermentation medium. We are observed similar variations of pH of the strain S3 relatively to total strain T. The pH is varied from 5.45 to 4.57 for S1, 5.45 to 4.49 for S2, 5.45 to 4.23 for S3 and 5.45 to 4.1 for the witness strain T. All yeast strain presentsa

remarkable diminution of their pH.

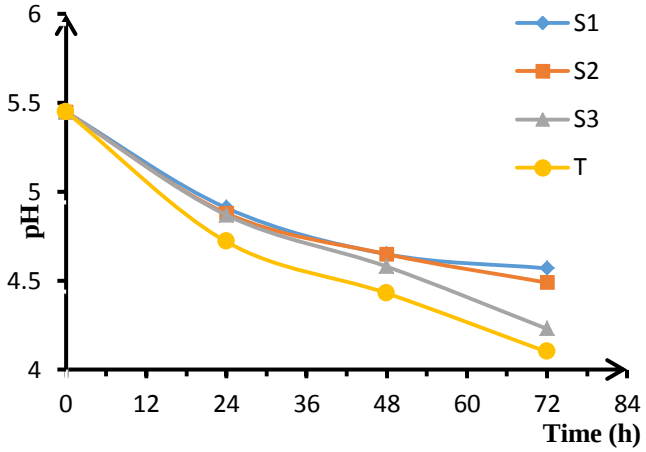

Figure 4: pH kinetics during the alcoholic fermentation

This result showed that the yeast strains can support most organic acids until pH 4.5. By against, their growth is inhibited by the high concentrations of lactic, citric and acetic acids and they are even more so with the sorbic and propanoic acids. Other stresses can modify this pH has been showed in[17], where the ethanoic stress causes a drop in cytoplasmic pH, which induces the death of cells. This intracellular decrease of pH can be due either to an influx of protons[18] or a proton intermediate accumulation of the chemical reaction such as acetic or glycerol acids[19].

Density

The Figure 5 showed a remarkable diminution of the density during the time for all strains studied from 1.075 to 1.029 (4.28%); 1.075 to 1.021 (5.023%) and 1.075 to 1.015 (5.58%) respectively. This diminution may be explained by the transformation of the glucose to the alcohol and the loss of the mass under CO_2 gas [20, 21].

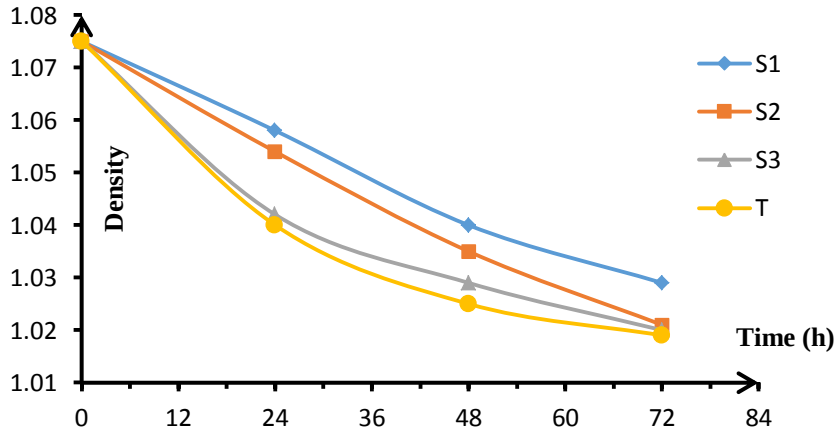

Figure 5: Density during the fermentation process

Total sugar tenor

The carbon compounds are of a great importance for the yeast, since they provide the necessary carbon for the biosynthesis of cells and the energy required for their operations. Carbohydrates are the most frequently used, especially mono-saccharides such as hexoses, disaccharides and tri-saccharides. Other abundant and inexpensive carbohydrates as pentoses and polysaccharides have been the subject of numerous studies in recent

years[22].Under anaerobic conditions, yeasts are capable to ferment glucose to produce ethanol, carbon dioxide, with glycerol co-production, from certain acids and esters[23]. In this metabolism, the final acceptor function of electrons is ensured by organic molecules wherein the yeast first uses Nicotinamide Adenine Dinucleotide NAD^+ as an intermediate electron acceptor which is reduced to NADH. The final reduction of an acetaldehyde into an ethanol and carbon dioxide and maintains the balance of the redox balance in the re-oxidant NADH produced during glycolysis, main degradation pathway to pyruvate sugar. The energy balance of this transformation is described by the following chemical reaction:

$$C_6H_{12}O_6 + 2Pi + 2ADP \rightarrow C_2H_6O + 2CO_2 + ATP \ (20kCal) \quad (1)$$

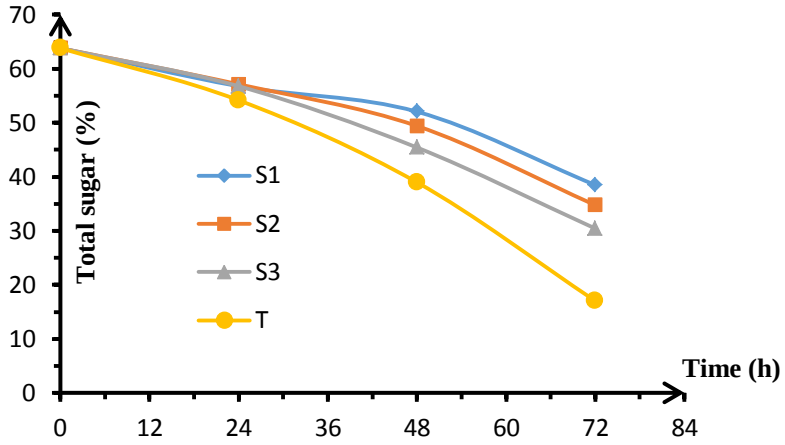

Figure 6: Total sugar during the fermentation process

The Figure 6 showed remarkable degradations of sugars, which is observed especially in the strain S3 relatively to the witness T, where the total sugar content decreases from 63.81% to 38.49% for S1; to from 63.81% to 34.77% for S2, from 63.81% to 30.44% for S3 and approximately from 63.81% to 17.05% for the strain witness T. The bioconversion process is active essentially during the first 48 hours for all strains studied due to the large assimilation of sugar and great production of biomass. This result is in good agreement with that reported by[24]. The evolution of the alcohol concentration during the fermentation process showed that the kinetics of the alcohol produced is proportional to the sugar content in dates fermented. These results are similar to those found by[25] since they are located between 60 and 80%.

Alcoholic degree

Yeast strains (Figure 7) do not exhibit the same sensitivity to the ethanol production. The ethanol tolerance depends on the composition of the cytoplasmic membranes of yeast cells and in particular its lipids. This composition depends itself from the culture medium and its temperature[26]. The decrease in microbiological parameters during fermentation showed that the fermentation kinetic strength of the strain S1 is better than those of the strains S2 and S3 relatively to witness T. This can be explained by the fact that S1 supports species that are more resistant to high alcohol concentration like the yeast species *Saccharomyces cerevisiae*.

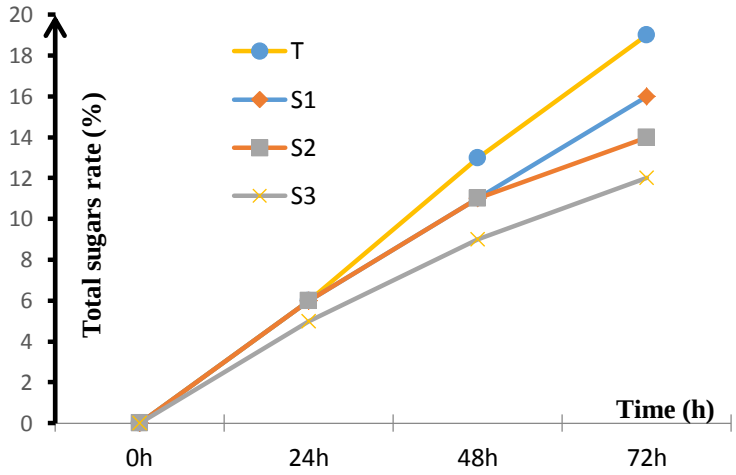

Figure 7: Alcoholic degree during fermentation process

During the alcoholic fermentation, the ethanol represents the principal cause of the strain and becomes toxic at concentrations ranging from 8 to 18% W/V during the culture which limits its production according to its physiological state and yeast strain used. Once, the concentration of ethanol increases in the culture medium, the rate of growth, cell viability, metabolic activity and yeast production capacity are decreased. Many studies have confirmed these phenomena, especially in *Saccharomyces cerevisiae* strain[27-30].

Conclusion

The common dates of low marketing value constitute a good biomass substrate for making an industrial ethanol process. Therefore, these scraps of dates can be successfully used for the manufacture of yeasts due to the richness of their mashes in sugar. By taking in account the simplicity of the biotechnological transformation process, suchindustry should be implemented immediately in phoenicical regions for helping to sustain in partly the national economy.

From the present study the following conclusions are noticed:

- We are able to isolate a new yeast strain from common dates (Tinaceur) originated of Adrar region in southern Algeria effective and more cheaper than that imported from foreigner countries based on fermentation process.
- Chemical and physical analysis of the mash of datescoming from the fermentation process of the variety “Tinaceur” in presence of the yeast strain naturally isolatedshowed remarkable biodegradable sugars which can be enhance the bioethanol produced compared to the using the witness yeast strain.
- Yeast strains separated in the present study have an optional effect. The yeast strain S1 presents a better production of ethanol compared to the other strains S2 and S3.So the strain S1 does not yet identified, therefore in perspective it is necessary to identify and analyze this strain S1 for better and further uses.

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