RESULTS

1. Isolation and Identification of Pseudomonas Pyocyanea Strain :-

The organism isolated from septic burn was identified as Pseudomonas pyocyanea according to Cruickshank (1975).

These results revealed that the developed colonies were of about 1-2 mm. in diameter, low convex , rough with entire edge. The medium turns greenish blue due to diffusible exopigments pyocyanin and fluorescin. The culture has a distinct musty smell.

Films prepared from the obtained growth, stained by Gram stain proved that it was Gram negative bacilli, about 2 x ½ U, non capsulated and non sporulated.

The isolated organism has the ability to grow on ordinary & Mac Conkey's media.

The other characteristic features of the isolated organism are demonstrated in Table (1) from which it could be concluded that the isolated organism was

Pseudomonas pyocyanea.

The degree of the bacterial growth was equal on both complete and minimal media.

Table (1)

Characteristic features of the isolated organism as Pseudomonas pyocyanea

Motility	Glucose fermentation	Oxidase production	Urease production	Indole production	M.R	V.P	Citrate Utilization	H_2S production	Growth at 42°C
motile	+ with acid only	+	+	-	-	-	-	-	+

II. Studies On Mutagenic Actions :

A. Lethal effect

- N-methyl-N-nitro-N-nitrosoguanidine (MNNG) :

The results obtained from the treament with MNNG applied in different concentrations at variable incubation periods are illustrated in Table (2) and Figure (1) .

Table (1) shows that a complete lethality was obtained after treatment of Pseudomonas pyocycenea with 10 or 5 mg MNNG/5 ml Pseudomonas cells suspension in saline with different incubation times.

MNNG in a dose of 2.5 mg/5 ml Pseudomonas suspension gives a survival percentage of 0.93, 0.05 after 15 and 30 minutes respectively, while at 45 minutes complete lethality was obtained for the Pseudomonas suspension.

As regards MNNG treatment with a concentration of 1.25 mg/5 ml Pseudomonas suspension, the survived

percentages of the organism after incubation times 15,30 and 45 minutes were 4.13, 1.24 and 0.032 respectively. From Figure (1) it was noticed that the lethality of 2.5 mg MNNG is higher than that of 1.25 mg MNNG.

Table (2)

Effect of MNNG on Pseudomonas pyocyanea using different concentrations at different periods of incubation.

Exp. No.	MNNG Conc.	Survivors of MNNG incubation period/minutes							
		0		15 minutes		30 minutes		45 minutes	
		No.of cells surviving	%	No.of cells surviving	%	No.of cells surviving	%	No.of cells surviving	%
1	10	620×10^5	100	0	0	0	0	0	0
2	5	550×10^5	100	0	0	0	0	0	0
3	2.5	320×10^5	100	300×10^2	0.093	160×10^2	0.05	0	0
4	1.25	290×10^5	100	120×10^4	4.13	360×10^3	1.24	94×10^2	0.032

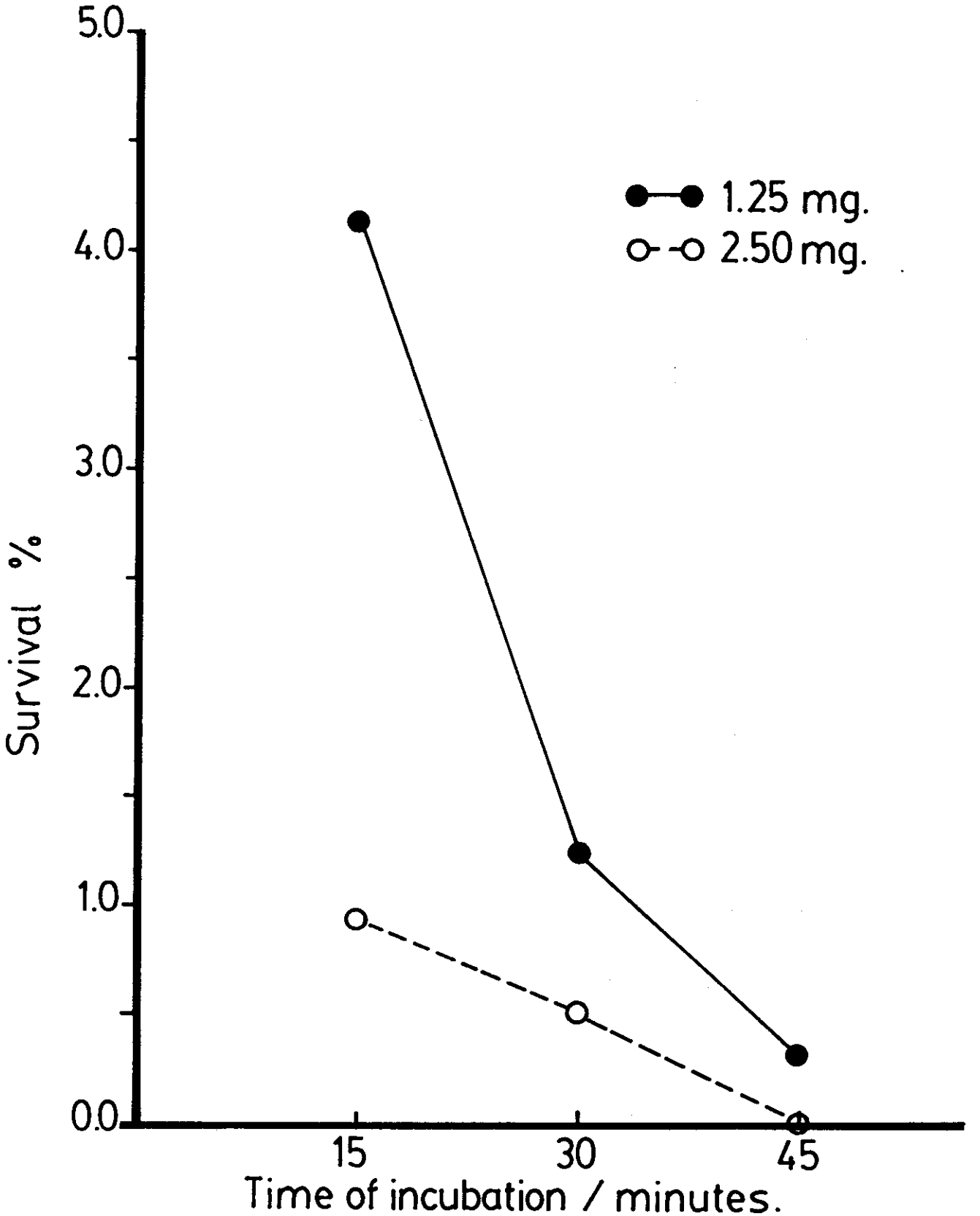

Fig. 1 :, The effect of 2.5 and 1.25 mg MNNG/5 ml suspension of the organism in saline.

Table (3)

The activity of Acriflavine on Pseudomonas pyocyanea suspension at different concentrations.

Exp. No. \ AC. Conc.	0		10		7.5		5		2.5	
	No. of survival	%	No. of survival	%	No. of survival	%	No. of survival	%	No. of survival	%
1	52×10^5	100	0	0						
2	37×10^5	100	0	0	0	0				
3	48×10^5	100	0	0	0	0	0	0		
4	40×10^5	100	0	0	0	0	0	0	40	0.001

Acriflavine :-

The results of acriflavine activity on the Pseudomonas suspension are illustrated in Table (3), which shows that a complete lethality was obtained for concentrations 10, 7.5 and 5 mg Ac/5 ml Pseudomonas suspension although the number of cells plated were 40 x 10^5/ml suspension. At concentration 2.5 mg Ac/5 ml Pseudomonas suspension, 40 colonies were survived and equal to 0,001 % .

Table (4)

Characterization of MNNG induced mutants and their frequencies.

Mutants requiring	MNNG incubation time			
	15 min		30 min	
	NO	%	NO	%
Arginine	3	30	2	28.57
Histidine	2	20	1	14.28
Thyronine	1	10	1	14.28
Tryptophan	1	10	0	0
Cystine	1	10	0	0
Isoleucine	0	0	1	14.28
Aspargine	0	0	1	14.28
Arginine-Histidine	1	10	0	0
Arginine-Aspargine	1	10	0	0
Glysine-Thyronine	0	0	1	14.28
Total NO	10		7	

B. Isolation of biochemical mutants :-

N-methyl-N-nitro-N-nitrosoguanidine mutants :-

No mutants were isolated after treatment with 2.5 mg MNNG although the number of colonies examined were 160 & 120 after 15 and 30 minutes incubation periods respectively.

As regards the concentration of 1.25 mg MNNG, after 15 minutes treatment, ten mutants were isolated after examination of 190 and the percentage equal to 5.26. After 30 minutes, seven mutants were isolated after examination of 175 colonies and with percentage of 4.0. While after 45 minutes incubation no mutants could be isolated after examination of 140 colonies.

Plate (1) shows the isolation of biochemical mutants.

Acriflavine mutants :-

Only one mutant was obtained after examination of 40 colonies survived after 2.5 mg Ac treatment with percentage equal to 0.001.

C. Characterization of biochemical mutants :-

Results obtained were illustrated in plates 2,3, 4,5,6,7,8,9,10 and Table (4).

Table (4) illustrated that arginine, histidine and thyronine requiring mutants were isolated from 15 and 30 minutes treatment with 1.25 mg MNNG, with a high frequency at 15 minutes for arginine and histidine requiring mutants. However, thyronine requiring mutant isolated with higher percentage equal to 14.28 at 30 minutes treatment. Meanwhile, tryptophan and cystine-requiring mutants obtained only after 15 minutes treatment but isoleucine and aspargine requiring mutants obtained only after 30 minutes treatment.

On the other hand, double auxotrophic mutants were obtained at 15 minutes incubation as arginine-histidine requiring mutant and glysine-thyronine requiring mutant obtained at 30 minutes treatment

- As regards to the Acriflavine mutant, it was found to be isoleucine - thyronine requiring mutant.

D. Comparative Studies Between Mutants And The Wild Strain :-

- Morphological changes :

Gram stained films for the wild strain of Pseudomonas pyocyanea and for different types of mutants proved that there is no change in the morphological background.

Other activities were illustrated in table (5), from which it could be concluded that : Arginine, cystine and histidine requiring mutants loss the characteristic feature of exopigment production & produce non pigmented growth.

Arginine, histidine and thyronine-isoleucine requiring mutants loss the ability of glucose fermentation.

All mutants and the strain are oxidase positive

Table (5)

Comparative studies between Pseudomonas and different types of mutants

Comparative studies / Pseud & mutants	Pigment production	Glucose fermentation	Oxidase production	Urease production
Pseudomonas pyocyenea	+	+	+	+
Arginine	-	-	+	-
Cystine	-	+	+	+
Histidine	-	-	-	+
Isoleucine	+	-	+	+
Thyronine	+	+	+	+
Aspargine	+	+	+	+
Tryptophan	+	+	+	+
Glysine thyronine	+	+	+	+
Arginine aspargine	-	-	+	-
Arginine histidine	-	-	-	-
Thyronine Isoleucine	+	-	+	+

but histidine requiring mutant was negative .

Arginine requiring mutant was urease negative in contrast to other mutants and the wild strain which were positive.

The antibiotic sensitivity changes were illustrated in table (6).

Table (6), shows that nebcin was the most effective antibiotic for the wild strain and all mutants, also nearly all mutants and the wild strain were resistant for cafatrexyl and macrodantin.

Table (6)

Antibiotic sensitivity of the wild strain and of the different mutants.

Antib.sensit.discs. / Pseud. and mutants	Nebcin	Macrodantin	Cefatrexyl	Amikin	Garamycin	Rifadin	Polymxin	Carbencillin
Pseudomonas	+++	+	-	+++	++	+	+	++
Arginine	+++	-	-	++	++	-	+	+
Thyronine	+++	-	-	+++	+	+	-	++
Histidine	+++	-	-	+	+	+	+	++
Cystine	+++	-	-	+++	++	+	-	-
Isoleucine	+++	-	-	+++	++	-	+	++
Aspargine	+++	-	-	++	++	++	+	+
Tryptophan	++	-	-	++	-	+	-	+
Glysine-thyronine	+++	-	-	-	++	-	+	-
Arginine-histidine	++	-	-	++	++	-	-	++
Arginine-aspargine	+++	-	-	++	++	+	+	+
Isoleucine-thyronine	+++	++	+	+++	++	++	++	++

+++ = High sensitive
++ = Moderate sensitive
+ = Weak sensitive
- = Resistant

a = CM b = MM

Plate (1)

Showing that the auxotrophic mutant are those which are unable to grow on MM and have the ability to grow normally on CM .

a = MM

b = MM + thyronine

c = CM

Plate (2)

Showing the characterization of 5 thyronine mutants which are unable to grow on MM but were able to grow on media containing thyronine.

a = MM b = MM + arginine

Plate (3)

Showing the characterization of 6 arginine mutants which are unable to grow on MM but were able to grow on media contaning arginine.

a = MM b = MM + histidine

Plate (4)

Showing the characterization of 5 histidine mutants which are unable to grow on MM but were able to grow on media containing histidine.

a = MM b = MM + glysine

Plate 5

Showing the characterization of 1 glysine mutant which is unable to grow on MM but was able to grow on media containing glysine.

a = MM b = MM + tryptophan

Plate (6)

Showing the characterization of 1 tryptophan mutant which is unable to grow on MM but was able to grow on media containg tryptophan .

a = MM

b = MM + cystine

Plate (7)

Showing the characterization of 1 cystine mutant which is unable to grow on M.M. but was able to grow on media containing cystine.

a = MM b = MM + aspargine

Plate (8)

Showing the characterization of 2 aspargine mutants which are unable to grow on MM but were able to grow on media containning aspargine.

a = MM b = MM + isoleucine

Plate (9)

Showing the characterization of 2 isoleucine mutants which are unable to grow on MM but were able to grow on media containing isoleucine.

a = MM b = MM + adenine

Plate (10)

Showing no characterized adenine mutants which were unable to grow on MM or media containing adenine.