

## Bacterial Flora of Dona Paula, India, Using Numerical Profiles

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Bacteriological studies were carried out at an estuarine beach over a period of six months. Maximum density was noted at 1 cm depth. Overall 155 isolates were identified into 13 genera. The predominant genus was *Coryneforms* (22.6%). The isolates were grouped into 43 by 3 digits sorts when profiled numerically. The separation values of the tests were high and thus point out the suitability of the tests for differentiating bacteria from this ecosystem. Maintenance of maximum test difference and higher diversity index at 1 cm depth, show that this depth sediment is more suited for studying bacterial variability in tropical estuarine beach.

Shore monitoring programmes to study the impact of industrial discharge into the sea or estuaries are carried out routinely on a temporal basis. The studies of microbial community structures have been limited in large part by extensive biochemical testing and the taxonomical difficulties involved in defining microbial species.<sup>1)</sup> The use of limited number of tests to generate numerical profiles of microorganisms described by Griffiths and Lovitt<sup>2)</sup> has been examined to study the bacterial flora of Dona Paula (Long. 73° 48'E Lat. 15° 27'N) sediment. Thus for the first time this method has been used in an estuarine beach and compared with the classical approach.

### Methods

Sediment samples were collected aseptically from the estuarine beach at low tide level using a plexiglass hand corer for a period of six months. The salinity and temperature of the area during the period of study ranged from 15-22‰ and temperature from 25-35°C. Sub-samples from 1, 3 and 5 cm were processed immediately in the laboratory. As the samples were estuarine in nature plating was done in nutrient agar (g/l peptone-5, beef extract 1.5, yeast extract, 1.5, pH 7.4±0.2) prepared in 50% aged sea water of salinity 35‰. After an incubation of 2-3 days at 28°C, the colony forming units were counted. About 10-15 isolates from each depth per month were isolated randomly, purified and stored for further studies.

Nine tests viz. gram reaction, cell morphology, catalase, motility, oxidase, gelatin liquefaction,

fermentation/oxidation and nitrate reduction were carried out. The isolates were differentiated solely on the basis of different patterns of test results. Tests were arranged in groups of three and scores 1, 2 and 4 assigned to them according to their position as already described by Griffiths and Lovitt.<sup>2)</sup> The comparison between sorts, test differences, percentage of isolates positive to different tests and the separation value were calculated using a mini computer (ABC-80). The isolates were subjected to some more tests so as to classify them upto generic level by the scheme of Schewan *et al.*<sup>3)</sup> Shannon diversity index  $H^{4-7}$  was calculated. The formula  $H' = C/N (N \log_{10} N - \sum n_i \log_{10} n_i)$  where  $C = 3.3219$ ,  $N$  = total number of individuals (*i.e.* total number of isolates in each sampling) and  $n_i$  = total number of individuals in  $i^{th}$  taxonomic grouping (total number of strain in each genus or sort as the case may be).

### Results

The depthwise variation in bacterial population during the period of study is shown in Table 1. As seen, the 1 cm depth samples recorded the maximum number throughout the study period except 1 month. The highest count ( $1.7 \times 10^4 \cdot g^{-1}$ ) was recorded in the second month and lowest in the fifth month at 5 cm depth ( $1.5 \times 10^2 \cdot g^{-1}$ ).

Overall 155 isolates were examined. Most of them were gram positive and motile, capable of liquefying gelatin and reducing nitrate. About 95% of the isolates could be identified upto the generic level. The dominant genus was *Corynebacterium* (22.6%) followed by *Moraxella* (14.8%),

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**Table 1.** Bacterial numbers of sediment samples at Dona Paula

| Months | Bacterial number (c.f.u. g <sup>-1</sup> ) |                       |                       |
|--------|--|-----------------------|-----------------------|
|        | 1 cm                                       | 3 cm                  | 5 cm                  |
| I      | 2.0 × 10 <sup>3</sup>                      | 4.8 × 10 <sup>3</sup> | 3.1 × 10 <sup>3</sup> |
| II     | 1.7 × 10 <sup>4</sup>                      | 8.1 × 10 <sup>3</sup> | 8.7 × 10 <sup>3</sup> |
| III    | 3.3 × 10 <sup>3</sup>                      | 2.2 × 10 <sup>3</sup> | 2.6 × 10 <sup>2</sup> |
| IV     | 5.4 × 10 <sup>3</sup>                      | 4.6 × 10 <sup>3</sup> | 3.8 × 10 <sup>3</sup> |
| V      | 3.9 × 10 <sup>3</sup>                      | 3.2 × 10 <sup>3</sup> | 1.5 × 10 <sup>2</sup> |
| VI     | 1.5 × 10 <sup>4</sup>                      | 6.1 × 10 <sup>3</sup> | 8.5 × 10 <sup>3</sup> |

**Table 2.** Generic distribution

| Genera                       | Percentage |
|------------------------------|------------|
| 1 <i>Staphylococcus</i>      | 0.6        |
| 2 <i>Micrococcus</i>         | 3.2        |
| 3 <i>Gaffkya</i>             | 4.5        |
| 4 <i>Diplococcus</i>         | 2.6        |
| 5 <i>Bacillus</i>            | 11.0       |
| 6 <i>Coryneformis</i>        | 22.6       |
| 7 <i>Vibrio</i>              | 11.6       |
| 8 <i>Pseudomonas</i>         | 10.3       |
| 9 <i>Alcaligenes</i>         | 5.8        |
| 10 <i>Enterobacteriaceae</i> | 5.8        |
| 11 <i>Moraxella</i>          | 14.8       |
| 12 <i>Flavobacterium</i>     | 1.9        |
| 13 <i>Acinetobacter</i>      | 1.2        |
| 14 Unidentified              | 4.5        |

*Bacillus* (11.0%) and *Pseudomonas* (10.3%) (Table 2). The number of genera ranged from 1 in the first month at 3 cm depth to 7 in the second month at 3 cm depth. The diversity indices ranged from 0.99 to 2.75. Higher indices were recorded at 1 cm depth in 5 out of 6 months (Table 3). When the isolates were numerically profiled they differentiated into 43 by 3 digit sorts and 25 by 2 digits sorts (Table 3). The diversity indices of the isolates from 1 cm depth are often higher than that from two depths. The highest diversity was seen in the fifth month at 1 cm depth with 11 sorts (3 digit) and 6 genera.

The efficiency of the tests used for the sort analysis is based on the separation values. As can be seen (Tables 4a & 4b) for all the 9 tests separation values around 50% and above were recorded depthwise, although a few exception was noted in the monthly values. Analyses of test differences (Table 5) showed that maximum number of sort pairs differed most often by 4 tests both depthwise and monthwise.

**Table 3.** Comparison of taximetric and numerical profiling methods

| Depths (cms)<br>Months | 1    |      |      |      |      |      | 3 |      |      |      |      |      | 5    |      |      |      |      |      |
|------------------------|------|------|------|------|------|------|---|------|------|------|------|------|------|------|------|------|------|------|
|                        | I    | II   | III  | IV   | V    | VI   | I | II   | III  | IV   | V    | VI   | I    | II   | III  | IV   | V    | VI   |
| Genera                 | 3    | 4    | 6    | 5    | 6    | 5    | 1 | 7    | 4    | 3    | 5    | 4    | 3    | 6    | 5    | 5    | 5    | 4    |
| Cumulative totals      | 3    | 6    | 8    | 11   | 12   | 13   | 1 | 8    | 9    | 12   | 12   | 12   | 3    | 6    | 9    | 11   | 11   | 12   |
| Diversity indices      | 1.41 | 1.36 | 2.45 | 2.24 | 2.13 | 1.81 | 0 | 2.75 | 1.98 | 0.99 | 1.61 | 1.32 | 1.06 | 2.40 | 2.25 | 2.01 | 2.19 | 2.02 |
| 2 digit sorts          | 5    | 5    | 7    | 5    | 10   | 8    | 1 | 4    | 6    | 7    | 6    | 4    | 8    | 8    | 5    | 5    | 5    | 3    |
| Cumulative totals      | 5    | 10   | 17   | 19   | 21   | 25   | 1 | 5    | 9    | 15   | 18   | 20   | 8    | 16   | 20   | 22   | 23   | 25   |
| 3 digit sorts          | 5    | 8    | 9    | 7    | 11   | 4    | 1 | 8    | 5    | 7    | 7    | 6    | 7    | 8    | 5    | 10   | 7    | 7    |
| Cumulative totals      | 5    | 13   | 22   | 27   | 37   | 43   | 1 | 9    | 13   | 20   | 26   | 32   | 7    | 15   | 20   | 30   | 36   | 43   |
| Diversity indices      | 2.11 | 2.79 | 3.12 | 2.81 | 3.39 | 2.85 | 0 | 3.00 | 2.32 | 2.72 | 2.63 | 2.45 | 2.77 | 2.95 | 2.59 | 3.14 | 2.72 | 2.64 |

### Discussion

The beach at Dona Paula does not have any previous record of pollution induced stress.<sup>3,6)</sup> Hence it is suited for any monitoring study. Being a sandy beach the bacterial population was not very high. Gram positive bacteria dominated during the study period. In this study 99.9% separation by gram reaction (depthwise) showed that these constitute 50% of the total. Gram positive bacteria are common in nearshore and terrestrial environs. *Coryneform*, a common soil

habitat was dominant.

The scoring method showed an increase in the number of sorts with time, with 1 cm depth showing the maximum diversity. In the conventional method of identification, it was generally the 1 cm depth that had higher number of genera and diversity over the period of study. The advantages of numerical profiling to study bacterial ecosystems have already been discussed by Griffiths and Lovitt.<sup>2)</sup> Moreover, the suitability of the tests used for the purpose of differentiation is a very important factor in scoring method. As shown in Table 4a and b the tests showed good variations and efficacy. Analyses of test differences showed that the mode of test differences was often with 4 tests. This shows that the population is quite heterogenous. The finding is also supported by the relatively high diversity indices throughout the period of study.

In cases of severe environmental stress such as an input of high concentration of toxic pollutants species diversity generally declines. Hence, any major changes in the environment would be seen not so much in the new sorts that emerge but in the major shift in the mode of test differences

Table 4a. Separation values as percentage of their theoretical maxima (depthwise)

| Depth (cm)           | 1    | 3    | 5    |
|----------------------|------|------|------|
| Gram positive        | 100  | 99.6 | 100  |
| Catalase             | 66.5 | 58.5 | 65.8 |
| Motility             | 99.7 | 100  | 99.1 |
| Rod shaped           | 53.2 | 39.5 | 56.4 |
| Oxidase              | 77.6 | 94.0 | 97.1 |
| Gelatin liquefaction | 91.1 | 58.5 | 98.3 |
| Fermentative         | 83.7 | 73.9 | 61.2 |
| Oxidative            | 91.1 | 96.0 | 99.1 |
| Nitrate reduced      | 97.5 | 96.0 | 99.1 |

Table 4b. Separation values as percentages of their theoretical maxima (monthly)

| Months               | I    | II    | III  | IV   | V    | VI   |
|----------------------|------|-------|------|------|------|------|
| Gram positive        | 83.2 | 69.1  | 88.8 | 69.1 | 94.4 | 13.1 |
| Catalase             | 22.2 | 96.6  | 88.8 | 60.3 | 0.0  | 73.2 |
| Motility             | 83.2 | 100.0 | 72.5 | 97.0 | 98.6 | 73.2 |
| Rod shaped           | 71.9 | 76.8  | 88.8 | 98.7 | 22.2 | 0.0  |
| Oxidase              | 58.0 | 88.8  | 88.8 | 96.5 | 50.2 | 97.0 |
| Gelatin liquefaction | 22.2 | 88.8  | 49.0 | 96.5 | 94.5 | 65.7 |
| Fermentation         | 99.7 | 50.4  | 72.5 | 27.4 | 22.2 | 90.4 |
| Oxidation            | 91.4 | 50.4  | 94.3 | 88.8 | 58.0 | 65.7 |
| Nitrate reduction    | 56.4 | 93.2  | 98.0 | 69.1 | 58.0 | 57.0 |

Table 5. Test differences of sorts

| Test differences | No. of depthwise sort pairs |             |             | No. of monthwise sort pairs |              |               |              |             |              | Most common sort pairs Total 92 |
|------------------|-----------------------------|-------------|-------------|-----------------------------|--------------|---------------|--------------|-------------|--------------|---------------------------------|
|                  | 1 Total 904                 | 3 Total 465 | 5 Total 904 | I Total 92                  | II Total 211 | III Total 137 | IV Total 277 | V Total 154 | VI Total 121 |                                 |
| 1                | 40                          | 26          | 34          | 5                           | 17           | 1             | 17           | 16          | 14           | 7                               |
| 2                | 109                         | 65          | 93          | 18                          | 40           | 16            | 48           | 30          | 20           | 16                              |
| 3                | 199                         | 94          | 200         | 17                          | 41           | 41            | 64           | 41          | 24           | 24                              |
| 4                | 238                         | 139         | 253         | 28                          | 65           | 32            | 71           | 36          | 26           | 20                              |
| 5                | 184                         | 79          | 170         | 11                          | 30           | 28            | 48           | 23          | 19           | 12                              |
| 6                | 89                          | 47          | 190         | 11                          | 15           | 13            | 21           | 6           | 15           | 10                              |
| 7                | 38                          | 12          | 37          | 1                           | 2            | 3             | 6            | 2           | 3            | 2                               |
| 8                | 6                           | 3           | 7           | 1                           | 1            | 3             | 2            | 0           | 0            | 1                               |
| 9                | 1                           | 0           | 1           | 0                           | 0            | 0             | 0            | 0           | 0            | 0                               |

as they showed a positive correlation with diversity index ( $r=+0.67$ ,  $df=18$ ,  $p<0.001$ ). Besides, the greatest advantage of this method is that none of the isolates is excluded from analyses as "unidentifiable" like in taxonomical approach. Hence, numerical profiling enabled the characterisation of bacterial flora and it is suggested that it could be used to monitor major environmental changes brought about by anthropogenic activities in tropical estuarine communities. Further experimental studies in the laboratory by artificially including certain pollutants would help in confirming the application.

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