WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 421-427. Research Article ISSN 2277– 7105

# DETERMINATION OF THE MOL% G+C VALUES OF BACTERIAL DNA ISOLATED FROM AL-BAIJY OIL REFINERY WORKERS, IRAQ

**Dr. Mohemid M Al-Jebouri*[1] and Ashwaq N Al-Doori[2]**

[1]Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq.

[2]Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq.

Article Received on
05 April 2015,

Revised on 25 April 2015,
Accepted on 17 May 2015

***Correspondence for Author**
**Dr. Mohemid Al-jebouri**
Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq.

## ABSRACT

**Background:** A number of air pollutants have been consistently linked to chronic obstructive pulmonary disease including asthma which has emerged as the principal occupational infectious lung diseases. The first unique feature of DNA that was recognized as having taxonomic importance was its mole percent guanine plus cytosine content (Johanson, 1981). Among the bacteria, the mol% G+C values range from 25-75 and the value is constant for a given organism. Closely related bacteria have similar mol% G+C values. **Methodology:** Genomic DNA was extracted from 15 unknown bacterial isolates from respiratory systems of oil refinery workers and standard strain of *Escherichia. coli* mj83 (*the mol % G + C of the DNA is*: 50.9) as control by the salting-out. **Results:** Five samples were with high purity ranged between 1.7-1.97, eight samples showed purity between 1.51- 1.61, two samples were 1.05 and 1.11 and one was 2.5. **Conclusions:** Three oxidative isolates showed low purity of DNA also showed unstable readings of UV absorbance at 260 nm, so that it was difficult to find the correct $T_m$ for each of them. This might be due to presence of pollutants which interact with the absorbance of DNA at 260 nm.

**KEYWORDS:** Bacteria, DNA G:C ratio, Oil refinery workers, Al-Baiji, Iraq.

## 1. INTRODUCTION

The first unique feature of DNA that was recognized as having taxonomic importance was its mole percent guanine plus cytosine content.[1] Among the bacteria, the mol% G+C values range from 25-75 and the value is constant for a given organism. Closely related bacteria have similar mol% G+C values. However, it is important to recognize that two organisms

that have similar mol% G+C values are not necessarily closely related; this is because the mol% G+C values do not take into account the linear arrangement of the nucleotide in the DNA. During the controlled heating of a preparation of double-stranded DNA in an ultraviolet spectrophotometer, the absorbance increases by ~ 40%. This is due to the disruption of the hydrogen bonds between the base pairs that link the two DNA strands. The temperature at the midpoint of the curve obtained by blotting temperature versus absorbance is called "melting temperature," or $T_m$. The $T_m$ is correlated in a linear manner with mol% G+C of content of the DNA.[2] The higher the $T_m$, the higher the mol% G+C of the DNA.[3]

## 2. MATERIALS AND METHODS

Isolation and identification of bacteria

Refinery description,bacterial isolation and identification were previously described elsewhere.[4]

***Determination of G+C %:***

**Extraction of genomic DNA:** Genomic DNA was extracted from 15 unknown bacterial isolates and standard strain of *E. coli* mj83 (*the mol % G + C of the DNA is*: 50.9) as control by the salting-out method of Pospiech and Neumann[5] that modified by Kieser[6]. Bacterial cultures were grown in 100 ml Brain Heart Infusion broth with rotating and precipitated at 8000 r.p.m. for 12 min at 4°C. Samples were resuspended in 5 ml SET buffer (75 mM NaCl, 25 mM EDTA pH 8, 20 mM Tris pH 7·5). 300µl Lysozyme (30mg $ml^{-1}$ concentration) was added and samples were incubated at 37 °C for 80 min. with shaking. 600µl SDS (10% w/v final concentration) then added and samples were incubated at 55°C for 1:50 h, 2 ml of 5 M NaCl was added; the samples were mixed and allowed to cool to less than 37°C. Chloroform (5 ml) then added and the samples were incubated at 4°C for 30 min with shaking. Cell debries was precipitated by centrifugation at 4500 r.p.m. at 4°C and supernatants transferred to a 15 ml tube. DNA was precipitated with 0·6 vol. 1 sopropanol at -20 °C for 18 min and recovered by centrifugation at 8000 r.p.m. for 15 min. DNA pellets were left to dry then re-suspended by 100 µl warm sterile distilled water.

**Estimation of DNA concentration:** The concentration was calculated from the optical density at 260 nm using UV-Visible Spectrophotometer and TE buffer as a control solution according to the following equation:

Unknown µg/ml = 50 µg/ml × Measured A260 nm × Dilution factor.[7]

Determination of DNA purity: Purity of isolated DNA was measured by dividing the absorbance value of diluted sample at 260 nm on the absorbance value at 280 nm.[8]

**Determination of $T_m$:** DNA was carefully dispersed in distilled water, and the thermal denaturation curve was determined stepwise by using an adjustable water bath, the temperature of the DNA solution was measured by using a thermometer placed in the liquid. A few temperature measurements versus absorbance readings were made by the UV Spectrophotometer (Aquareius, Germany) till reaching the melting temperature ($T_m$).[9]
The %G+C was calculated from the equation
%G+C = 2.44 ($T_m$ - 69.4).[10]

## 3. RESULTS AND DISCUSSION

***Determination of the mol% G+C values of Bacterial DNA***

DNA preparation was tested for purity and concentration because when working with DNA, it is often important to know its concentration, and to prepare a concentration ranges between 5 – 20 µg/ml for each tested sample suitable for measuring melting temperature. The easiest means for determining DNA concentration is through spectrophotometric analysis. Since nitrogenous bases absorb UV light, the more concentrated the DNA solution, the more UV light it will absorb a wave length of 260 nm with use of TE buffer. The concentration of pure double-stranded DNA with an $A_{260}$ of 1.0 is 50µg/ml. Thus, the following formula was used to determine the DNA concentration of a solution.
Unknown (µg/ml)/ Measured $A_{260}$ = 50 (µg/ml)/ 1.0 $A_{260}$.[11]

Calculation of DNA purity was done by measuring the ratio $A_{260}/A_{280}$. The most common purity calculation is the ratio of the absorbance at 260 nm divided by the absorbance at 280 nm. Good-quality DNA will have a ratio of 1.7–2.0. A reading of 1.6 does not render the DNA unsuitable for any application, but lower ratios indicate more contaminants are present.[12]

Table 8 shows the DNA concentration, purity, melting temperature and correlating mol% G+C values for the strains tested. Five samples were with high purity ranged between 1.7-1.97, eight samples showed a purity between 1.51- 1.61, two samples were 1.05 and 1.11 and one was 2.5. According to Oswald[13] a good quality of DNA samples should have a $A_{260}/A_{280}$ ratio of 1.7- 2, but since the sensitivity of different techniques to these contaminants varies, these values should only be taken as a guide to the purity. If the quotient is between 1.8- 2,

the purity is 70 -95%, indicating highly purified DNA.[14] If a sample has an $A_{260}$ / $A_{280}$ of less than 1.8, it is usually considered to be contaminated by protein.

Both DNA and RNA absorb maximally at 260 nm, while most proteins absorb strongest at 280 nm. However, most proteins can absorb strongly at 260 nm (the absorbance varies, depending on the protein). Thus, it can be difficult to accurately measure the concentrations of DNA, RNA and protein in complex mixtures and they will contribute to an increased $A_{260}$ / $A_{280}$ reading leading to an overestimation of the DNA concentration.[13,15]

The first unique feature of DNA that was recognized as having taxonomic importance was its mole percent guanine plus cytosine content (mol% G+C).[3] In the current study, mol% G+C values were initially determined by thermal denaturation method. During the controlled heating of a preparation of double-stranded DNA and measuring of absorbance in ultraviolet spectrophotometer, the absorbance increases by ~ 40%. This is due to the disruption of the hydrogen bonds between the base pairs that link to the two DNA strands. The temperature at the midpoint of the curve obtained by blotting temperature versus absorbance is called "melting temperature," or $T_m$. The $T_m$ is correlated in a linear manner with mol% G+C of content of the DNA.[2] As shown in the table 8, mol% G+C value along with biochemical tests carried out for each isolate was compared with Tables of Bergey's Mannual of Systematic Bacteriology[15] and so, isolates were classified. For standard strain of *E. coli* the mol% G+C value measured was 50.3 while it was 50.9. This might be because of the temperatures used in this study did not include the decimal parts of centigrade. The highest mol% G+C value were recorded for *Micrococcus nishinomiyaensis* reached 67.3% which agreed with Kocur, *et al*[16] who found 32 isolates of *Micrococcus nishinomiyaensis* have mol% values of the G+C content in DNA ranged from 66.4 to 71.1% (average, 68.8%). The G+C content of the DNA for seven strains was high as 57.6%, 58.8% and 60.0% (average, 58.8%) those were classified as *Stomatococcus mucilagenosus*. The results agreed with what was found by Bergan and Kocur[17] who studied *Stomatococcus mucilagenosus,* probably a normal inhabitant of the mouth and upper respiratory tract of humans, with guanine-plus-cytosine content of its deoxyribonucleic acid varies between 56 and 60 mol%. The mol% G+C values determined for *Actinomyces israelii*, *Rhodococcus equi*, *Eikenella corrodens,* and *Aerococcus viridans* were 62.5%, 58.8%, 56.4% and 36.8%, respectively.

According to Bergey's Mannual of Systematic Bacteriology[15,18] the mol% of G+C for DNA of these organisms is as follows: *Actinomyces israelii* (57- 65)%, *Rhodococcus equi* (56-

60)%, *Eikenella corrodens* (56- 58)%, and *Aerococcus viridans* (35- 40)%. The three oxidative isolates that showed low purity of DNA also showed unstable readings of UV absorbance at 260 nm, so that it was difficult to find the correct $T_m$ for each of them. This might be due to presence of pollutants which interact with the absorbance of DNA at 260 nm or incorrect preparations.

**Table 8.Determination of DNA concentration, purity, melting temperature and correlating mol% G+C values**

| Sample | A 260 | A 280 | A 260/ A 280 | DNA conc. ng/µl | $T_m$ | % G+C | Bacterial species |
|---|---|---|---|---|---|---|---|
| control | 0.376 | 0.201 | 1.87 | 18.80 | 90.0 | 50.3 | *E. coli* mj83 |
| H1 | 0.169 | 0.109 | 1.55 | 08.45 | 93.5 | 58.8 | *Rhodococcus equi* |
| H5 | 0.260 | 0.144 | 1.80 | 13.00 | 97.0 | 67.3 | *Micrococcus nishinomiyaensis* |
| R8 | 0.208 | 0.129 | 1.61 | 10.40 | 94.0 | 60.0 | *Stomatococcus mucilagenosus* |
| R22 | 0.577 | 0.339 | 1.70 | 28.85 | 92.5 | 56.4 | *Eikenella corrodens* |
| R70 | 0.414 | 0.163 | 2.54 | 20.70* | * | * | Still unknown strain |
| R71a | 0.103 | 0.059 | 1.75 | 05.15 | 84.5 | 36.8 | *Aerococcus viridans* |
| R98a | 0.092 | 0.060 | 1.53 | 04.60 | 95.0 | 62.5 | *Actinomyces israelii* |
| R117 | 0.427 | 0.385 | 1.11 | 21.35* | * | * | Still unknown strain |
| R119 | 0.214 | 0.111 | 1.97 | 10.70 | 93.0 | 57.6 | *S. mucilagenosus* |
| R122 | 0.213 | 0.138 | 1.54 | 10.65 | 94.0 | 60.0 | *S. mucilagenosus* |
| R123 | 0.211 | 0.140 | 1.51 | 10.55 | 93.5 | 58.8 | *S. mucilagenosus* |
| R167 | 0.220 | 0.139 | 1.58 | 11.00 | 94.0 | 60.0 | *S. mucilagenosus* |
| R175 | 0.207 | 0.136 | 1.52 | 10.35 | 93.0 | 57.6 | *S. mucilagenosus* |
| R179 | 0.210 | 0.136 | 1.54 | 10.50 | 93.0 | 57.6 | *S. mucilagenosus* |
| R180 | 0.572 | 0.544 | 1.05 | 28.60* | * | * | Still unknown strain |

* error value was read by instrument

## 4. CONCLUSIONS

The mol% of G+C for DNA of these organisms is as follows: *Actinomyces israelii* (57- 65)%, *Rhodococcus equi* (56- 60)%, *Eikenella corrodens* (56- 58)%, and *Aerococcus viridans* (35- 40)%. The three oxidative isolates that showed low purity of DNA also showed unstable readings of UV absorbance at 260 nm, so that it was difficult to find the correct $T_m$ for each of them. This might be due to presence of pollutants which interact with the absorbance of DNA at 260 nm or incorrect preparations.

## REFERENCES

1. Johnson, J L. Genetic characterization, p. 450472. In: P. Gerhardt, R.G.E. Murray, R.N. Costilow, E.W. Nester, W.A. Wood, N.R. Krieg, and G.B. Phillips (ed.), Manual of

methods for general bacteriology. American Society for Microbiology; 1981, Washington, D.C.

2. Marmur, J, Doty, P. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J Mol Biol, 1962; 5: 109-118.
3. Johnson, J L. Genetic characterization, p. 450472. In: P. Gerhardt, R.G.E. Murray, R.N. Costilow, E.W. Nester, W.A. Wood, N.R. Krieg, and G.B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, 1981; Washington, D.C.
4. Al-Jebouri,MM, Al-Doori,A N. (2014) Estimation of immunoglobulin E among infected asthmatic patients workers in Al-Baiji oil refinery of Iraq. World J Pharm Pharmaceut Sci, 2014; 3(6): 1747-1762.
5. Pospiech, A. ,Neumann, B A versatile quick-prep of genomic DNA from Gram-positive bacteria. Trends Genet, 1995; 11(6): 217-8.
6. Kieser, T. Preparation and analysis of genomic and plasmid DNA. Jhon center, Nerwich, 1995; NR47 UH. UK.
7. Cesarone, C F, Bolognesi C, Santi, L Improved microfluorometric DNA determination in biological material using 33258 Hoechst. NCBI Anal Biochem, 1979; 100(1): 188-97.
8. Sambrook, J, Fritsch, E, Maniatis, T. Molecular and laboratory manual. 2nd ed. Cold Spring Harbour Laboratories, 1989; New York.
9. Garvie, E I. Lactic dehydrogenases of strains of the Genus *Leuconostoc*. J Gen Microbiol, 1974; 58: 85-94.
10. De Ley, J. Reexamination of the association between melting point, buoyant density and chemical base composition of deoxyribonucleic acid. J Bacteriol, 1969; 101: 738-754.
11. Finn, K K. Wizard midiprep kit for plasmid purification. 3rd ed. Promega Corporation, 2003.
12. Lozano, AL C, Bautista, M A, Dussan, G J, Vives-Flórez1, M J. DNA Extraction from Heavy oil contaminated Microcosms and Rpob gene PCR Amplification. Actual Biol, 2008; 30(88): 7-14.
13. Oswald, N. Tips Quick reference: Determining DNA Concentration & Purity. Science Squared, Ltd, 2007.
14. Teare, J M, Islam, R, Flanagan, R, Gallagher, S, Davies, M G, Grabau, C. Measurement of nucleic acid concentrations using the DyNA Quant and the GeneQuant. Biotechniques, 1997; 22: 1170-1174.

15. Holt, J G,Sneath, P H, Krieg, N R. Bergey's Manual of Systematic Bacteriology, Vol. 2. Lippincott Williams and Wilkins, 1986; 972-1010.
16. Kocur, M, Schleifer, K H, Kloos, W E. Taxonomic status of *Micrococcus nishinomiyaensis* Oda 1935. Int J Syst Bacteriol, 1975; 25: 290–293.
17. Bergan, T.; Kocur, M. "*Stomatococcus mucilaginosus* gen. nov., sp. nov., ep. rev., a member of the family Micrococcaceae," International J Systematic Bacteriol, 1982; 32(3): 374–377.
18. Krieg, N R, Brenner, D J, Staley, J T. Bergey's manual of systematic bacteriology: The proteobacteria, 2005; Springer. ISBN 978-0-387-95040-2.