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RESEARCH ARTICLE

COMPARATIVE STUDY OF TWO TECHNIQUES CONTROL OF DNA FROM YEAST ISOLATES PRODUCING PECTINASE

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Abstract

Polymerase Chain Reaction (PCR) is a widely used technique in the field of molecular biology to rapidly make very large number of copies of a specific DNA sample for detailed studies. The success of the technique however is dependent on the on the quality, i.e purity of the extracted DNA specimen. The aim of this study was to evaluate the quality of extracted DNA from pectinase producing yeast to determine the suitability of the extraction method to produce pure extract without non-inhibiting substances.In this study, DNA extracts from six (06) isolates of pectinase-producing yeasts were quantitatively and qualitatively analyzed using NanoDrop spectrophotometry and agarose gel electrophoresis methods. These analyses showed that the concentration of DNA extracts from the isolates evaluated by the NanoDrop spectrophotometric method ranged from 403.8 to 1082.4 ng/µL and the purity index A 260/280 was between 2.03 and 2.11. In sum, agarose gel electrophoresis showed that the intensity of the DNA bands was irregular and not necessarily in line with the data provided by the NanoDrop spectrophotometry.

Introduction:-

Polymerase Chain Reaction, (PCR) was developed in 1983 by Karry Mullis and remains an essential tool in the field of molecular biology which is an in vitro method for the amplification of specific DNA sequences. In most molecular biology studies, the extraction and purification of nucleic acids (DNA and RNA) remain key steps (Somma, 2004). The objective of nucleic acid extraction methods is to obtain purified nucleic acids in order to increase the probability of success of the polymerase chain reaction (PCR).

Nucleic acid quality and purity are among the most critical factors for PCR analysis (Somma, 2004). In order to obtain highly purified nucleic acids free of contamination, the application of appropriate extraction methods is essential. Contaminants such as SDS, Phenol, Ethanol, Isopropanol, Sodium Acetate, Sodium Chloride, EDTA, mM Hemoglobin, Heparin, Urea may inhibit the PCR reaction (Somma, 2004). "The quality of extracted DNA is not always optimal because of secondary metabolites that interfere with DNA isolation procedures and PCR reactions (Raoudha et al., 2012). In order to ensure the absence of PCR-inhibiting substances in the sample, nucleic acid quality control assays are mandatory.

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In the case of the molecular identification of microorganisms, particularly yeasts, by PCR-RLPF, the quality of the extracted DNA remains a determining factor (Lopez et al., 2001). In order to ensure the success of PCR, a comparative quality control study of DNA extracted from pectinase-producing yeasts isolated from cocoa mucilage juice was performed. The quality of the extracted DNA was evaluated using two methods, namely Nanodrop Spectrophotometer and agarose gel electrophoresis.

Materials and Methods:-

The microbial material is composed of six (06) yeast isolates from the cocoa mucilage juice. These strains were isolated on YPDA solid medium (10 g/L yeast extract, 10g/L pepton, 10g/L D-glucose and 10g/L agar). Pectinase production was revealed by the method described by Jaafar et al (2006) on pectin agar using copper II acetate.

Methods:

DNA extraction:

The DNA of the six yeast isolates was extracted as follows. A culture of the yeast isolates in YPD liquid medium (10g/L yeast extract; 10 g/L pepton; 10 g/L D-glucose) at 30°C was made and left overnight. The cultures were then transferred into 1.5 mL Eppendorf tubes and centrifuged at 14000 rpm for 10 min. After centrifugation, the supernatants were removed and 200 µL lysis buffer (150 mM ammonium chloride; 10 mM potassium bicarbonate; EDTA 0.1 mM at pH 7) was added to the remaining pellets and the tubes homogenized by vortexing. The tubes were then subjected to thermal shock by incubating at 96°C for 15 min and placing at -20°C for 10 min. The heat shock was repeated twice. After which 200 µL of chloroform was added to the tubes and centrifuged at 13,000 rpm for 10 min to separate DNA from proteins by precipitation. . The supernatants were recovered and 200 µL isopropanol was added and incubated at -20°C for 30 min to precipitate DNA in the form of "pellets". The DNA precipitation was repeated and 300 µL of 70% ethanol was added to the pellets to purify the DNA by solubilizing undesirable impurities. The solution was centrifuged at 12,000 rpn for 10 min and the supernatants removed. . The tubes were dried at 37°C for 30 min after which 50 µL of Tris-EDTA buffer was added and the tubes stored at -20°C (Figure 1).

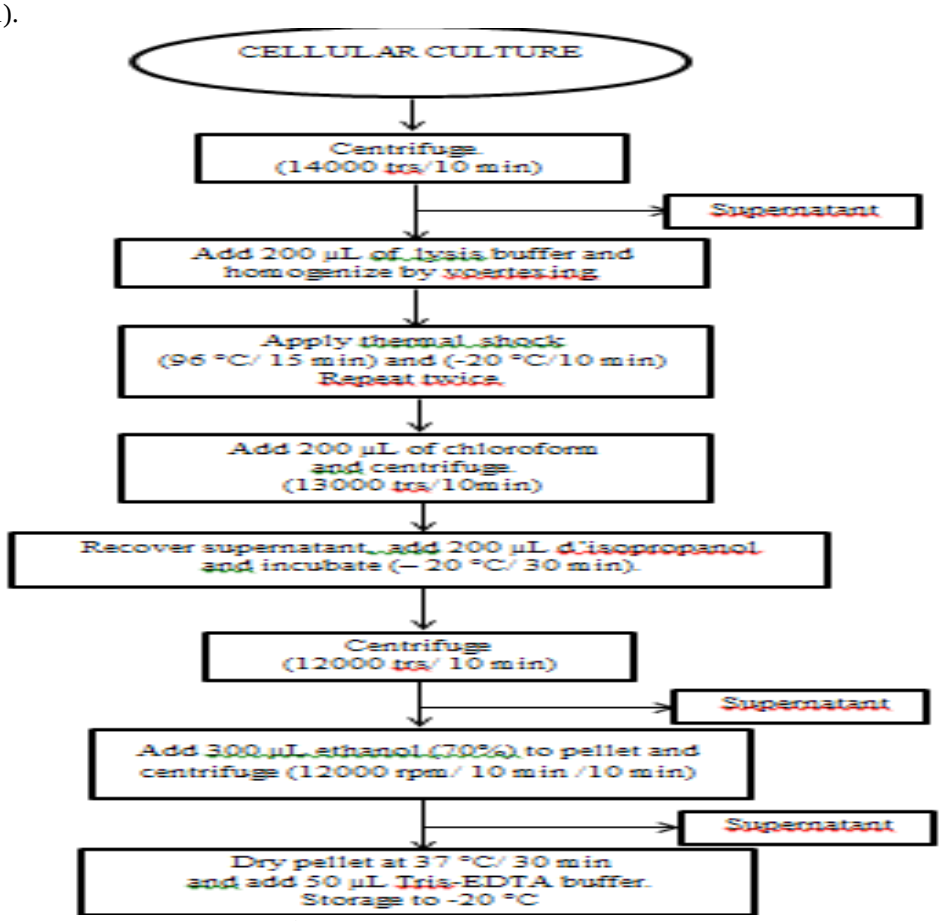

Figure 1:- DNA extraction process.

Determination of extracted DNA:
The quantity of DNA extracted as well as the verification of its quality were made by Nanodrop spectrophotometry to obtain DNA concentration (ng/µL), DO260/DO280 ratio (ratio estimating the purity of the DNA) and observation on agarose gel and staining with ethidium bromide.

NanoDrop spectrophotometry:
Proteins are absorbed at a wavelength of 280nm while nucleic acids are absorbed at a wavelength of 260nm. The absorbance value at 280nm will allow the determination of any contamination of protein origin. For an A260/280 ratio value between 1.8 and 2, DNA is considered pure. An A260/A280 ratio is less than 1.8, indicates contamination of protein origin and a value greater than 2 indicates contamination due to RNA. These measurements were performed using a NanoDrop Spectrophotometer (Thermo Scientific 2000c) to produce micro-volume absorbance spectra without a cuvette or capillary to enable measurements over a wide range of wavelengths (190-850 nm) for the analysis of spectra in the visible and UV. The spectrophotometer was coupled to a computer for data acquisition (Figure 2). The measurement was carried out as follows: raise the sampling arm and pipette one microliter of DNA extract was pipetted directly onto the lower measurement pedestal of NanoDrop spectrophotometer. Lower the sampling arm and initiate a spectral measurement using the software on the PC. The sample column is automatically drawn between the upper and lower pedestals and the measurement is made. When the measurement is complete, raise the sampling arm and wipe the sample from both the upper and lower pedestals using a paperback. The tris-EDTA buffer was used as blank. The data were automatically recorded by computer.

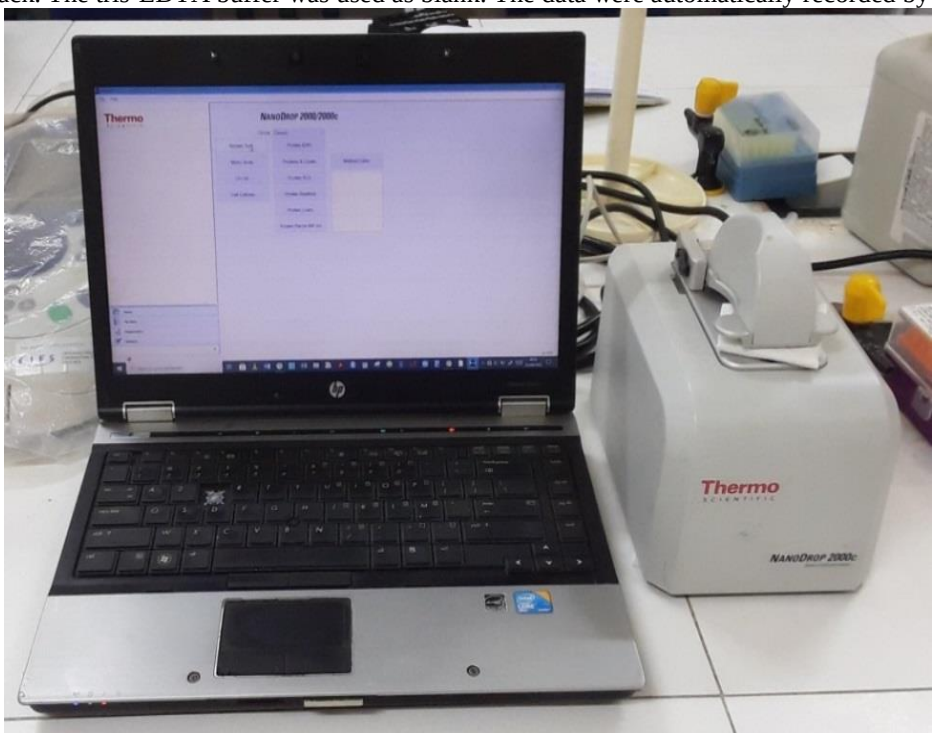

Figure 2:- NanoDrop spectrophotometer associated with computer.

Agarose gel electrophoresis:
To judge if the extraction is satisfactory or not, an electrophoresis was made to study the intensity of the bands to determine the relative order of magnitude of quantity of extracted DNA as proposed by Sonnier, 1999.

The required agarose level (0.8%) was suspended in 50 mL of 0.5X TBE buffer [45 mMTris-Base (Sigma), 45 mM boric acid (Sigma), 1 mM EDTA (Sigma)]. The suspension was heated to boiling and the liquid gel allowed to cool down to 50 °C. Ethidium bromide, 0.4 µg/mL was added before pouring into a gel carrier. Once solidified, the gel was immersed in an electrophoresis vessel containing 0.5X TBE. Aliquots of 10 µL of DNA were spiked with 2 µL of loading buffer (50% TE pH 8, 50% glycerol, bromophenol blue) before being deposited in the gel wells. The gel was first stained in an ethidium bromide solution (2 drops of ethidium bromide in 0.5 X TBE) for 10 min and visualized (Bentchop 2UV Transilluminator UVP, USA) under UV illumination after migration at 100 V for 15 min.

Results:-

NanoDrop spectrophotometry:

The quantity and purity of the extracted DNA are given Table 1. DNA concentrations ranged from 403.8 to 1082.4 ng/µL are relatively high. DNA purity values were slightly higher than 2 and were between 2.03 and 2.11. The lowest concentration (403.8 ng/µL) was observed in isolate 1 while the highest concentration (1082.4 ng/µL) was obtained in isolate 3.

Table I:- DNA extracts concentrations and purity index.

Sample	Concentration ADN (ng/µL)	Abs 260	Abs 280	A 260/280
1	403.8	8.077	3.959	2.04
2	877.1	17.641	8.409	2.09
3	1082.4	21.647	10.434	2.07
4	743.9	14.878	7.311	2.03
5	462.4	9.247	4.515	2.05
6	567.7	11.374	5.374	2.11

Agarose gel electrophoresis:

The analysis of the relative quantity and quality of DNA extracted on agarose gel is presented in Figure 3. This analysis was done by comparing the relative intensity and pattern of migration bands (Table II). An irregularity in the intensity of the bands was observed. The highest band intensity was recorded with isolate 3 which is justified by the higher DNA concentration (1082 ng/µL). On the other hand, isolate 2 with a DNA concentration of 877.1 ng/µL (A 260/280 =2.09) showed low band intensity in contrast to isolate 6 with a DNA concentration of 567.7 ng/µL and a purity index of 2.11.

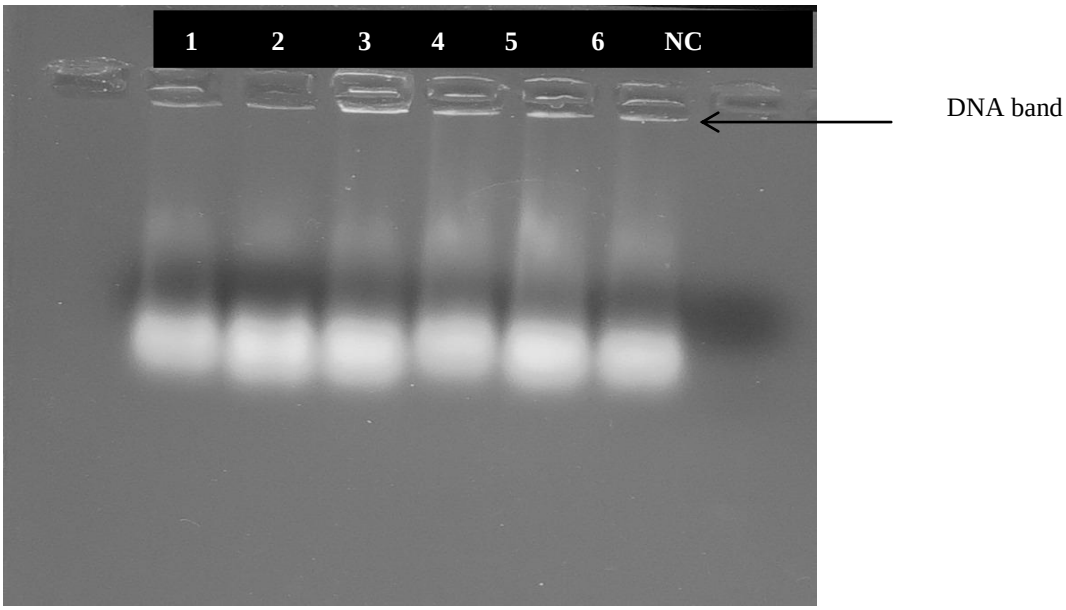

Figure 3:Yeast DNA electrophoresis on 0.8% agarose gel.
1 to 6: Yeast isolates; NC: Negative control

Table II:- DNA bands intensity.

Sample	1	2	3	4	5	6
Intensity of bands	+	+	+++	++	++	++

+ : low intensity
++ : medium intensity
+++ : high intensity

Discussion:-

Quantitative and qualitative analysis of DNA extracts by NanoDrop spectrophotometric methods revealed that high concentrations of DNA (between 403.8 and 1082.4 ng/µL) were obtained but the DNA extracts showed some level of impurities. The purity index values (A 260/280) of the samples being slightly higher than 2, indicate a low contamination of the DNA extracts most probably by the presence of RNA. Indeed, Glasel (1995) pointed out that for an A260/280 ratio of 1.81, the sample contained 60% proteins and 40% nucleic acids. However, Wilfinger et al, (1997) pointed out that a significant variation in this ratio could be a function of pH and ionic strength and that it was therefore important to take these parameters into account in the interpretation of this ratio. Also the A 260/280 ratio highest in this study could indicate that the DNA extracts contained most nucleic acid than proteins. From this evaluation using Agarose gel electrophoresis, it appears that the intensities of the bands observed on the gel are well parallel to the concentration values of extracted DNA for the different samples precisely for isolates 2 and 6. The same observation was made by Sonnier (1999), where the values of the concentrations of DNA extracted from Anopheles dirus and the purity index determined by spectrophotometry were parallel to the intensity of the bands observed on the agarose gel.

Conclusion:-

Quantitative and qualitative comparison of DNA extracts using NanoDrop spectrophotometry and agarose gel electrophoresis methods showed that the results were not always in agreement. These discrepancies could be due to several factors (manipulation, the physiological state of the microorganisms...). Therefore, it is important to carry out extraction optimization studies in order to guarantee a better quality and a significant quantity of extracted DNA. The purity of extracted DNA can be optimized by treating the extracts with the enzyme RNAaseand Proteinase K to digest RNA and protein that may be present in the DNA samples.

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