RESEARCH ARTICLE

The DNA Extraction from Chlorella Algae, and its Preparation for Real Time-polymerase Chain Reaction

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ABSTRACT

Our current research involved the extraction of DNA from chlorella algae for prepare it to real-time polymerase chain reaction (RT-PCR) After the collection of algal samples from the riverine quarter environment, they were placed in test tubes, about (1-mL) and centrifuged at (3500 rotation for 5 minutes), with placed a control negative sample for certain the success of the practical steps sequentially of the experiment, and to know the effectiveness of the lytic enzymes, and the reagents which used. When the microalgae are visible down each test tube by watching its green color, 400 µL of the lytic solution add to the remaining sample with mixing, and 10 µL of an enzyme proteinases K are added to them, which helps to break down the walls and membranes of the cells. The incubated of samples at a temperature of 65°C for 1-hour, followed by the addition 400 µL of chloroform phenol (it plays the role of breaking down DNA-related proteins) with shaking, and the samples are centrifuged again to take the upper layer as it represents the DNA of the algal samples, they are withdrawn and transferred to a new pentroph. A 5 µL RNA-ase enzyme is added to them to destroy the RNA and preserve the DNA. Added (500 mL of isopropanol), with mixing well by hand, and centrifuged at 12,000 rotation, for 10 minutes, alcohol removed and DNA extracted prepared for the PCR reaction.

Keywords: Chlorella algae, DNA extraction, real-time polymerase chain reaction.

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INTRODUCTION

Algae are living organisms that live in water of various kinds, whether fresh or salty, and algae are distinguished by their green color because they contain the green pigment, whereas the rest of the algae have different pigments depending on the nature of the water in which they live. Extracting DNA in the aquatic environment differs from that in the terrestrial environment, as extracting it from terrestrial algae (especially those developed in the desert) is difficult. The reason for this is the development of their enlarged cell walls when they are adapted to the same terrestrial environments.[1] Typically, the methods that are used to improve the goodness of extracted DNA have concentrated on purification, as many parameters have the most effect on the success of useful applications (activity of restriction enzyme, hybridizations, polymerase chain reaction (PCR). The purification of DNA from the algal samples can be increased by some extraction protocols that depend on the cetyl-trimethyl-ammonium bromide (CTAB) extraction method[2] or by the selection of species-appropriate extraction buffers.[3] Some Chlorella algae live in clean water, which is free of various kinds of waste and organic matter as well, and some beneficial environmental transformations occur through their presence in the water, called self-purifying transformations. chlorella algae is one of the extreme trade-used species.[4,5] This algae have a stimulation effect on the growth in of agricultural plants[6,7] and can also inhibit the growth of pathogenic microorganisms.[8] To obtain chlorella's biomass in a short time for use in research experiments and scientific, medical, and industrial applications, it is necessary to develop optimal methods for its cultivation and preservation. Biodiesel production, in addition to the possibility of using it as an organic fertilizer,[9] and in the (treatment, and transformation) of wastewater that contains very high percentages of ammonia gas through its reaction with other materials and other compounds that eventually lead to the existence of suitable solutions for this and other medical purposes. Other industries that can be used.[10] Algae are one of the most primitive organisms found on earth, with some evidence of fossils dating back nearly three billion years ago. Algae include prokaryotic and eukaryotic organisms,

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Table 1: The amounts of solutions and reagents, or measurements of the samples with the additives, through the practical steps.

Details of steps	*Amounts of solutions and reagents, or measurements of the samples through the practical steps.*				
1- Lytic solution	Chlorella algae µL	(100) (-)	(200) (-)	(300) (-)	(400) (+)
	Control negative sample	(-)	(-)	(-)	(-)
2- Proteinases K enzyme	Chlorella algae µL	(4) (-)	(6) (-)	(8) (+)	(10) (+)
	Control negative sample	(-)	(-)	(-)	(-)
3- Chloroform phenol	Chlorella algae µL	(100) (-)	(200) (-)	(300) (-)	(400) (+)
	Control negative sample	(-)	(-)	(-)	(-)
4- RNAase enzyme	Chlorella algae µL	2 (-)	3 (-)	4 (-)	5 (+)
	Control negative sample	(-)	(-)	(-)	(-)
5- Isopropanol	Chlorella algae mL	200 (-)	3 (-)	400 (+)	500 (+)
	Control negative sample	(-)	(-)	(-)	(-)

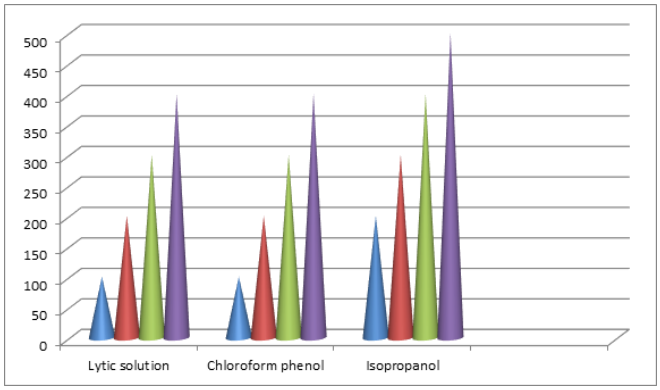

Figure 1: Represents the amounts added for each of (lytic solution, chloroform phenol, isopropanol) during the practical steps of the experiment, which include DNA extraction from chlorlla algae samples.

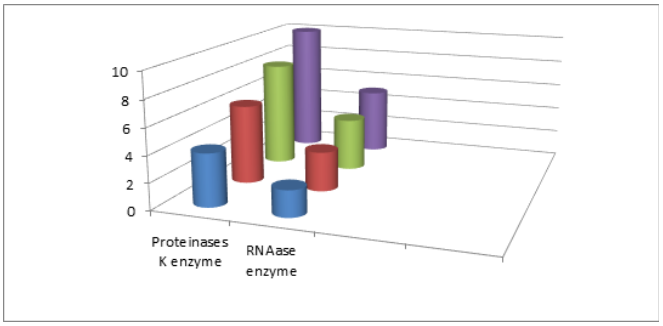

Figure 2: Represents the amounts added for each of (Proteinases K enzyme, and RNAase enzyme), during the practical steps of the experiment, which include DNA extraction Chlorlla algae samples.

but they are currently considered the only eukaryotes in the current classification. There are many aspects that require the development of applied research on algae in general, whether marine or riverine ones, in order to reach products that enrich the economic reality as they contribute to various discoveries, including what is useful in the health food industry because the source is considered natural because algae are plants, and including what is useful in the manufacture of various medicines, since algae contain the most important elements and nutritional compounds needed to build the human body, such as unsaturated fats and other vitamins rich materials, continuing research work, using modern technologies enables us to ensure the conservation of the species and to monitor the most important changes that occur to it due to the increase in environmental pollutants, and the different nature of the environment in which the algae live and its relationship with the rest of the competing organisms over time.

MATERIALS AND METHODS

Materials

Each test tube contains (1-mL) sample of chlorella algae, negative sample, plastic test tubes, centrifuge, lytic enzymes, proteinase K enzyme, incubator, chloroform phenol, shaker, RNAase enzyme, isopropanol, PCR reaction device.

Methods

- Chlorella algae collected from the riverine quarter environment of Karbala city, and 20 test tubes were taken, each one is full of 1-mL of the chlorella algae sample.
- Centrifuged them at about (3500 rotation for 5 minutes), with placing a control negative sample to determine the success of the practical steps of the experiment, and the validity of the effectiveness of the lytic enzymes, and the used reagents.
- After that, the presence of microalgae is checked (by watching its green color), then the supernatant liquid is disposed of and added to the remaining precipitate (400 µL) of a lytic solution, the remaining sample is mixed with the solution as well as the control negative sample also is mixed with the solution by vibrating for (10 minutes).
- Then (10 µL of an enzyme proteinase K) is added to them, which helps to break down the walls and membranes of the cells. The samples are then placed in the incubator at a temperature of (65°C for 1-hour) to allow the enzyme to perform.
- Then, (400 µL) of chloroform phenol is added (it plays the role of breaking down DNA-related proteins), and the contents are shaken, then placed in the shaker for (10 minutes), and the samples are centrifuged again.

Table 2: shows the correlation between Proteinases K enzyme and RNAase enzyme. Its significant at about the 0.01 level (2-tailed).

		P	*R*
P	Pearson correlation	1	1.000**
	Sig. (2-tailed)		.000
	Sum of squares and cross-products	20.000	10.000
	Covariance	6.667	3.333
	N	4	4
R	Pearson correlation	1.000**	1
	Sig. (2-tailed)	.000	
	Sum of squares and cross-products	10.000	5.000
	Covariance	3.333	1.667
	N	4	4

- Upper layer be taken as it represents the DNA of the algal samples. They are withdrawn and transferred to a new pentroph, and 5 µL RNAase enzyme is added to it to destroy the RNA and preserve the DNA. And for the precipitation of the DNA, we add 500 µL of isopropanol, then mix well by hand, then centrifuge the component in (12,000 rotations, for 10 minutes) for the DNA to be deposited. The alcohol is removed from it and preserved for preparation for the PCR reaction (it's a modification of normal PCR to the extracted DNA), then measuring the amount of DNA of the sample during PCR process.

RESULTS AND DISCUSSION

Some different methods and techniques can be carried out in the laboratory to obtain the pure DNA of the chlorella algae, including this method that we are about to study to reach the desired results. This method includes preparing the DNA to be ready to carry out the real-time PCR reaction, through which the knowing the viability of the chlorella algae alive in the aquatic environment, and the ability or possibility of the algae to withstand (toxins, and pollutants, contaminants) in the water, and the preservation of the species through which it is possible to build biological, and industrial facilities leading to the production of various products that serve in developing the economic reality in the country, and achieving sufficiency self-promotion, activating industrialization and marketing it to other countries. By collecting chlorella algae (the well-known green color) from the rivers of the city of Karbala, which represents fresh water, its purity and cleanliness must be preserved to ensure that there are no impurities attached to it or accompanying it. Workable samples are taken in 20 test tubes so that each tube contains (1-mL) of algae culture. This method is used for a piece of DNA that is known to almost algal species. The genomic DNA extraction from the chlorella algal specimens and the interesting location is (amplified and estimated) by real-time polymerase chain reaction (RT-PCR). A diluted sample in the amplifying of DNA is heated to break the hydrogen bonds, giving two-single strands of DNA. In this method, the single nucleotide polymorphisms (SNP) genotype was determined successfully.[11] The main purpose of the centrifugation process is to separate the components that make up the algae sample, so we take what is deposited at the bottom of the tube and discard the liquid that separated to the top. Table 1 shows in detail all the ideal ratios of solutions and reagents that are required to be available in the extraction process within successive stages, including what was clarified in Figure 1 as positive results; (lytic solution 400 µL, chloroform phenol 400 µL, isopropanol 400 µL), respectively, and what was clarified in Figure 2 as positive results (proteinases K enzyme 10 µL, RNAase enzyme 5 µL), respectively. These proportions and quantities if they serve the purpose in their interaction if it was less than it is, while the control negative is still inactive as an indicator of the activity of solutions and reagents through the processing of the DNA extraction from the chlorella algal samples. The correlation between proteinase K enzyme and RNAase is significant, as shown in Table 2, because it is at about 0.01 level, and the descriptive statistics are shown in the statistical analysis (range, minimum, maximum, mean, Std. deviation, and variance) as shown in Table 3, to know all the effect of each amount through the practical side in the experiment. The long of DNA fragments is crucial for high-quality libraries preparation and, so far efficient genomes assembly using long readings.[12] Almost studies agree that there are many advantages of chlorella algae. Among them is the production of algal bio-diesel, which is an energy-intensive process.[13-15] They also do not have a common ancestor. Often when he mentions algae, an image of green swamps or flowers that are poisonous to algae comes to mind.[16] In RT-PCR, the measurement of DNA occurs after each cycle by fluorescent dyes, which yield an increased fluorescent signal in the direct proportions to the numerous PCR product molecules (in amplification) generated. The data collected in the exponential phases through the reaction yields quantitative information on the starting quantity of the amplification target. There are many advantages to real-time PCR, such as; the ability to monitor the signs of progress of the PCR as it occurs in real-time. The precise measurement of the number of pieces at each cycle allows widely accurate quantification of the starting materials in specimens, increasing the dynamic range of many detections (amplification and detection) that occurs in a single tube and eliminating the post PCR manipulations. The interest in culturing the alga chlorella by methods and techniques chosen by the researcher himself enables us to address many of the environmental problems that aquatic nature suffers from, such as removing some microorganisms and the survival of others that feed on algae.[17] Some disadvantages can be mitigated by careful selection of strains that can be grown in wastewater at low temperatures.[18] Chlorella algae is characterized by its ability to rid the body of various types of toxins as it acts as a detox. Many scientific studies on animals have shown that chlorella alga can remove heavy metals to which the body is exposed, such as cadmium and lead, in addition to reducing the excess of the most important minerals in the body, such

Table 3: To show the; Range, minimum, maximum, mean, std. deviation and variance as a descriptive statistics of proteinases K enzyme, RNAase enzyme.

	N	*Range*	*Minimum*	*Maximum*	*Mean*		*Std. deviation*	*Variance*
	Statistic	*Statistic*	*Statistic*	*Statistic*	*Statistic*	*Std. error*	*Statistic*	*Statistic*
P	4	6.00	4.00	10.00	7.0000	1.29099	2.58199	6.667
R	4	3.00	2.00	5.00	3.5000	64550	1.29099	1.667
Valid N (listwise)	4							

as iron. copper is toxic when it is in high concentrations. In addition to that, chlorella reduces the amount of many harmful compounds found in basic human foods, and chlorella helps to support, stimulate and enhance the work of the immune system and works to stimulate the production of as many bodies as possible. In addition, it reduces cholesterol levels in the blood because it contains fibers that act as antioxidants (carotenoids and niacin). It also works to prevent and fight some chronic diseases through powerful antioxidants, such as in (vitamin C) (chlorophyll, lycopene). Chlorella algae accumulate high levels of useful lipids as tri-acyl-glycerols (TAG).[19] There are special techniques that can be carried out on chlorella algae that lead to the production of products to protect the arteries from hardening and maintain the level of blood pressure within the normal limit, in addition to maintaining the health of the heart and kidneys, improving the health of people with respiratory diseases, and reducing the level of sugar in the blood and insulin regulation.

CONCLUSION

We conclude from our current research study that the algae chlorella can be classified as a medicinal plant, one of the most important medicinal plants that can be benefited from at all levels, because the research work includes various advanced techniques on this type of algae enable us to manufacture various products that are used in daily life and improving the economic reality of the country: So, we consider it our current research as applied research that contains constructive ideas that lead us to broad aspirations in the field of science and knowledge.

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