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# Extracellular Deoxyribonuclease Activity of *Pseudomonas aeruginosa**

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*Pseudomonas aeruginosa* cultured on deoxyribonucleic acid (DNA) media containing Nissan's heart extract or chicken heart extract could produce extracellular deoxyribonuclease, while on other DNA media with heart extracts from cow, pig and mouse could not. Variation in the kind of peptones in the DNA media did not make significant difference in this activity, although some peptones caused cloudiness of the media. *P. aeruginosa* did not need cation activators of chloride compounds to produce extracellular deoxyribonuclease. On the modified Eiken's DNA medium, gram negative bacilli other than *P. aeruginosa* and *Serratia marcescens*, i.e. *Klebsiella*, *Salmonella*, *Shigella*, *Escherichia coli* and *Alcaligenes* did not produce deoxyribonuclease. The production of extracellular deoxyribonuclease activity on the modified Eiken's DNA medium can be used as a supporting test for biochemical identification of *P. aeruginosa*.

Bacterial extracellular deoxyribonuclease is useful in bacterial identification and test for pathogenicity[3,7,9]. Thus, the deoxyribonuclease test is widely used in clinical laboratories. Only a few species have been reported to produce extracellular deoxyribonuclease, such as *Staphylococcus aureus*,[1] Group A streptococci,[8] *Corynebacterium diphtheriae*,[7] *Serratia marcescens*[2] and *Bacillus megaterium*.[4] Rothberg[5] examined production of extracellular deoxyribonuclease of enterobacterial species cultured on Difco's DNA testing medium and obtained the following results: 100% of 58 strains of *S. marcescens*, 8% of 117 strains of *Proteus* and one of 7 strains of *Alcaligenes* produced extracellular deoxyribonuclease, 3 out of 59 strains of *Pseudomonas aeruginosa* showed only slight extracellular deoxyribonuclease activity and all other bacilli did not have this activity.

No satisfactory medium has ever been found for the production of extracellular deoxyribonuclease by *P. aeruginosa*, therefore the authors have attempted to analyze the conditions of extracellular deoxyribonuclease production, and found that *P. aeruginosa* cultured on the deoxyribonucleic acid (DNA) medium containing special ingredients could produce extracellular deoxyribonuclease. This provides a very useful guide for identifying *P. aeruginosa*.

## MATERIALS AND METHODS

The medium used for extracellular deoxyribonuclease production was a modified Eiken's

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DNA medium. Other commercially available DNA media such as Difco's and Eiken's were also used for comparison. The major difference between Eiken's and modified Eiken's DNA medium is that the latter contains Nissan's heart extract instead of the heart infusion. The ingredients of modified Eiken's DNA medium are: heart extract (Nissan Chemicals) 1%, poly-peptone (Kyokuto Chemicals) 1%, DNA (N. B. C.) 0.2%, NaCl 0.5%, $CaCl_2$ 0.04% and agar (Difco) 1.5%. All the ingredients were dissolved in distilled water, autoclaved at 121°C for 15 min and poured in petri dishes to make plates. *P. aeruginosa* was cultured on plates by band-inoculation and after 24-hr incubation at 37°C, the cultures were flooded with 1.5 N HCl. A clear zone around the colony streak greater than 2 mm from the edge of colony indicates a positive reaction. Adjacent to the clear zone a white hazy area is always present which results from the action of HCl on the salts of DNA in the medium.

To study the influence of heart extract on the extracellular deoxyribonuclease production of *P. aeruginosa*, heart extracts from cow, pig, chicken and mouse were made by the method usually used for making beef extract.[6] One percent of laboratory-made heart extracts was substituted for the Nissan's heart extract in modified Eiken's DNA medium.

To study the effect of various peptones on the production of extracellular deoxyribonuclease, 1% of various peptones was substituted for poly-peptone in the same medium.

One half percent of 0.01 M solution of various chloride compounds was used instead of calcium chloride in the same medium to find the relation between the cation of chloride and extracellular deoxyribonuclease production by *P. aeruginosa*.

## RESULTS AND DISCUSSION

Nineteen strains of *P. aeruginosa* selected from 246 strains kept in our department were cultured on modified Eiken's DNA medium, Eiken's DNA medium and Difco's DNA testing medium, respectively. All the 19 strains on the modified Eiken's DNA medium produced extracellular deoxyribonuclease, while only 2 strains on the Eiken's were positive and all the strains on the Difco's were negative. This difference is probably due to the fact that Difco's DNA testing medium did not contain heart extract or infusion, and though Eiken's DNA medium contained heart infusion, its origin was different from that of the modified one. This suggests that Nissan's heart extract probably contains some substance that stimulates *P. aeruginosa* to produce extracellular deoxyribonuclease.

The results of substituting various heart extracts from different animals for the Nissan's heart extract in the modified Eiken's DNA medium (Table 1) show that only the heart extract made from chicken could induce extracellular deoxyribonuclease production by *P. aeruginosa*. It seems that both Nissan's and chicken's heart extract may contain the same special components

Table 1. Effect of various heart extracts on deoxyribonuclease production

| Sources | Result | | |
|---|---|---|---|
| | + | ± | − |
| Heart extract (Nissan) | 8 | 0 | 0 |
| Beef heart for infusion (Difco) | 0 | 6 | 2 |
| Pig heart extract | 0 | 8 | 0 |
| Chicken heart extract | 8 | 0 | 0 |
| Mouse heart extract | 0 | 0 | 8 |

+ zone of clearing greater than 2 mm from edge of colony
± zone of clearing 1 to 2 mm from edge of colony
− no clearing

which are necessary for extracellular deoxyribonuclease production.

When we substituted various peptones for the poly-peptone in the modified Eiken's DNA medium, some of the peptones such as Bacto-peptone, proteose-peptone, neo-peptone and Bacto-soytone, became cloudy after autoclaving but poly-peptone and Kyokuto-peptone did not (Table 2). Although there was difference in the cloudiness of media, this did not affect the extracellular deoxyribonuclease production. When 1.5 N HCl was added to the cloudy medium, clear zone appeared showing the positive result, and the clear media became white precipitate but clear zone still remained to show positive reaction. This indicates that the kind of peptones did not make difference to the extracellular deoxyribonuclease production of *P. aeruginosa.*

Table 2. Effect of various peptones on deoxyribonuclease production

| Species | No. of strains tested | Number of positive strains | | | | | |
|---|---|---|---|---|---|---|---|
| | | Bacto-peptone | Proteose-peptone | Neo-peptone | Poly-peptone | Kyokuto-peptone | Bacto-soytone |
| *P. aeruginosa* | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| *Serratia marcescens* (positive control) | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| *Escherichia coli* (negative control) | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Turbidity | | ++ | +++ | +++ | − | − | + |

The results of using various chloride compounds in the modified Eiken's DNA medium showed that *P. aeruginosa* could produce extracellular deoxyribonuclease without cation or other activators (Table 3). With some cations the production of deoxyribonuclease was increased whereas in some others the production decreased.

Some gram-negative bacteria kept in our laboratory such as *P. aeruginosa* (246 strains including 5 strains of achromogens), *Klebsiella* (45 strains), *Salmonella* (36 strains), *Shigella* (104 strains), *Serratia marcescens* (36 strains), *Escherichia coli* (4 strains) and *Alcaligenes* (6 strains) were

Table 3. Effect of cations on deoxyribonuclease production in 13 strains of *Pseudomonas aeruginosa*

| Chemicals | Deoxyribonuclease* | | | Chemicals | Deoxyridonuclease* | | |
|---|---|---|---|---|---|---|---|
| | + | ± | − | | + | ± | − |
| $MnCl_2 \cdot 4H_2O$ | 12 | 1 | 0 | LiCl | 8 | 3 | 2 |
| $AlCl_3$ | 10 | 3 | 0 | $CCl_3 \cdot COOH$ | 7 | 4 | 2 |
| $MgCl_2 \cdot 6H_2O$ | 9 | 4 | 0 | $HgCl_2$ | 3 | 2 | 8 |
| $CaCl_2$ | 12 | 1 | 0 | $FeCl_3 \cdot 6H_2O$ | 0 | 7 | 6 |
| KCl | 10 | 2 | 1 | $ZnCl_2$ | 0 | 1 | 12 |
| $NH_4Cl$ | 10 | 2 | 1 | $BaCl_2$ | ** | ** | ** |
| $PbCl_3$ | 9 | 2 | 2 | None | 10 | 2 | 1 |
| $SnCl_4 \cdot 5H_2O$ | 9 | 3 | 1 | | | | |

0.5% of 0.01 M of chloride compounds was used.

* + zone of clearing greater than 2 mm from edge of colony
± zone of clearing 1 to 2 mm from edge of colony
− no clearing

** no growth

cultured on the modified Eiken's DNA medium to examine their extracellular deoxyribonuclease production. Bacteria other than *P. aeruginosa* and *Serratia marcescens* showed negative results (Table 4). This suggests that the deoxyribonuclease activity can be used for separating *P. aeruginosa* from other bacteria. This is especially useful for non-pigment-producing strains of *P. aerugionsa* to differentiate from gram-negative bacteria which do not ferment lactose or ferment slowly. Thus, the extracellular deoxyribonuclease production of *P. aeruginosa* can be used as a supporting test for biochemical identification of this bacterium.

Table 4. Survey of gram-negative bacteria for presence of deoxyribonuclease

| Organism | No. of strains tested | Deoxyribonuclease | | |
|---|---|---|---|---|
| | | + | ± | − |
| *P. aeruginosa* | 246 | 243 | 3 | 0 |
| *Klebsiella* | 45 | 0 | 0 | 45 |
| *Salmonella* | 36 | 0 | 0 | 36 |
| *Shigella* | 104 | 0 | 0 | 104 |
| *Serratia marcescens* | 36 | 34 | 2 | 0 |
| *Escherichia coli* | 4 | 0 | 0 | 4 |
| *Alcaligens* | 6 | 0 | 0 | 6 |

+ zone of clearing greater than 1.5 mm from edge of colony
± zone of clearing 1 to 1.5 mm from edge of colony
− no clearing

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# 綠膿桿菌之細胞外去氧核醣核酸酶之活性

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綠膿桿菌之細胞外去氧核醣核酸酶尚未被詳細報告過。在含有特別成分之去氧核醣核酸之培養基內，發現本菌可以分解去氧核醣核酸產生澄清之分解環，亦即可以產生細胞外之去氧核醣核酸酶。僅就其產生之條件加以分析，結果如下：

(1)在去氧核醣核酸之培養基內若添加日水出品之心抽出物或鷄心抽出物則可以引誘本菌產生此種細胞外酶，但其他動物如牛，猪及鼠等之心抽出物則否。

(2)雖然部份蛋白胨會影響培養基之混濁度，但並不影響本菌之細胞外去氧核醣核酸酶之活性。

(3)本菌之細胞外去氧核醣核酸酶之產生並不絕對需要氯化合物之陽離子的活化。但培養基若含有鎂，鈣，鉀及銨等離子則對部份菌株可以得到較佳的結果。

(4)革蘭氏陰性桿菌培養於本培養基時，除綠膿桿菌及靈菌外，其他如傷寒桿菌，赤痢桿菌，肺炎桿菌，大腸桿菌及產鹼桿菌等都呈現陰性結果。

綠膿桿菌之細胞外去氧核醣酸酶之活性，不但可鑑定本菌之色素產生株，亦可應用於非色素產生株與其他乳糖醱酵較慢或不醱酵之其他革蘭氏陰性桿菌間之鑑定。