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**EFFECT OF IRON CONCENTRATION ON THE PRODUCTION
OF VIRULENCE FACTORS IN *Aeromonas hydrophila* AND
*Vibrio cholerae***

BY

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ABSTRACT

Aeromonas hydrophila and *Vibrio cholerae* are well known human pathogens. A total of 43 *A. hydrophila* isolates derived from human diarrheal samples (23 isolates) and freshwater (20 isolates) were used in this study. In addition, 40 *V. cholerae* O1 El Tor isolates were obtained from fecal samples of cholera patients. The objective of the study was to screen for the ability of the isolates to produce virulence-associated factors, namely, siderophores, hemolysins and proteases, under iron-deficient and iron-rich conditions at the growth temperatures of 28°C and 37°C.

In both *A. hydrophila* and *V. cholerae*, the siderophore production increased significantly ($p < 0.01$) under iron-limiting conditions at both 28°C or 37°C, suggesting that siderophore production was one of the main iron-acquisition mechanisms of these bacteria.

The role of plasmids of *A. hydrophila* in moderating siderophore production was analyzed. Treatment with acridine-orange for 24 hours removed the larger plasmids but smaller plasmids (< 5 Mda), more common in environmental isolates, were resistant to curing. No significant difference ($p > 0.05$) was found in the siderophore producing ability of the isolates before and after curing. This observation suggests that siderophore production in *A. hydrophila* was not influenced by plasmids.

Almost half of the *A. hydrophila* isolates were non-hemolytic. Among the hemolytic strains, a significant increase ($p < 0.01$) in the hemolytic activity was detected under iron-limiting conditions, indicating that the hemolysins of *A. hydrophila* could be involved in iron-acquisition process. In contrast, the *V. cholerae* isolates were non-hemolytic at 37°C. However, at 28°C, 13 of the 40 isolates possessed low-

hemolytic activity. The hemolysin production of the hemolytic *V. cholerae* isolates at 28°C. decreased under iron-limiting conditions, indicating that hemolysins of *V. cholerae* are not involved in iron-acquisition. The protease production of both *A. hydrophila* and *V. cholerae* isolates did not increase under iron-limiting conditions, implying that proteases have no role in iron-acquisition in these bacteria.

The production of virulence-associated factors in these two species was also affected by growth temperature. The *V. cholerae* isolates produced more siderophores at 37°C compared to 28°C. Clinical isolates of *A. hydrophila* produced more hemolysins at 37°C than 28°C. In general, the *A. hydrophila* isolates produced more proteases at 28°C than 37°C. However, siderophore production of the *A. hydrophilic* isolates and protease production of the *V. cholerae* isolates were not affected by the growth temperature.

In *A. hydrophila*, the production of these factors was also dependent upon the source of the isolates. The clinical isolates consistently produced more siderophores and proteases than did the environmental isolates. In contrast, the environmental isolates produced more hemolysins than the clinical isolates.

The results of this study indicate that both siderophores and hemolysins are involved in the iron-acquisition process of *A. hydrophila*. In contrast, in the *V. cholerae* El Tor isolates tested, siderophore production was probably the main iron-acquisition mechanism.

ABSTRAK

Aeromonas hydrophila dan *Vibrio cholerae* adalah patogen manusia yang terkenal. Sebanyak 43 isolate *A. hydrophila* yang diambil daripada sampel cirit-birit manusia (23 isolate) dan air tawar (20 isolate) digunakan dalam kajian ini. Selain dari itu 40 isolate *V. cholerae* O1 E1 Tor telah diambil daripada sampel najis pesakit kolera. Tujuan kajian ini ialah untuk menskrin keupayaan isolate-isolate ini untuk menghasilkan faktor berhubung virulen, khasnya siderophore, hemolisin dan protease di bawah keadaan kekurangan zat besi dan kelebihan zat besi pada suhu tumbesaran 28°C dan 37°C.

Dalam kedua-dua *A. hydrophila* dan *V. cholerae*, penghasilan siderophore bertambah dengan bererti ($p < 0.01$) di bawah keadaan kekurangan zat besi pada kedua-dua 28°C dan 37°C, menyarankan penghasilan siderophore sebagai salah satu mekanisme pemerolehan zat besi yang utama dalam bakteria ini.

Peranan plasmid-plasmid *A. hydrophila* dalam pengawalan penghasilan siderophore juga dikaji. Perlakuan dengan acridine-orange selama 24 jam menyingkirkan plasmid-plasmid besar tetapi plasmid-plasmid yang lebih kecil (< 5 Mda), adalah lebih resistan terhadap penyingkiran. Tiada perbezaan ($p > 0.05$) didapati dalam keupayaan menghasilkan siderophore sebelum dan selepas penyingkiran plasmid. Pemerhatian ini mencadangkan bahawa penghasilan siderophore dalam *A. hydrophila* tidak dipengaruhi oleh plasmid.

Hampir separuh daripada isolate-isolate *A. hydrophila* didapati tidak hemolitik. Di kalangan isolate-isolate hemolitik, suatu pertambahan bermakna ($p < 0.01$) didapati di bawah keadaan kekurangan besi, mencadangkan bahawa hemolisin *A. hydrophila* mungkin terlibat dalam pemerolehan besi. Sebaliknya, semua isolate-isolate *V. cholerae* tidak hemolitik pada 37°C. Bagaimanapun, pada 28°C, 13 isolate daripada 40 isolate ini

mempunyai aktiviti hemolitik yang rendah. Penghasilan hemolisin oleh *V. cholerae* yang hemolitik berkurangan di bawah keadaan kekurangan besi, mencadangkan bahawa hemolisin *V. cholerae* tidak terlibat dalam proses pemerolehan besi. Penghasilan protease oleh kedua-dua *A. hydrophila* dan *V. cholerae* tidak bertambah di bawah keadaan kekurangan besi, mencadangkan bahawa protease tidak terlibat dalam pemerolehan besi kedua-dua spesis ini.

Penghasilan faktor berhubung virulen oleh kedua-dua spesis ini juga dikawal oleh suhu tumbesaran. Isolate-isolate *V. cholerae* menghasilkan lebih banyak siderophore pada 37°C berbanding dengan 28°C. Isolate-isolate klinikal *A. hydrophila* menghasilkan lebih banyak hemolisin pada 37°C berbanding dengan 28°C. Umumnya, isolate-isolate *A. hydrophila* menghasilkan lebih banyak protease pada 28°C berbanding dengan 37°C. Bagaimanapun penghasilan siderophore oleh *A. hydrophila* dan penghasilan protease oleh *V. cholerae* didapati tidak dipengaruhi oleh suhu tumbesaran.

Dalam *A. hydrophila*, penghasilan faktor-faktor ini juga didapati bergantung kepada sumber pengasingan. Isolate-isolate klinikal secara konsisten menghasilkan lebih banyak siderophore dan protease berbanding dengan isolate-isolate persekitaran. Sebaliknya, isolate-isolate persekitaran menghasilkan lebih banyak hemolisin berbanding dengan isolate-isolate klinikal.

Keputusan kajian ini mencadangkan bahawa kedua-dua siderophore dan hemolisin adalah terlibat dalam proses pemerolehan besi dalam *A. hydrophila*. Sebaliknya, dalam isolate-isolate *V. cholerae* O1 El Tor yang dikaji, besar kemungkinan penghasilan siderophore adalah mekanisme pemerolehan besi yang utama.

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