Bacterial Flora of Positive Coliform Tests of Pacific Oysters from Polluted and Clean Regions of Vancouver Island

by F. R. Bernard
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BACTERIAL FLORA OF POSITIVE COLIFORM TESTS OF PACIFIC OYSTERS FROM POLLUTED AND CLEAN REGIONS OF VANCOUVER ISLAND

by

F. R. Bernard

Pacific Biological Station, Nanaimo, B.C.

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INTRODUCTION

The concept that coliform bacteria, particularly faecal coliforms, indicate a health hazard because of possible presence of pathogenic organisms is essentially an offshoot of a late nineteenth century concept and has culminated in the coliform-group pollution indicator. Controversy has surrounded the advantages and disadvantages of the use of particular biotypes. The greatest problem is in the differentiation between faecal and non-faecal coliform groups and to date an acceptable and uniform technique of separation has not been realized. Coliform bacteria include aerobic and facultative anaerobic non-spore forming gram-negative rods which ferment lactose with gas production at 35 °C in 48 ± 3 hours. Currently the faecal group is characterized by lactose fermentation and gas production within 24 hours at 44.5 °C (± 0.5 °C). It is accepted that spurious results are obtainable, particularly from biotypes having an IMViC reaction --++ (Klebsiella group).

It is generally held that the faecal group predominates in polluted areas. This study was undertaken to determine the IMViC characterization for oysters cultured in isolated localities far from any human pollution and comparison to individuals collected from a heavily polluted area, as a preliminary step to establish if there is a characteristic coliform flora of polluted and non-polluted waters.

METHODS

Three areas on the isolated west coast of Vancouver Island where raft-grown Pacific oysters were left beached 7 months or more were selected for this study. In each case two 12-oyster samples were removed and standard method five-tube decimal dilution MPN techniques applied (lactose broth incubated at 35.3 °C for 24 and 48 hours, confirmation of gas-positive tubes in brilliant green bile broth in conjunction with E. coli determination by use of E.C. medium incubated at 44.5 °C for 24 hours). Randomly selected positive lactose broth tubes were streaked upon Tryptone glucose extract agar and incubated at 35.5 °C for 24 hours. All positive E.C. tubes were picked to E.M.B. agar. Fifty randomly selected discrete colonies were taken from each group and inoculated into lactose broth incubated at 35.5 °C for 24 hours. IMViC determinations were applied to inoculates from the lactose broth tubes.

The uninhabited west coast of Vancouver Island localities were Quaite Bay, Kyuquot Sound, and an isolated locality in Nootka Sound. Comparison was made with Ladysmith Harbour, a heavily polluted inlet on the southeastern coast of Vancouver Island.

The experiments were undertaken in June during heavy rains after a 2 to 3 week rainless period. Due to the high runoff, the numbers of heterotrophic bacteria in the sea were expected to be rather high.
RESULTS AND DISCUSSION

This study concerns itself only with the characterization of the bacterial organisms responsible for positive reactions in the standard multiple tube method for coliform evaluation of shellfish. No actual counts are reported, but in the west coast locations a high inoculate had to be used to obtain positive results, except for Nootka, where the oysters would not have met the Public Health criteria for the commercial marketing of shellfish. A possible explanation may lie in local faecal contamination by indigenous mammals.

A summary of percentage occurrence of the various "coliform reacting" bacteria is presented in Table 1, which is a summary of the various varieties of each group shown in Table 2.

Although no methodology exists for the absolute differentiation of non-faecal coliform type bacteria from those of intestinal origin, it is generally accepted that a positive elevated temperature test is a clear indication of recent faecal contamination. Examination of Table 1 demonstrates that this is probably true in areas of known and heavy sewage pollution. (All the positive elevated temperature tubes from Ladysmith Harbour checked out as E. coli, but for regions of no human pollution only 65% of the positive tubes were E. coli.)

Many workers have reported Klebsiella aerogenes to be the dominant soil coliform type. In this study these four IMViC biotypes constituted 32 to 36% of the flora contained in the presumptive lactose tubes and decreased to 24 to 28% of the colonies sampled from the E.C. elevated temperature medium.

K. aerogenes variety IV (IMViC -++)), which is elevated temperature medium positive, accounted for 2 to 14% of the presumptive tube flora and rose to 18 to 20% of the E.C. medium colonies characterized by IMViC procedures. The E. coli group consisted of 24 to 46% of the primary lactose tubes and 60 to 74% of the E.C. positive tubes. The Citrobacter group accounted for 12 to 24% of the presumptive tubes and insignificantly in the E.C. confirmed positive tubes (0 to 6%).

The specificity of the E.C. elevated temperature test for the determination of E. coli is widely accepted, particularly by European workers. However, other biotypes, notably E. coli type II (IMViC -++) and K. aerogenes type IV (IMViC -++) are also gas positive. Thomas et al. (1955) reviewed previous studies and concluded that elevated temperature gas producers not being E. coli type I organisms are rare in most habitats, but Henriksen (1954) reported a much higher proportion of biotypes capable of gas production from Eijkman medium at 44 C. Tennant and Reid (1961) noted that 6 to 7% of non-faecal organisms, representing 10 coliform biotypes, were gas-positive at 44 C. Tennant et al. (1961) examined the specificity of the E.C. confirmation test for E. coli and reported the mean fidelity of this procedure to be around 90%; paradoxically, the percent recovery of E. coli was higher in unpolluted than in polluted areas.
The IMViC characterization of the presumptive tubes and again after inoculation into the E.C. elevated temperature tubes allowed an indication of the proportions of the various biotypes to be gained. The results confirmed the report by Tennant et al. (1961) that E.C. gas negative and gas positive biotypes could occur when mixed with "faecal" cultures with the E.C. confirmatory medium. This would preclude further tests directly applied to the E.C. tubes without first replating and colony selection. Further study of the competition and interaction of various biotypes within one culture tube is required. It is evident that many coliforms are able to survive at least 24 hours in the E.C. elevated temperature medium and probably some multiplication occurs. In Quait Bay the presumptive lactose tubes yielded 8% E. coli type I, while in the E.C. medium they consisted of 42%; however, one other elevated temperature lactose fermenter and six other negative biotypes were isolated from the same culture. In the study "faecal coliforms" (IMViC characterization, gas production at 44.5 °C) accounted for 35 to 42% of all colonies tested. Of particular interest was one culture from Kyuquot which was negative in brilliant bile green yet E.C. positive.

The distribution pattern of IMViC types is quite different in the heavily polluted Ladysmith Harbour, where 80% of the presumptive tubes contained E. coli, and all the elevated temperature tests were due to faecal bacteria. These results may be explained by the speculation that polluted areas have a decreased load of organisms other than faecal, but it is unlikely that a large influx of E. coli would result in the depression of the numbers of other representatives of the group. The probable explanation lies in species competition within the growing medium. A large inoculate of E. coli may inhibit the development of Citrobacter, and any organism compatible to the inhibitory properties of the medium, that predominated in the inoculate will gain the upper hand and numerically exceed other forms. Such a hypothesis would explain that more even distribution of the IMViC types in the unpolluted areas.

It may be concluded from the wide spectrum of biotypes present in the pollution-free areas and the resultant high counts both of general coliform and E. coli that great care in interpreting MPN evaluations must be exercised, particularly in regions demonstrably free of human faecal pollution. In Kyuquot the number of E.C. positive tubes from two samples dropped from 18 to 8 after screening out non-faecal E.C. positive biotypes, reducing the faecalMPN. It can be concluded that MPN procedures have acceptability only as routine monitoring techniques and must be preceded by IMViC characterization of the coliform group and overall sanitary survey and hydrographic studies of the areas in question.

REFERENCES


Table 1. Percentage occurrence of various IMViC types in positive tubes from oysters collected from various localities.

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<th>IMViC group</th>
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<th>Kyuquot</th>
<th>Ladysmith Harbour</th>
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*P. = Presumptive test (production of gas in lactose broth).
C. = Confirmed test (production of gas in brilliant bile green broth).
+ = *E. coli* test (production of gas in Eijkman medium at 44.5 C).
Table 2. Percentage distribution of group varieties determined by IMViC procedure on randomly chosen bacterial colonies from oysters collected at various localities.

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