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Anaerobiosis of Pathogenic *Pseudomonas aeruginosa*: Suppression of *Tox A* Gene

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ABSTRACT *Pseudomonas aeruginosa* PAO_1 and PA 103 strains were cultured in TSBD medium in the presence or absence of oxygen with low or high iron medium. Both the strains showed higher cell densities with high iron medium under aerobic and anaerobic conditons suggesting that iron is a very important factor for bacterial cell growth. The exotoxin-A assay showed that there was 45% and 79.5% reduction in the toxin yields of PAO_1 and PA 103 strains respectively when cultured in iron restricted TSBD medium under anaerobic condition, indicating the suppression of *Tox A* gene, a situation comparable to Cystic Fibrosis (CF) lung infections of *pseudomonas*.

Pseudomonas aeruginosa is an opportunistic bacterial pathogen (Vasil, 1986) and secretes exotoxin - A (ETA) into the culture supernatant under aerobic condition. ETA is a 68 kDa protein and considered as one of the most virulence contributors of *Pseudomonas*. It is an ADP - ribosyl transferase enzyme which inhibits the eucaryotic protein synthesis at the level of translation, by covalently combining with the elongation factor-2 (Iglewski and Kabat, 1975; Iglewski et al., 1977). ETA is similar to diphtheria toxin. Being an important virulence factor, its structure, function and regulation in the presence of oxygen have been reviewed in detail (Wick et al., 1990). The *CFTR* gene responsible for cystic fibrosis (CF) disease in Caucasians has been identified and sequenced (Kerem et al., 1989). This genetic defect leads to defective electrolyte transport in several epithelial cells and ultimately to the secondary, fatal infections with *Pseudomonas aeruginosa*. Recently it has been reported that *Pseudomonas aeruginosa* encounters anaerobic conditions in CF lungs (Elizabeth, 1993, 1997) and *las B* gene encoding the normal virulence factor, the elastase was suppressed (Elizabeth, 1996) under anaerobic condition *in vitro*. Therefore the present investigation was carried out to study the expression of *tox A* gene under anaerobic condition, a situation comparable to CF lung infection of this bacterium.

MATERIAL AND METHODS

Bacterial Culture Conditions: Pseudomonas aeruginosa PAO_1 and PA 103 strains (obtained from MTCC, India and USA) secrete ETA, of which PA 103 strain was a hyper toxin producer (Wick et al., 1990). These two strains were cultured aerobically at 37°C for 18 h in low and high iron TSBD medium (Trypticase Soy broth dialysate, treated with chelex-100, Sigma-USA). The high iron medium was supplemented with 1 µl of $FeCl_3$ stock solution contains 10.76 µg of iron. The low iron medium did not receive any additional iron. This was the primary culture which was used to inoculate the secondary aerobic and anaerobic cultures with low and high iron media, incubated at 37°C for 24 h. The anaerobic cultures were supplemented with 50 mM sodium nitrate ($NaNO_3$) as a terminal electron acceptor and cultured in Coy anaerobic chamber (Elizabeth, 1997). The culture samples were collected at every 2 h until 10 h of incubation period and at 24 h from a single culture. The cell densities were measured at 540 nm. The 24 h samples were spun for 4 min at 14,000 rpm and different aliquotes of supernatants were used for exotoxin assay (Iglewsky and Kabat, 1975).

RESULTS AND DISCUSSION

The higher cell densities shown by PAO_1 and PA 103 strainst (Table 1) when cultured in high iron media, both under aerobic and anaerobic conditions suggest that iron is the most important factor for bacterial growth. However, PA 103 strain grew much better than PAO_1 strain in TSBD medium, indicating the dependence of

Table 1: The cell yields of *P. aeruginosa* PAO_1 and PA 103 strains cultured under aerobic and anaerobic conditions in low and high iron TSBD medium

Incubation period (h)	*OD 540 nm. of PAO_1 strain*				*OD 540 nm. of PA 103 strain*			
	Low Fe ($+0_2$)	*Low Fe (-0_2)*	*High Fe ($+0_2$)*	*High Fe (-0_2)*	*Low Fe ($+0_2$)*	*Low Fe (-0_2)*	*High Fe ($+0_2$)*	*High Fe (-0_2)*
2	0.006	0.003	0.010	0.004	0.013	0.010	0.021	0.032
4	0.065	0.005	0.082	0.011	0.062	0.009	0.112	0.021
6	0.211	0.013	0.237	0.026	0.222	0.006	0.416	0.014
8	0.333	0.016	0.559	0.046	0.408	0.026	0.720	0.020
10	0.477	0.085	0.834	0.096	0.614	0.018	1.008	0.026
24	0.548	0.077	0.678	0.061	0.562	0.176	0.960	0.188

$+0_2$ = cells grown in the presence of oxygen; -0_2 = bacterial cells grown under anaerobic condition.
Note the higher cells densities of both strains in high iron media under aerobic and anaerobic conditions suggesting that Fe is important factor for bacterial growth.
PA 103 strain grew well than PAO_1 strain.

cell growth on the strain. In both PAO_1 and PA 103 strains, the cell yield was higher in high iron medium but the yield of ETA was less in the same medium under aerobic and anaerobic conditions. The bacterial cell growth was less in low-iron-medium (where the available iron was chelated *i.e.* iron concentration was zero) and the ETA production was higher in the presence or absernce of oxygen in both the strains. These results indicate an inversely proportional relationship between the bacterial cell growth and yield of ETA of *Pseudomonas aeruginosa* PAO_1 and PA 103 strains. The low cell densities observed in 24 h cultures than those of 10 h cultures can be attributed to either exhaustion of nutrients or toxic effect after 10 h of incubation. In the present study there was 45% and 79.5% reduction in the exotoxin-A yields of PAO_1 and PA 103 strains, respectively (Table 2) when cultured in low iron medium without oxygen, which indicated the suppression or low expression of *Tox A* gene under anaerobic condition. This situation may be similar even in CF lung infections where *pseudomonas* encounters iron limitation and there is a possibility for novel virulence factors to express under anaerobic condition (Elizabeth, 1997).

Table 2: The aerobic and anaerobic yields of exotoxin-A of *P. aeruginosa* PAO_1 and PA 103 strains

Strains	*Low Fe ($+0_2$)*	*Low Fe (-0_2)*	*High Fe ($+0_2$)*	*High Fe (-0_2)*
1. PAO_1	6,713	3.701 (45%)	442	40
2. PA 103	112,793	23.125 (79.5%)	1,567	1,510

$+0_2$ = cultures grown in the presence of oxygen; -0_2 = cultures grown under anaerobic condition.
The figures in the table represent mean C^{14} counts/1 ml of 24 h culture spuernatant of toxin A. The figures in parenthesis indicate % reduction in toxin yields under anaerobic condition in comparison to those of aerobic, suggesting the suppression of *tox A* gene in the absence of oxygen.

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