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# MOLECULAR IDENTIFICATION OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTED DAIRY PRODUCT

**HASSAN ZMR[1], EFFAT BAM[2], MAGDOUB MNI[1], TAWFIK NF[2], SADEK ZIM[2] AND MABROUK AMM[2]***

[1]Food Science Dept., Faculty of Agriculture, Ain Shams Univ., Shoubra El-Khaima, Cairo, Egypt

[2]Dairy Science Dept., Microbiology Laboratory, National Research Centre, Dokki, Cairo, Egypt

***Corresponding author's E Mail: mabrouk455@hotmail.com**

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## ABSTRACT

The objective of this study was to identify four strains of lactic acid bacteria (LAB) isolated from traditional fermented dairy product (Laban Rayeb). The isolates were identified by using molecular biology technique of polymerase chain reaction (PCR) and DNA sequencing analysis. After finishing the PCR amplification reaction, the purified DNA of tested strains were sequenced and determined for arrayed nucleotides using capillary electrophoresis hit by laser beam. Sequenced data of isolates were analyzed by using software application. The sequences of tested strains were compared with the known data deposited in the NCBI Gen-Bank by using basic local alignment search tool (BLAST).The results showed that the isolated strains were genetically identified and confirmed as *Lactobacillus casei* NRC AM2, *Lactobacillus rhamnosus* NRC AM6, *Pediococcus pentosaceus* NRC AM4 and *Pediococcus acidilactici* NRC AM8. Thereby, concluded that the genotypic techniques are very rapid, accurate, more sensitive and depend on the stability of the genomic DNA.

**Keywords: DNA Sequencing, Electropherogram, Laban rayeb, Lactic acid bacteria Polymerase chain reaction**

## INTRODUCTION

Laban Rayeb is one of traditional fermented dairy products that have been known and popular in rural regions in Egypt. It is produced by spontaneous fermentation of buffalo's or cow's milk by natural flora in milk. This product contains mixed cultures of lactic acid bacteria which produce natural organic acids, flavour compounds, exopolysaccharides and antimicrobial metabolites which play an essential role in fermented products and health benefits **[1,2].** Isolation of LAB from fermented dairy products is highly interesting because of these microflora had a good technological functions **[3]**.Traditional Egyptian fermented dairy products are good valuable sources of LAB with new important technological and genetic biodiversity **[4,5,6].** Lactic acid bacteria are a group of related bacteria that produce lactic acid as a result of carbohydrate fermentation, gram positive, catalase negative, usually non motile and acid tolerant microorganisms. Several species of these genera occur naturally in milk, dairy products, fermented foods and the human gut **[7].** Generally, LAB have a long history of safe use in a variety of fermented food products all over the world. Members of the genera *Lactococcus, pediococcus, Lactobacillus* and *Bifidobacterium* are generally recognized as safe (GRAS) **[8]**.

Nowadays, rapid molecular methods have been applied for identification of various species of LAB associated with food and dairy products. These methodologies are based on primers that target different partial or complete genome sequences, such as the 16S ribosomal RNA (rRNA) gene. Ribonucleic acid (RNA) is a biologically important type of molecule that consists of a long chain of single stranded nucleotide units. Ribosomal RNA (rRNA) is a type of non coding RNA that is involved in translating messenger RNA (mRNA) into proteins in prokaryote and acts as the catalytic component of the ribosome. The 16S rRNA sequence is used for phylogenetic studies as it is highly conserved between different species of bacteria **[9]**. Polymerase chain reaction is a DNA replication process that allows for the exponential amplification of target DNA. This technique play an important role in routinely used for rapid detection, identification and differentiation of lactic acid bacteria **[10,11,12]**. Hence, the aim of this study is to isolate and identify of some strains of LAB isolated from Rayeb milk.

## MATERIALS AND METHODS

### Samples collection

A total of 8 samples of Laban Rayeb (traditional fermented milk) were collected in sterile cups and transferred to the laboratory under aseptic cooled conditions.

### Sample preparation and isolation

Ten grams of each sample were homogenized with 90 ml of sterile physiological saline (0.85 % NaCl) **[13].** The resulting homogenate was serially diluted up to $10^{-8}$ using one milliliter from each dilution was plated onto sterile petri dishes after that; De Mann Rogosa-Sharpe (MRS) agar were poured. All plates anaerobically incubated at 37° C for 48-72 h (BBL Gas Pak Anaerobic System, USA), and separate colonies randomly selected from plates. The selected colonies were characterized phenotypically (cell morphology, gram staining, physiological and biochemical tests **[6]**. Purified selected strains were kept in sterile reconstituted skim milk (12.5 % w/v) supplemented with 1% yeast extract and 25-30 % (w/v) glycerol then stored at -20 °C in deep freezer until used.

### Genotypic identification

### Genomic DNA extraction

The Genomic DNA of four isolates was extracted from overnight cultures according to the procedure of MicroSeq® 500 DNA bacterial isolation kits protocol. The strains were grown on MRS broth medium at 37º C for 24 h (OD at 620 nm reached to 1-1.5). One ml of each overnight culture was added into a sterilized micro centrifuge tube and the samples were centrifuged at (5000 rpm /10 min at 4 ºC). The supernatant was discarded and the pellets were washed with buffer pH 8 and centrifuged for obtaining pure pellets. Under aseptic condition 200 µL of Prep Man Ultra Sample Preparation Reagent was added then the tubes closed tightly and vortexed at 10 min for 30 sec then heated to 100 ºC for 10 min in a water bath. The tubes were cooled and centrifuged at (16000 x g for 3 min at 4 ºC). The supernatant which include the extracted DNA was transferred into new micro centrifuge tube for using in the following step.

### Polymerase chain reaction (PCR)

The amplification of extracted DNA was carried out in (a veriti™ thermal cycler, applied biosystems, Singapore, Indonesia). The reaction volume for thermal cycling is 20 µL and the program of polymerase chain reaction are presented in table (1). The PCR products were stored at - 20 ºC until use in the following step.

Table 1: The program of polymerase chain reaction (PCR)

| Initial step | Each of 30 cycles | | | Final extension | Final step |
|---|---|---|---|---|---|
| Hold | Cycle | | | | Hold |
| | Melt | Anneal | Extend | Hold | |
| 95/10 min | 95/30 Sec | 60/30 Sec | 72/45 Sec | 72/10min | 4 ºC |

**Purification and sequencing the PCR product**

Polymerase chain reaction products were purified for removing the unused dNTPs and primers from the PCR mixture before sequencing. The purified PCR products were automatically sequenced according to the method of **[14].** and the sequencing files were exported to the MicroSeq analysis software. The obtained nucleotide sequences were analyzed by using applied biosystems® 3500 genetic analyzer. Finally, the data were compared with the known data in basic alignment search tool (BLAST), NCBI (National Center of Biotechnology Information), www.ncbi.nlm.nih.gov.

**RESULTS AND DISCUSSION**

**Genotypic identification of strains**

Genotypic techniques are very important tools for analysis of microorganisms isolated from foods and other biological materials. The nucleotide sequences of four strains were analyzed by software application and data were compared with the known data. Molecular results showed that, the isolates symbolized by codes NRC AM2, NRC AM4, NRC AM6 and NRC AM8 were genetically identified as *Lactobacillus casei* with accession number NR075032.1, *Pediococcus pentosaceus* with accession number NR075052.1, *Lactobacillus rhamnosus* with accession number NR113332.1 and *Pediococcus acidilactici* with accession number NR042057.1 respectively. The results of molecular identification by using 16S rRNA gene are display in table 2. The graphical representation nucleotide sequence illustrated in figures 1,2,3 and 4. From the obtained genotypic identification results, it's clear that, the genotypic modern techniques are very rapid, accurate, more sensitive and reliable compared with phenotypic identification methods. The genotypic identification depending on the stability of the genomic DNA **[12, 15, 16, 17].**

**Table 2: Genotypic identification of four isolates**

| Isolates | Genotypic identification (16S rRNA gene) | % | Accession Number |
|---|---|---|---|
| NRC AM2 | *Lactobacillus casei* | 100 | NR075032.1 |
| NRC AM4 | *Pediococcus pentosaceus* | 97 | NR075052.1 |
| NRC AM6 | *Lactobacillus rhamnosus* | 97 | NR113332.1 |
| NRC AM8 | *Pediococcus acidilactici* | 99 | NR042057.1 |

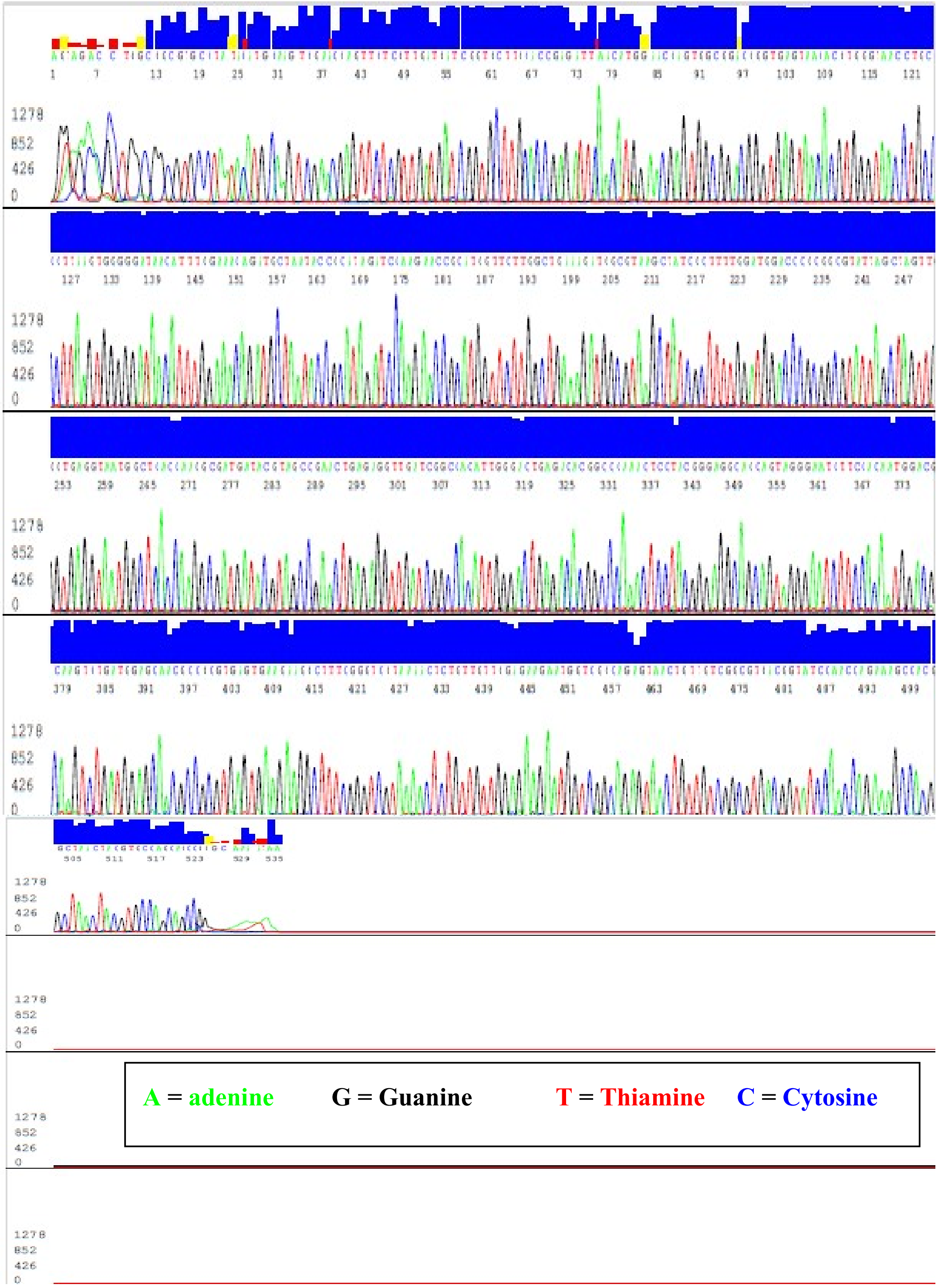


**Figure 1: Electropherogram of 16S rRNA PCR Product of *Lactobacillus casei***

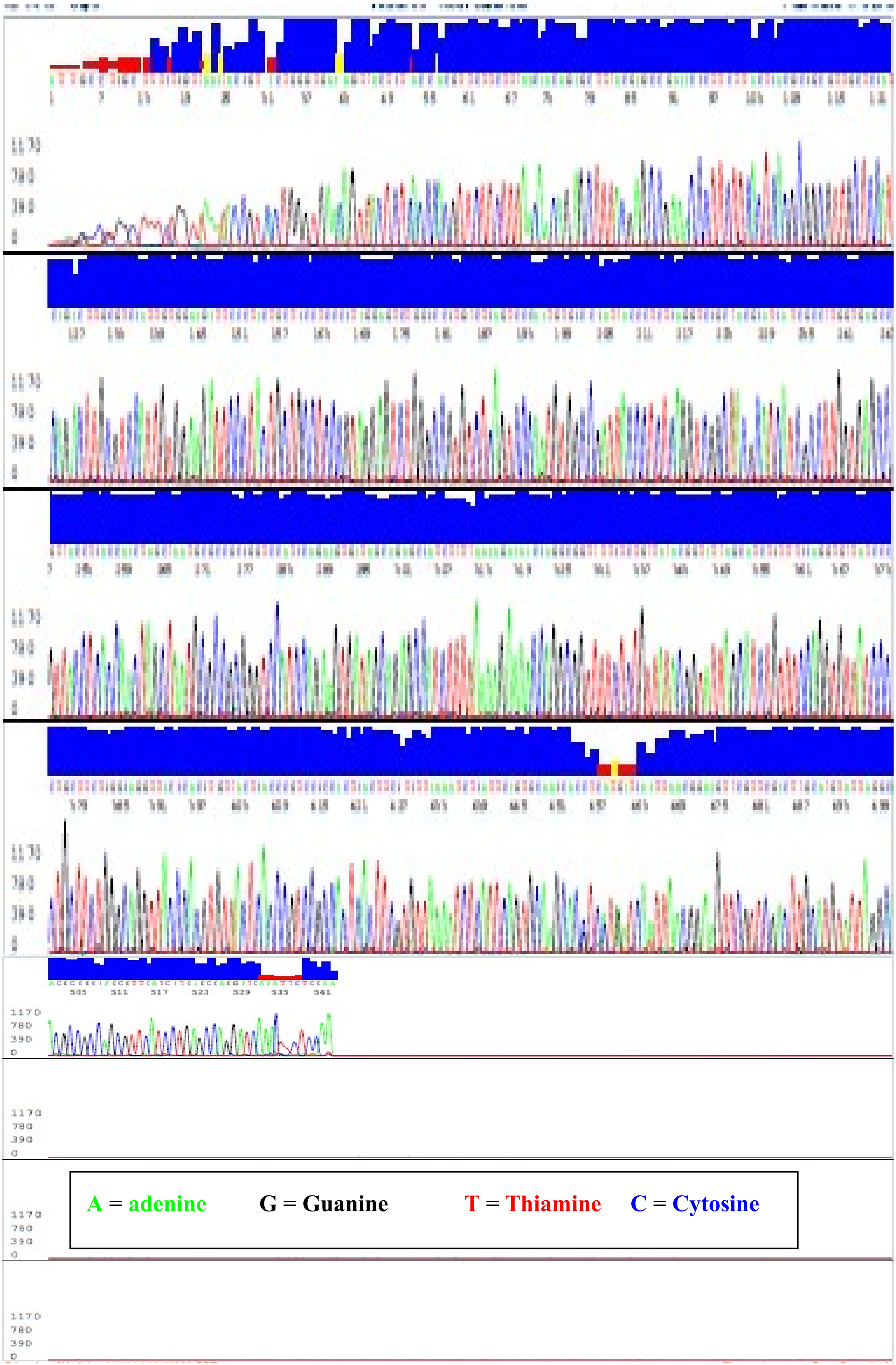


**Figure 2: Electropherogram of 16S rRNA PCR Product of *Pediococcus pentosaceus***

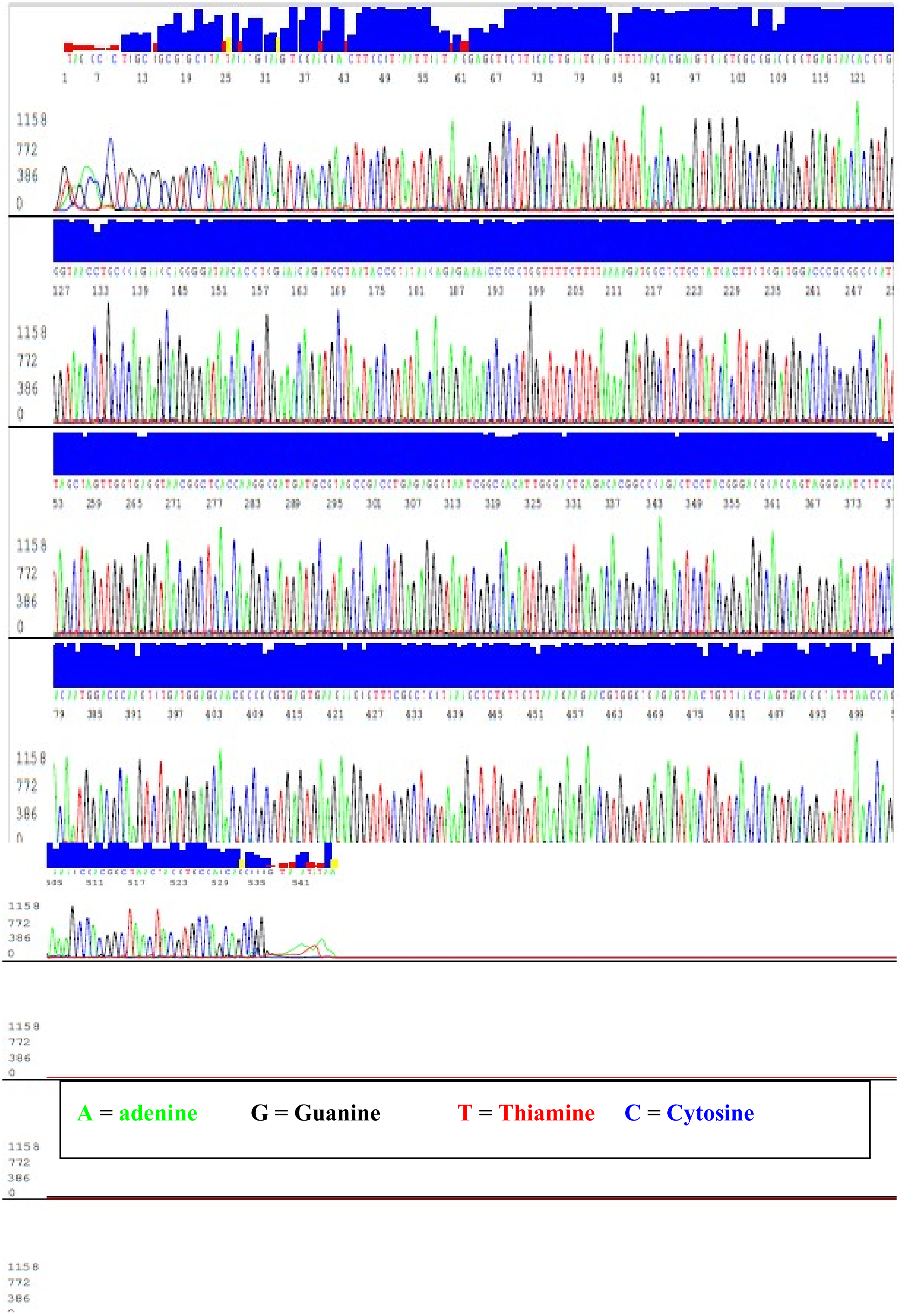


**Figure 3: Electropherogram of 16S rRNA PCR Product of *Lactobacillus rhamnosus***

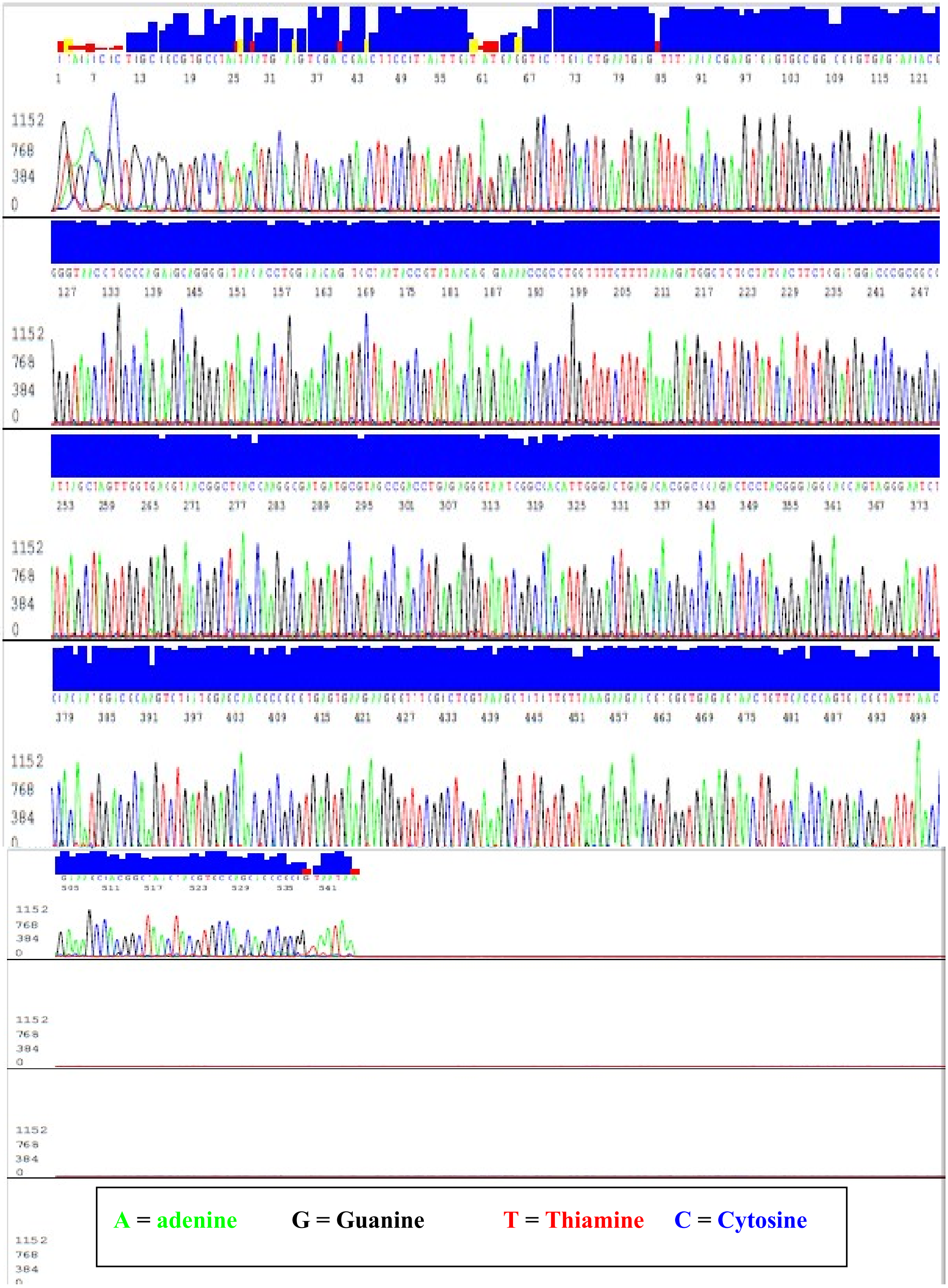


**Figure 4: Electropherogram of 16S rRNA PCR Product of *Pediococcus acidilactici***

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