PLASMID-MEDIATED IRON SEQUESTERING SYSTEMS IN PATHOGENIC STRAINS OF VIBRIO ANGUILLARUM AND ESCHERICHIA COLI


Department of Microbiology and Immunology
School of Medicine
Oregon Health Sciences University
3181 S.W. Sam Jackson Park Road
Portland, Oregon 97201

INTRODUCTION

Properties of the host vertebrate and invading bacteria define the conditions that precipitate the onset of an infectious process. In disseminated infections, an important bacterial attribute is the ability to grow in the host vertebrate's body fluids and tissues.

An essential element for bacterial growth is iron, which is found as a component of many bacterial cellular processes (29). However, in vertebrate fluids, iron is bound by high affinity iron-binding proteins and thus the concentration of available iron is far too low for bacterial utilization (6). Certain pathogenic isolates of Vibrio anguillarum and Escherichia coli have developed very efficient plasmid-mediated iron-sequestering mechanisms (7,8,31). These systems encoded by pJM1 in V. anguillarum and pColV-K30 in E. coli, responsible for the high-virulence phenotype of these bacteria, have been genetically characterized (9,11,12,28,32). The analysis of mutants in these 2 systems led to the demonstration of the existence of 2 components: a siderophore and a receptor for the iron-siderophore complexes. In the case of pColV-K30 the siderophore was identified as aerobactin (30), a hydroxamate compound originally found in culture supernatants of Aerobacter aerogenes (14). The receptor was determined to be a 74 kilodalton (kd) outer membrane protein (4,15). Recently, the genetic determinants for aerobactin and its receptor have been cloned (3).

In the case of V. anguillarum, concomitant with efficient plasmid-mediated iron uptake, there was an induction of the synthe-
sis of 2 novel outer membrane proteins. One of them, the 86 kd OM2 protein was associated with the presence of pJM1 (10). Analysis of transposition-generated mutants suggested that OM2 is a receptor for complexes of iron with a siderophore produced by V. anguillarum cells harboring an intact pJM1 plasmid (28).

The present work describes the molecular cloning and expression of genetic determinants of the pJM1 plasmid-mediated iron-uptake system of V. anguillarum. A component of this system, the iron-regulated outer-membrane protein OM2, is encoded by the pJM1 plasmid and may play a role as a receptor for complexes of iron with the V. anguillarum siderophore anguibactin.

It is also reported here that although the pJM1 and pColV-K30 plasmid-mediated iron uptake systems are phylogenetically unrelated (27), they share a common feature: the iron uptake regions in both plasmids are flanked by repeated sequences. These characteristics may have played a role in the epidemiological spread of these virulence factors.

RESULTS AND DISCUSSION

Cloning of pJM1 DNA Genes Involved in the Iron Uptake Process.

The pJM1 DNA fragments obtained by partial digestion with the restriction endonuclease XhoI were ligated with the vector pVK102 (17) cleaved with the same enzyme. The ligated DNA was in vitro packaged and the phage particles were used to transduce E. coli HB101. Based upon previous knowledge of the iron-uptake region in pJM1 (28), clones carrying DNA fragments associated with the iron-uptake sequences were selected for further studies. The pJM1 DNA fragments contained in the clones pJHC-T2, pJHC-T7, pJHC-T11, and pJHC-T2612 are shown in Fig. 1. Each of these clones was conjugated into different iron uptake-deficient low-virulence V. anguillarum strains as previously described (24) by using the helper plasmid pRK2013 (13). The V. anguillarum recipients were the plasmidless H775-3, the 775::Tnl-5 strain harboring the Tnl insertion derivative pJHC-91 and 775::Tnl-6 carrying the deletion plasmid pJHC9-8 (Fig. 1). The plasmids carried by each V. anguillarum exconjugant are listed in Tab. 1. Analysis of the production of siderophore activity was carried out by testing culture supernatants in bioassays using as indicator strain V. anguillarum 775:Tnl-5 (28). The results shown in Tab. 2 indicated that exconjugants harboring either the cosmid pJHC-T7 or pJHC-T2612, in addition to an indigenous pJM1 derivative such as pJHC-91 or the deleted plasmid pJHC9-8 exhibited a level of siderophore activity comparable to that produced by the wild-type V. anguillarum 775. Exconjugants carrying only the clone pJHC-T7 or pJHC-T2612 produced a very low level of siderophore activity. No siderophore activity was detected in V. anguillarum har-