Studying the resistant capacity of wild type and mutant type of Bacillus brevis bacteria

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Abstract:

N-methyl-n-nitro-n-nitrosoguanidin (MNNG) and acridin orange (A.O.) was used as mutagenic agents for generation of gramicidin negative mutants of Bacillus brevis. Three gramicidin negative mutants were obtained by using MNNG (1000mg\ml) and ten gramicidin negative mutants were obtained by using A.O. (3mg\ml). A relationship between streptomycin resistance and suppression of gramicidin production was noticed. The resistance to streptomycin was increased five folds in mutant strain as compared to the wild strain.

دراسة المقاومة البكتيرية المضاد الحيوي streptomycin بين بكتريا bacillus brevis المحلية والمطفرة

الخلاصة:

تم استعمال N-methyl-n-nitro-n-nitrosoguanidin (MNNG) and acridin orange (A.O.) كعوامل مطفرة للبكتريا المحلية Bacillus brevis المنتجة للمضاد الحيوي Gramicidin (s)، وذلك للحصول على بكتريا غير منتجة لهذا المضاد الحيوي.

تم الحصول على ثلاث عزلات مطفرة من البكنريا الغير منتجة للمضاد الحيوي عند استخدام تركيز 1000mg\ml للعامل المطفر MNNG، بينما تم الحصول على عشر عزلات مطفرة من البكنريا الغير منتجة للمضاد الحيوي عند استخدام تركيز 1000 mg\ml للعامل المطفر .A.O.

لقد تم دراسة العلاقة بين مقاومة البكتريا للمضاد الحيوي streptomycin وتثبيط انتاج المضاد الحيوي Gramicidin الذي تنتجه البكتريا المحلية.

وقد وجدت انه هناك علاقة بين الاثنين، حيث زادت مقاومة البكتريا المطفرة امضاد الحيوي streptomycin خمس مرات اكثر مقارنة مع البكتريا البرية الغير مطفرة.

Introduction:

Gramicidin (s) is synthesized by Bacillus brevis with participation of two enzymes:

1- Gramicidin synthetase I.
2- Gramicidin synthetase II.

And this occurs in the presence of ATP and Mg+2 ions. This antibiotic is representing a decapeptide of ring like structure, and it is contain 2 peptide chains. Gramicidin (s) is composed from the following amino acids:

D-phenylalanine, L-proline, L-valine, L-ornithine, L-leocine, (Hori and Kurotsu, 1977).

Gramicidin synthetase I is called (heavy enzyme) and its molecular weight is 280000 dalton.This enzyme is activating the following amino acids: Proline, valine, ornethine , and leocine.

Gramicidin synthetase II is called (light enzyme), and its molecular weight 100000 dalton. This enzyme is activating phenylalanine (Katz and Demain, 1977).

These 2 enzymes have been coded by an operon genes, its size is 5.9 Kb.pand called (grs). This operon consists of 3 genes found on the same chromosome (Kratzschmar et. al., 1989, Karaus and Moraheil, 1988).

ISSN 2072-3875

This research is studying the relationship between streptomycin resistance and suppression of gramicidin production.

Materials and methods:

1. Preparation of spore suspension:

A spore suspension of Bacillus brevis was prepared according to Piriet and Demain (1983). This has been achieved by culturing the bacteria in a flask of 300 ml. The flask contains 40 ml of Hansons sporulation broth media.

The media inoculated with pure colony and incubated at37C°\300 rpm for 24hr. after the incubation period the culture were transferred to 2000 ml conical flask contains 200 ml Hansons sporulation broth media, incubated at the same conditions for 7 days.

The spores precipitated by using the centrifuge at 5000 rpm for 30 min at room temperature and spores were collected and washed by d.w. for 4 times and suspended by double d.w

2. Studying the optimum concentration of the MNNG:

For this purpose it was used method of Iwaki and Shimura,(1972), Shimura et al, (1974).

This was achieved by inoculating 3 test tubes that each tube contains 9 ml of nutrient broth media and 0.1 ml of the bacteria spore suspention, incubated at 37 C °\250 rpm for 24hr.

Seven test tubes were contained with 9 ml, 1 ml of diluted media and different concentration of MNNG (100, 250, 500, 1000, 1250 mg\ml), incubated at 37 C °\250 rpm for 1hr. Tow test tubes were left as a control.

The cells were precipitated at 5000 rpm for 30 min, the cells washed by adding minimal media which, and rewashed 3 times and the cells re-suspended by adding 5 ml of minimal media.

Then other test tubes were contained 9 ml of nutrient broth and 1 ml of mutant spore suspension, incubated at 37 C ° for 24hr.

And the viable cell count was achieved by using plate counting method, using nutrient agar media. The best concentration of MNNG was selected according to the ratio of cells that resistant to killing.

3. Studying the optimum concentration of the A.O.:

For this purpose it was used method of Iwaki and Shimura, (1972), Shimura et al, (1974).

This was achieved by inoculating 3 test tubes that each tube contains 9 ml of nutrient broth media and 0.1 ml of the bacteria spore suspension, incubated at 37 C °\250 rpm for 24hr. Seven test tubes were contained with 9 ml, 1 ml of diluted media and different concentration of A.O. (1, 2, 3, 4, 5 mg\ml), incubated at 37 C °\250 rpm for 1hr. Tow test tubes were left as a control. The cells were precipitated at 5000 rpm for 30 min, the cells washed by adding minimal media which, and rewashed 3 times and the cells re-suspended by adding 5 ml of minimal media. Then other test tubes were contained 9 ml of nutrient broth and 1 ml of mutant spore suspension, incubated at 37 C ° for 24hr.

ISSN 2072-3875

Then the viable cell count was achieved by using plate counting method, using nutrient agar media. The best concentration of A.O. was selected according to the ratio of clles that resistant to killing.

4. Indicating the mutant isolates:

It was using Carlton and Brown, (1981) method. The tested strain was staphylococcus aurous. The production of gramicidin antibiotic was noticed by the presence of a clear zone.

5. Susceptibility of wild and mutant isolates to streptomycin antibiotic and indicating the MIC (minimal inhibitory concentration):

Seven test tubes were contained 9ml of nutrient broth and different concentrations of streptomycin (10, 20, 50, 100, 150, 200, and 250 mg\ml) and inoculated with wild isolate. Other seven test tubes were contained the same contents except they inoculated with mutant strain. Then the MIC estimated from disappearance of the growth.

Results and discussion:

The optimum concentration of MNNG and A.O.:

Bacteria were obtained from Baghdad university\department of biology, which isolated from Iraqi soil and diagnosed as having ability to produce gramicidin (s) antibiotic.

The effect of different concentrations of MNNG and A.O. on production of gramicidin (s) antibiotic from the local isolate Bacillus brevis were studied. This study were revealed that the best concentration of MNNG was 1000 mg\ml, which was the killing ratio more than 50% (table 1), and the resistant cell ratio that lack the ability to produce gramicidin was 3.6%. This result was agreed with that of Iwaki and Shimura, 1972, Shimura et. al, 1974. In the case of A.O. the concentrations of 2 and 3 mg\ml give closely related results to killing ratio while 4 and 5 mg\ml give a little ratio of cells resistant to killing (table 2), this result was agreed with that of Marahiel and his group of workers in inducing mutation in cells of Bacillus brevis . The best concentration of A.O. was 2 mg\ml (Table 2).

Table (1): Different concentration of MNNG and the percentage ratio of cells resistant to killing

MNNG concentration mg\ml	Ratio of cells resistant to killing%
100	71.3
250	63.9
500	53.2
1000	3.6
1250	2.23

ISSN 2072-3875

Table (2): Different concentration of A.O. and the percentage ratio of cells resistant to killing

A.O. concentration mg\ml	Ratio of cells resistant to killing%
1	14.2
2	4.9
3	3.9
4	0.11
5	0.04

Identification the mutant isolates:

The mutant isolates that have mutation by the action of MNNG and A.O. was identified. This was done by screening the isolates by using replica plating method, and the bacteria used in this test were staphylococcus arouse. The screening revealed that some mutant isolates have been reduced their ability to produce the gramicidin, were others were lack their ability to produce the antibiotic. The phenotype, biochemical, and microscopic results revealed that the mutant isolates were related to Bacillus brevis. Then these isolates (which lack their ability to produce the gramicidin) have been isolated in order to test their resistant capacity against streptomycin antibiotic.

Gradient streptomycin plating method:

13 mutant isolates that lack their ability to produce the gramicidin were cultured on petteridishes containing streptomycin gradient media. All these isolates revealed their ability to grow on this media; this result is agreed with that of Mukherjee and Paulus. 1977 in which all mutant isolates of Bacillus brevis lacking gramicidin (s) production revealed resistant to high concentration of streptomycin antibiotic, while wild isolate which is producing to the gramicidin (s) is sensitive.

The obtaining results indicated that the using of 2 mutagenic agents (MNNG and A.O.) has been induced a genetic mutation that make the mutant isolate either reduced or lacked their ability to produced gramicidin (s) antibiotic. This result agreed with that of Marahiel et al, (1979).

Measurement of streptomycin MIC against the wild isolate and the mutant isolate of Bacillus brevis:

It was found that 20 mg\ml of streptomycin is so enough for inhibit the growth of wild isolate of the bacteria, while in mutant isolate higher concentration of streptomycin was needed to inhibit the growth of mutant isolate of the bacteria. 200 mg\ml of streptomycin was identified as the best concentration for inhibition the growth of the mutant bacteria, that is mean the resistance to streptomycin was increased five folds in mutant strain as compared to the wild strain.

ISSN 2072-3875

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ISSN 2072-3875