Quantitative determination of some Extracellullar Compounds by Cyanobcteria *Nostoc* Sp. and *Rivalloria* Sp. in Outdoor Cultures

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Abstract

Two N_2 – fixing cyanobacteria species (*Nostoc Sp.* and *Rivalloria Sp.*) had been isolated, purified and identified from sediment samples of Tigris river. Carbohydrates (98.0 μg/ml), total lipid (28.2%), protein (87.5 μg/50mg dry wt.) and nucleic acid (RNA) (67.5 μg/20mg dry wt.) contents were quantitatively determined. The free amino acids and lipids of the cyanobacteria were identified as well.

Results show high lipid content of two species. Due to the high protein and lipid contents, these cyanobacteria might be used as food substitutes.

التقدير الكمي لبعض المركبات المطروحة من قبل السيانوبكتريا *Nostoc sp.* و *Rivalloria sp.* إلى الوسط الزرعي

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الخلاصة

تم عزل وتنقية وتشخيص صنفين من السيانوبكتريا المثبتة للنتروجين (*Nostoc sp.* و *Rivalloria sp.*) من عينات من نهر دجلة. كذلك تم التقدير الكمي للكاربوهيدرات (98 مايكروغرام لكل مل)، الليبيدات الكلية (28.2%)، البروتينات (87.5 مايكروغرام لكل 50 مليغرام وزن جف) والحامض النووي RNA (67.5 مايكروغرام لكل 20 مليغرام وزن جاف) المطروحة إلى الوسط الزراعي، بالإضافة إلى تشخيص الأحماض الأمينية الحرة والدهون المطروحة إلى الوسط الزرعي.

وقد أشارت النتائج إلى وجود الدهون بنسب عالية بالنوعين المعزولين. ونظراً لاحتواء ذين النوعين على تراكيز عالية من الدهون والبروتينات لذا يمكن استخدمهما كبدائل غذائية .

Introduction

The importance of blue – green aglae as nitrogen fixers is well established (Carr and Whitton, 1982 ; Wolk, 1980). The nitrogen – fixing, heterocystous, filamentous cyanobacteria are particularly attractive for the photoproduction of phycobiliproteins and other chemicals (Borowitzka, 1988 ; Rodriguez et al., 1989, 1991, 1992). They do not require the addition to the culture media of nitrogen fertilizer, since they can grow using atmospheric nitrogen as the sole nitrogen source. The lack of combined nitrogen in the culture media, aside from its economical implication, restricts the problem of contamination by other organisms. Moreover, the filamentous nature of these organisms facilitates separation of biomass from

the medium. Despite these obvious advantages for mass production, available information regarding outdoor production of filamentous N_2 – fixing cyanobacteria is scarce. (Fontes et al., 1987 ; Boussiba, 1993 ; Moreno et al., 1995 ; Al-Saadon, 1997 ; Abbas and Heraib, 1998 ; Al-Timari et al., 2001).

In this paper, carbohydrate, proteins, nucleic acid (RNA) and total lipids contents have been evaluated from two N_2 – fixing cyanobacteria isolated from Tigris river and their free amino acids and lipids were identified as well.

Material and Methods

Collection of Sample:

The sediments were sampled by Van Veen grab samples from Tigris river at Tikrit in April 1996. Sub. Samples (the top 5 cm) were transferred into polyethylene bottles and brought to the laboratory.

Cultures:

Pure cultures of the two N_2 – fixing cyanobacteria (*Nostoc sp.* and *Rivalloria sp.*) which had been isolated, purified and identified from sediment samples in were maintained aerobically on ASM-1 medium free combined nitrogen (Gallon et al., 1978).

Acetylene Reduction (N_2 – Fixation)

Nitrogennase (EC:1.18.2.1) activity was measured as acetylene reduction (Gallon and Hamdi , 1984) by using PERKIN – ELMER (GC.900) gas chromatograph fitted with a para pack R column (at 50 C°) and flame ionization detector (at 100 C°), using helium as carrier gas.

Determination of Carbohydrate:

2-10 ml samples of the cell culture were filtered immediately using a filter paper (GF/F). Carbohydrate was analyzed by the phenol – sulfuric acid

method (Elisabeth and Annegret, 1988) with glucose in 1M NaOH as standard.

Determination of Protein Concentrations:

50 mg of excreted matter of the collected cyanobacteria resuspended in trichloroacetic acid (TCA) and left 0 – 4 C° for 18 – 24 hr. . The suspension was centrifuged and the supernatant used for determination the protein . Protein was estimated by modification of the lowry method (Baily , 1963) using bovine serum albumin in 1M NaOH as standard.

Determination of Nucleic acids (RNA & DNA)

100 mg of excreted matter of the collected cyanobacteria resuspended in ethanol and left at 0 – 4 C° for 18 – 24 hr. .The ethanolic suspension was centrifuged and the supernatant used for determinations the nucleic acids .For the RNA determination, the lipid – free precipitated was resuspended in 0.3M KOH at 30C° for 18 – 24 hr. and the orcinol method (Schenider ,1945) was followed. For the DNA determination, the lipid free precipitated was resuspended in 0.5 M HclO4 and incubated at 70C° for 45 min. and the diphenylamine method (Schenider ,1945) was followed .

Determination of Total Lipids:

100 mg of excreted matter of the collected cyanobacteria was extracted overnight with 50ml of benzene: methanol (1:1), in a continuous extraction apparatus of the soxhlet type (Pearson, 1971). Then the mixture was dried using rotary evaporator and the yeild was considered as percentage of total lipids in the excreted matter.

Separation of Lipids by Thin Layer Chromatography(One Dimensional).

Spots of the extract was put on a thin layer chromatography plate (silica gel)(20 x 20 cm). The strips were developed in ascending

chromatography using hexane: diethylether: glacial acetic acid (80:20:1v/v) as the solvent extractor (Ivor and Feinbery, 1965). The development of the ascending chromatograms was stopped when the front had moved 17.5cm from the points of applications. The Rate of flow (R_f) values that obtained were zero, 0.05 , 0.2 , 0.4 – 0.45 and 0.75 – 0.8 which corresponding to the: phospholipids, cholesterol, free fatty acid, triglyceride and cholesterol esters respectively (Fig.1).

Separation of Lipids by Thin Layer Chromatography (Two Dimensional).

Spots of the extract was put on a thin layer chromatography plate (silica gel)(20x20cm) in solvent I (chloroform: methanol : ammonium hydroxide) (65 : 25: 4 v/v) and solvent II (chloroform : acetone: methanol: acetic acid : water) (50:20:10:10:4 v/v) as eluent (williams, 1978). TLC plate was dried and stained with phosphate spray (Vaskovsky and Kostetsky, 1968) to detect the lipids as shown in Fig (2a,2b).

Separation of Amino Acids by Thin Layer Chromatography (Two Dimensional).

Spots of the extract was applied on a thin layer chromatogtaphy plate (silica gel)(20x20cm). Ascending the plate was carried out for 6 hours, using a mixture of solvent consist of (butanol: acetic acid: water) in proportions (20:80:75 v/v) as eluent (Al-Riahi, 1992).

TLC plate was dried and stained with ninhydrin – cadmium stain (Heathcote et al., 1972) to detect the amino acids as shown in Fig (3a,3b).

Results and discussion

Table (1) and (Fig.4,5) show protein production of the two cyanobacteria Nostoc *sp.* and *Rivalloria sp.* with time. The results show an increase excreted protein with time along the growth period. The photoproduction of protein by heterocysts leads to harboring the filamentous

cyanobacteria. The reductant required for N_2 – fixation is photosynthetically generated in the vegetative cells, which perform a photosynthesis of the water – splitting type. This reductant (most probably, a reduced carbon compound) migrates to the heterocysts – specialized cells of the filaments in which nitrogenase is confined and N_2 – fixation take place where it provides the reducing power for the reduction of N_2 to ammonia. ATP, which is also required in the reaction, is generated in the heterocyst by cyclic photophosphorylation. The resulting ammonia is incorporated to the glutamate imported from the vegetative cells, which is then converted into glutamine. Glutamine is exported from the heterocysts to the vegetative cells where, via glutamate, provides reduced nitrogen to be combined with different carbon skeletons photosynthetically generated in these cells. In this way, a variety of nitrogenous compounds, which includs protein as the major product, are synthesized by the cells in the filaments of the heterocystous cyanobacteria. Protein production by this group of N_2 – fixing cyanobacteria can thus be summarized by the following synoptic equation:

$$N_2 + CO_2 + H_2O \xrightarrow{\text{light}} \text{proteins} + O_2$$

The levels of excreted nucleic acid RNA (Table 2, Fig 6,7) decrease with time along the growth period. No results for excreted DNA was obtained.

Table (3) and Fig (8,9) show maximal production of carbohydrate in first seven days along with increasing nitrogenize activity (Rippka and Stanier, 1978). However, a later decrease in carbohydrates is followed the seventh day of growth. This indicates that carbohydrates which were no longer accumulated were consumed for biosynthesis. Nitrogenize synthesis and activity are depends on the degradation of an organic substrate which serves both as a general source of carbon for amino acid synthesis, and as a source of reductant (Ernst et al., 1984 ; Gallon and Chaplin, 1988 ; Quesada et al., 1992).

Lipids present in cyanobactria composed mainly of glycolipids and take part in the structure of the hetrocysts(Wolk,1982;Ahlgren et al., 1992). Table(4) shows the percentage of the total lipids in the excreted matter of the isolated cyanobacteria. The high lipid contents may be related to the presence of hetrocycts in the two studied species. According to our results on one – dimensional chromatography cholesterol ester (CE), triglyceride (TG), free fatty acid (FA), Cholesterol (C) and phospholipids (PL) were obtained (Fig.1). On two dimensional chromatography (Fig.2a,2b) pigments and neutral lipids (pig + NL), monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG) and phosphatidylcholine (PC) were obtained as excreted lipids. Their results are correlated with lipids and fatty acids produced by *Anacystis aidulans*, *Microcystis aeruginosa*, *Oscillatoria rubesceus* and *Spirulina platensis* (Margret et al., 1984).

The results of free amino acids of the investigated two blue – green algae (*Nostoc sp.* and *Rivalloria sp.* are presented in Fig. (3a,3b). The most abundant excreted amino acids present in a free state in the two studied species (*Nostoc sp.* and *Rivalloria sp.* are glutamic acid, aspartic acid, phenylalanine, methionone, tyrosine and threonine. The latter two amino acids are absent in cyanobacterium *Rivalloria sp.* Pomiluiko and Stetsenko (1973) denoted that blue-green algea contained predominetally aspartic acid, glutamic acid, isoleucine, leucine, tyrosine and valine. Also, Dusheiko et al., (1969) determined the amino acid composition of protein in blue – green algae using an automated amino acid analyzer. Their results showed the presence of essential amino acids and thus they concluded that the proteins of algea might be considered biological valuable for animal feeding as well as for microbiological purposes.

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supply.

Table (1): Concentration of protein in the excreted matter of the two isolated species of cyanobacteria (μg/50 mg dry wt.).

Species	Age of culture (days).				
	1	3	5	7	10
Nostoc sp.	18.2	31.5	50.0	71.5	81.0
Rivalloria sp.	11.0	22.5	37.5	59.5	87.5

Table (2): Concentration of nucleic acid (RNA) in the excreted matter of the two isolated species of cyanobacteria (μg/20 mg dry wt.).

Species	Age of culture (days).				
	1	3	5	7	10
Nostoc sp.	45.7	67.5	61.0	35.2	21.0
Rivalloria sp.	39.5	41.7	33.5	21.5	17.7

Table(3): Concentration of carbohydrate in the excreted of the two isolated species of cyanobacteria (μg/ml).

Species	Age of culture (days).				
	1	3	5	7	10
Nostoc sp.	53.5	69.5	87.2	98.0	40.0
Rivalloria sp.	36.5	39.1	47.5	62.5	19.0

Table(4): Percentage of total lipids in the excreted matter(as gm/100 g).

Species	Total lipid (%)
Nostoc sp.	28.2
Rivalloria sp.	26.4

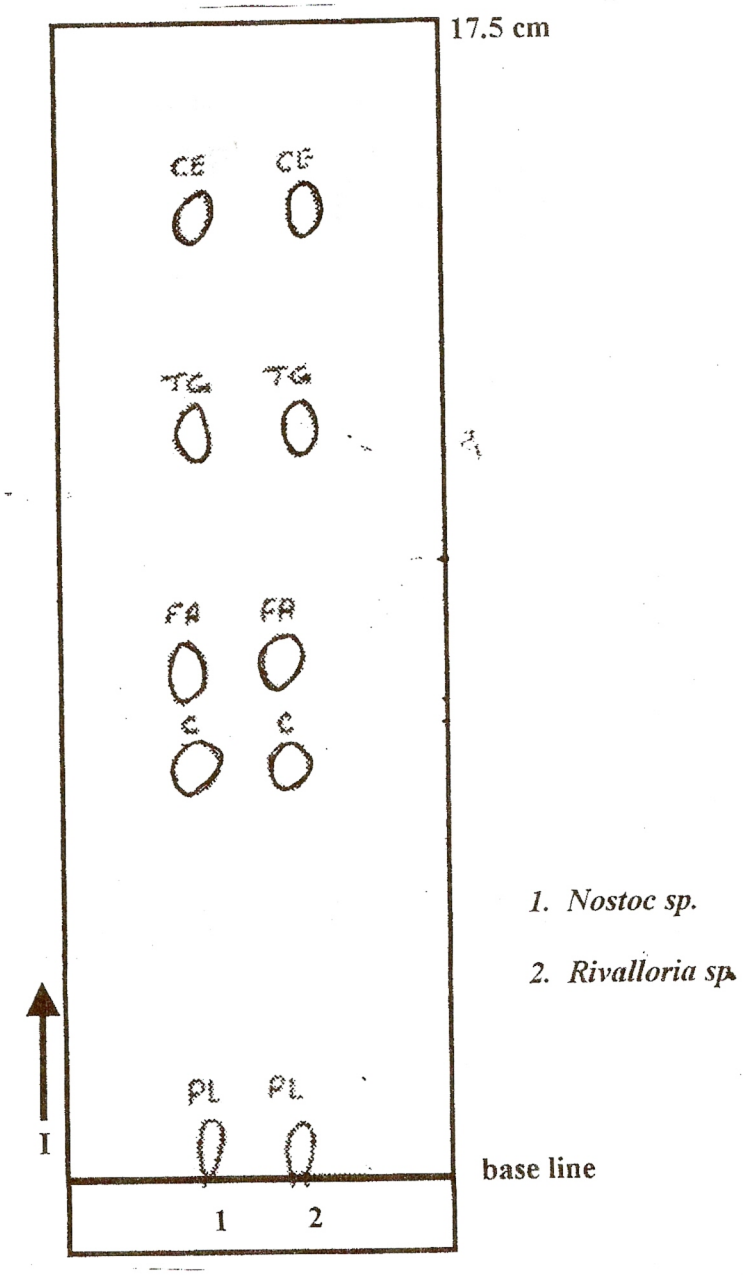


Fig.(1): Chromatogram of lipid found in the excreted matter of the two cyanobacteria *Nostoc sp.* and *Rivalloria sp.* by one – dimensional chromatography on thin layer silica gel plate.

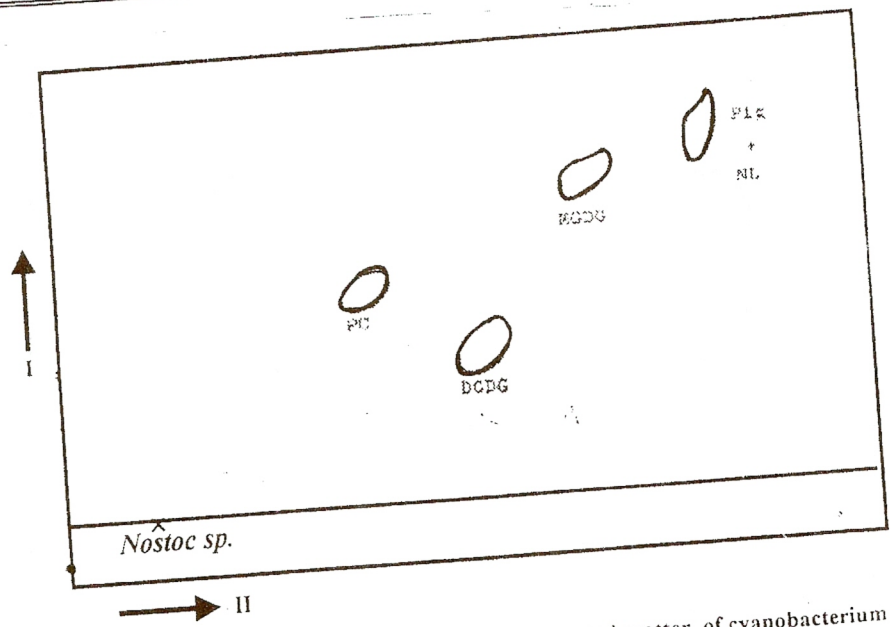


Fig.(2a): Chromatogram of lipid found in the excreted matter of cyanobacterium *Nostoc sp.* by two – dimensional chromatography on thin layer silica gel plate.

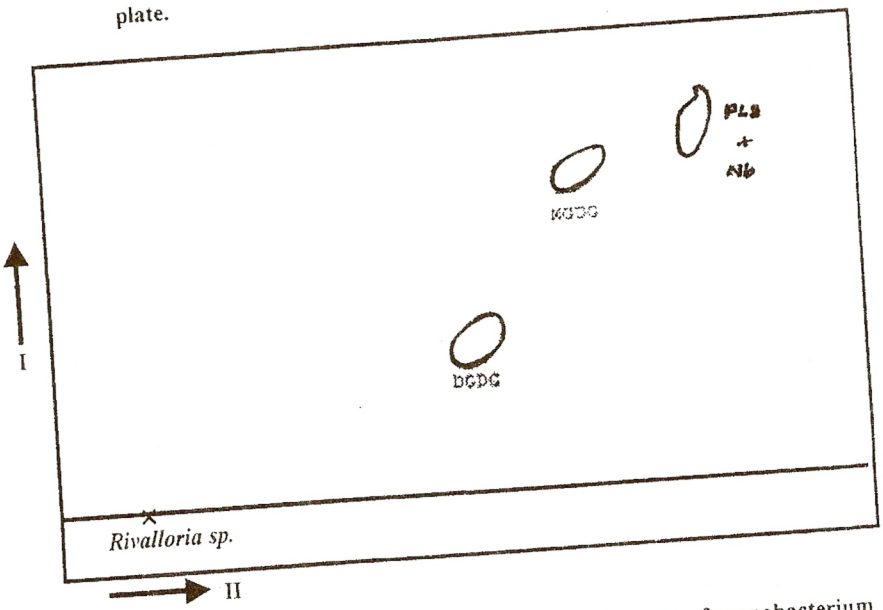
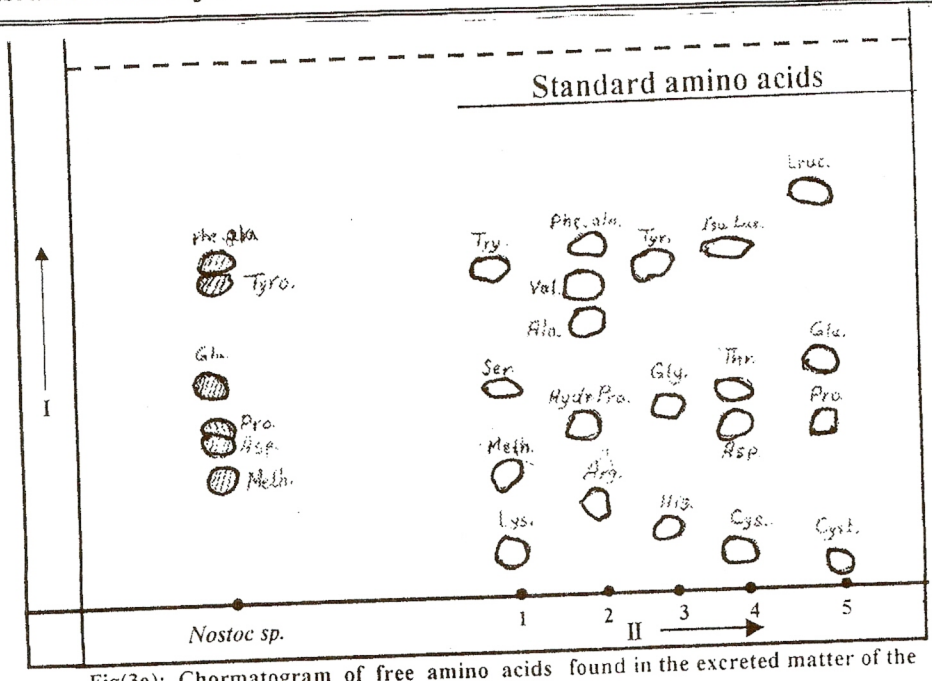
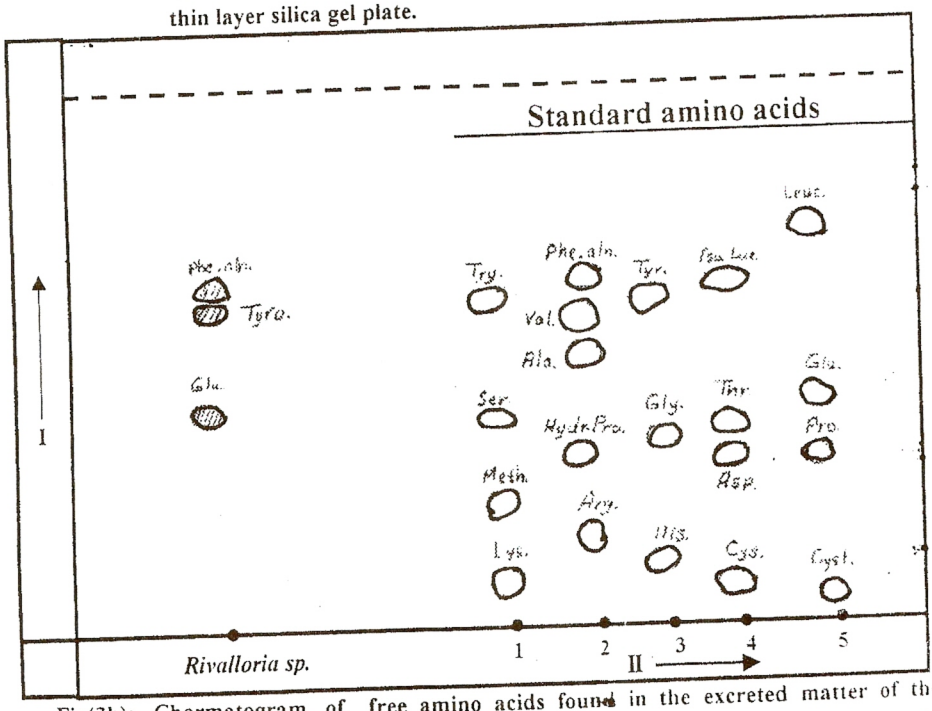


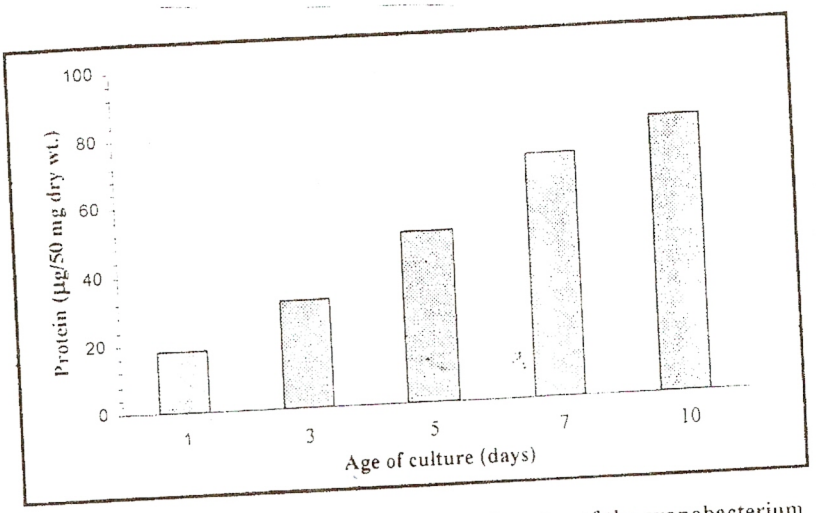
Fig.(2b): Chromatogram of lipid found in the excreted matter of cyanobacterium *Rivalloria sp.* by two – dimensional chromatography on thin layer silica gel plate.



Fig(3a): Chormatogram of free amino acids found in the excreted matter of the cyanobacterium *Nostoc sp.* by two – dimensional chromatography on thin layer silica gel plate.

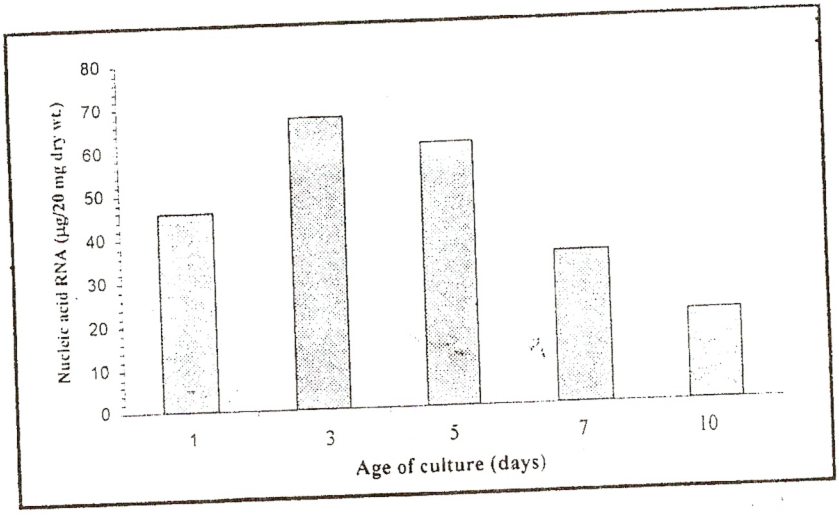


Fig(3b): Chormatogram of free amino acids found in the excreted matter of the cyanobacterium *Rivalloriasp.* by two–dimensional chromatography on thin layer silica gel plate.

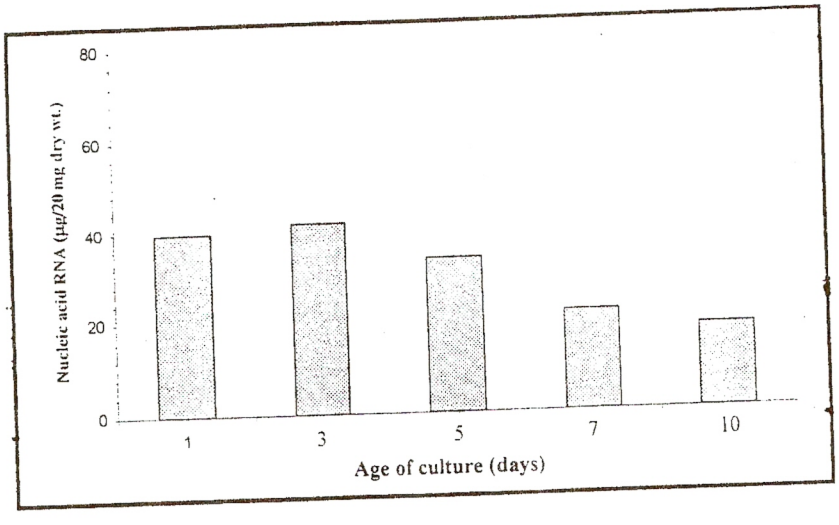


Fig(4): Concentration of protein in the excreted matter of the cyanobacterium *Nostoc sp.*

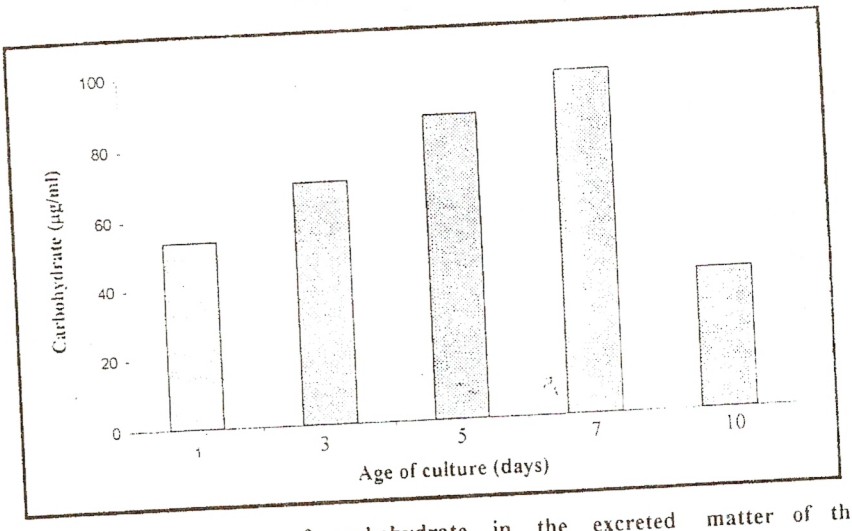
Fig(5): Concentration of protein in the excreted matter of the cyanobacterium *Rivalloria sp.*



Fig(6): Concentration of nucleic acid (RNA) in the excreted matter of the cyanobacterium *Nostoc sp.*



Fig(7): Concentration of nucleic acid (RNA) in the excreted matter of the cyanobacterium *Rivalloria sp.*



Fig(8): Concentration of carbohydrate in the excreted matter of the cyanobacterium *Nostoc sp.*

Fig(9): Concentration of carbohydrate in the excreted matter of the cyanobacterium *Rivalloria sp.*

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