

## Isolation of tRNA From Uropathogenic Escherichia Coli

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### ABSTRACT:

#### BACKGROUND:

Transfer RNA is a type of RNA which during protein synthesis act as an adaptor molecule, matching amino acids to their codons on mRNA. tRNA also functions in the formation of cross links during peptidoglycan synthesis.

#### AIM OF THE STUDY:

The aim of this study is that, extraction of tRNA from uropathogenic *Escherichia coli* then detect the presence of such molecules after extraction and measure the purity of the tRNA extract solutions.

#### METHODS:

Thirty uropathogenic *E.coli* isolates were isolated from hospitalized and non hospitalized patients, complaining of urinary tract infections, of Al-Kadhymia Teaching Hospital and subjected to tRNA extraction. A method of tRNA extraction was modified by adding sodium dodecyl sulfate (SDS) instead of urea. Polyacrylamide gel electrophoresis and two methods of staining, ethidium bromide staining and silver staining, as well as spectrophotometric detection were used.

#### RESULTS:

Ethidium bromide stained gel reveals bands with molecular weight less than yeast rRNA. Silver stained gel shows bands with molecular weight less than ova albumin (45000 dalton) but with approximate molecular weight of chymotrypsinogen (24000 dalton).

#### CONCLUSION:

The tRNA extracts were relatively pure with the modified method of extraction. In the present study, a modification of polyacrylamide gel electrophoresis to detect tRNA and to determine their molecular weight was applied.

**KEY WORDS:** tRNA isolation, uropathogenic *e.coli*

### INTRODUCTION:

Transfer RNA is a type of RNA which during protein synthesis act as an adaptor molecule, matching amino acids to their codons on mRNA<sup>(1,2,3)</sup>. tRNA also functions in the formation of cross links during peptidoglycan synthesis<sup>(4)</sup>.

Many studies had been performed to determine the components of tRNA and were found that tRNA composed of polynucleotide chain, each nucleotide consists of four major nitrogen bases which are

guanine, cytosine, adenine and uracil as well as phosphoric acid and ribose sugar<sup>(5, 6)</sup>.

The tRNA tend to give rise to internal base pairs and are therefore folded to some degree (cloverleaf structure). Various modified nucleotides are found in tRNA, occupying specific 75-85 nucleotides long and the molecular weight of tRNA about 25000 dalton and positions in polynucleotide chain and affect secondary structure and interact with cellular component<sup>(7)</sup>.

The molecules of tRNA were extracted before by different ways<sup>(8, 9)</sup>. In the present study, extraction of tRNA from uropathogenic *E.coli* and detection of the presence of such molecules with our modified polyacrylamide gel electrophoresis and agarose gel electrophoresis was planned.

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## MATERIALS AND METHODS:

Uropathogenic *E.coli* isolates: Thirty Uropathogenic *E.coli* isolates were isolated from hospitalized and non hospitalized patients, complaining of urinary tract infections, of Al-Kadhymia Teaching Hospital their ages ranging from 6 months to 70 years.

Extraction of tRNA : Transfer RNA extraction solutions were prepared according to Varshney(1991)<sup>(10)</sup>. These solutions were resuspended buffer (a mixture of 0.3 M of sodium acetate-Analar\UK-and 10 mM EDTA –BDH\UK- PH=4.5) .Phenol (BDH\UK) – chloroform (Surechem\UK) 5:1,PH=4.5 ,sodium acetate solution (10 mM sodium acetate ,PH=4.5 ) and 8M lithium chloride solution (BDH\UK).The tRNA was extracted according to Varshney(1991)<sup>(10)</sup>.Using spectrophotometer, the quality was checked by measuring the absorbance at 260\280.

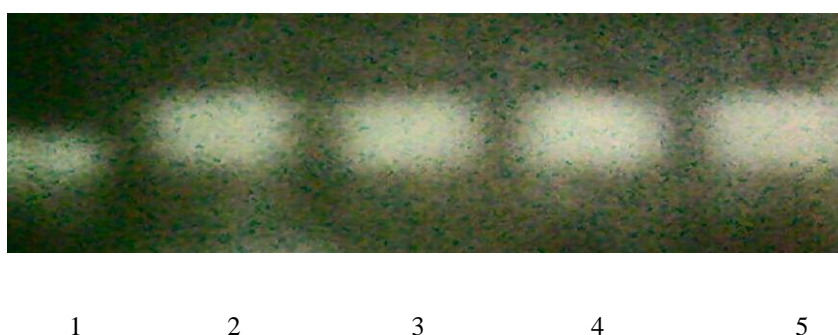
Electrophoresis of tRNA: Six and half percent of polyacrylamide gel was prepared as described by Abelson and Simon (1990)<sup>(11)</sup>but with a modification of Varshney(1991)<sup>(10)</sup>.Polymerized gel contains 10% sodium dodecyl sulfate (BDH\UK) as well as 1M sodium acetate .The extracts of tRNA were concentrated approximately 10 times with superfine sephadex G-25 (Pharmacia\USA) before electrophoresis . Ova albumine , chymotrypsinogen and yeast rRNA (BDH-UK) serve as markers during electrophoresis

The gel after electrophoresis was divided into two parts, the first contains yeast rRNA was submitted to ethidium bromide staining and the second which contain, protein markers, submitted to silver stain procedure.

Gel staining : The gel soak in 0.05% of ethidium bromide (BDH-UK)and the bands were visualized by gel exposure to 260nm UV light .The revealed bands photographed by a paloroid MP-4 camera with type 667 film <sup>(12)</sup> .The second part of gel was stained with silver stain .The staining solutions and staining procedure were accomplished according to Abelson and Simon (1990)<sup>(10)</sup>.Five percent and 10% ethanol(Merck\Germany) and 5% acetic acid ,10% glutaraldehyde,ammoniacal-silver nitrate (BDH\UK) and formaldehyde (Fluka-Switzerland)were prepared for such procedure.

## RESULTS:

Ethidium bromide stained gel reveals bands with molecular weight less than yeast rRNA(figure-1).Silver stained gel shows bands with molecular weight less than ova albumin(45000 dalton)but with approximate molecular weight of chymotrypsinogen (24000 dalton), figure-2. The ratio of the absorbance of tRNA extracts at 260 nm and at 280 nm was between 1.54 – 2.0.



**Figure-1: Ethidium bromide stained polyacrylamide gel electrophoretogram of tRNA .**  
**Lane 1: rRNA molecular marker, Lanes 2,3,4,5 : represent tRNA bands of uropathogenic E.coli.**

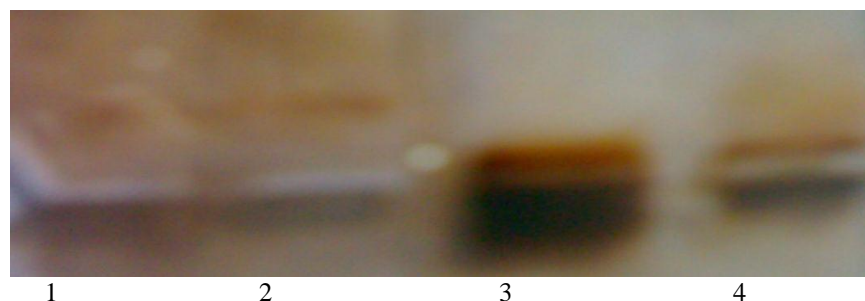


Figure -2: Silver stained polyacrylamide gel electrophoreticogram of tRNA .

Lanes 1,2: are tRNA bands of uropathogenic *E.coli*

Lanes 3,4: molecular markers, ova albumin (45000 dalton) and chymotrypsinogen (24000 dalton).

## DISCUSSION:

Nucleic acids are extracted with the aid of substances that denature the proteins with which they are associated with sodium dodecyl sulfate (SDS) or water saturated phenol depends on the type of nucleic acid (RNA or DNA) and on the nature of the contaminants (whether lipid containing or not) .Analysis are facilitated by the fact that RNA is completely hydrolyzed by concentrations of alkali that don't hydrolyze DNA<sup>(7,13)</sup>. According to these facts we recommended acidic PH during tRNA extraction in spite of that tRNA molecules are relatively resistant to be hydrolyzed by alkaline PH and also by ribonucleases .This feature is due to the presence of methyl group or occasionally other groups and these modifications make possible to folded and conformational flexibility<sup>(14, 15)</sup>.

In the present study , polyacrylamide gel electrophoresis was modified by substituting 8M of urea that has been used in the original reference by SDS and by adding 1M sodium acetate .Two methods of nucleic acid staining procedures were used, ethidium bromide staining and silver staining .The first used to visualized the fluorescence feature of tRNA under UV light and compare their size with the size of yeast rRNA, while silver stain used to reveal both tRNA and protein markers and facilitate a comparison between their molecular weight with that of tRNA.

Proteins are usually the major contaminants of nucleic acid extracts and these have maxima absorbance at 280 nm .The ratio of absorbance at 260 and 280 nm between 1.8 and 2.0 is indicative of fairly pure nucleic acid preparations ,but values less than 1.8 signify presence of proteins as impurities<sup>(16)</sup>.In our study 260/280 ratio was between 1.54 -2.0 this probable indicative to the presence of amino

acids ,in some tRNA extracts ,that are bind to the tRNA molecules during protein synthesis .

## CONCLUSION:

relatively pure tRNA were extracted from uropathogenic *E.coli* . Using two types of electrophoresis , agarose gel electrophoresis and sodium dodecyl sulfate polyacrylamide gel electrophoresis , tRNAs were detected and determined their molecular weight. The purity is checked for all tRNA extracts using spectrophotometer. On the base of these facts ,the extracted tRNAs were relatively pure and can be used for further studies.

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