Systematic and Applied Microbiology 36 (2013) 224–234

Contents lists available at SciVerse ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Natural transformation as a mechanism of horizontal gene transfer among environmental *Aeromonas* species☆

Jennifer R. Huddleston[a,*], Joshua M. Brokaw[a], John C. Zak[b], Randall M. Jeter[b]

[a] *Biology Department, ACU Box 27868, Abilene Christian University, Abilene, Texas 79699, USA*
[b] *Department of Biological Sciences, Box 43131, Texas Tech University, Lubbock, Texas 79409, USA*

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form 15 January 2013
Accepted 20 January 2013

Keywords:
Aeromonas
Natural transformation
Horizontal gene transfer
Environmental bacteria

ABSTRACT

Aeromonas species are common inhabitants of aquatic environments and relevant as human pathogens. Their potential as pathogens may be related in part to lateral transfer of genes associated with toxin production, biofilm formation, antibiotic resistance, and other virulence determinants. Natural transformation has not been characterized in aeromonads. DNA from wild-type, prototrophic strains that had been isolated from environmental sources was used as donor DNA in transformation assays with auxotrophs as the recipients. Competence was induced in 20% nutrient broth during the stationary phase of growth. Optimal transformation assay conditions for one chosen isolate were in Tris buffer with magnesium or calcium, pH 5–8, and a saturating concentration of 0.5 μg of DNA per assay (3.3 ng of DNA μl^{-1}) at 30 °C. Sodium was also required and could not be replaced with ammonium, potassium, or lithium. The maximal transformation frequency observed was 1.95×10^{-3} transformants (recipient cell)$^{-1}$. A survey of environmental *Aeromonas* auxotrophic recipients ($n = 37$), assayed with donor DNA from other wild-type environmental aeromonads under optimal assay conditions, demonstrated that 73% were able to act as recipients, and 100% were able to act as donors to at least some other aeromonads. Three different transformation groups were identified based on each isolates' ability to transform other strains with its DNA. The transformation groups roughly corresponded to phylogenetic groups. These results demonstrate that natural transformation is a general property of *Aeromonas* environmental isolates with implications for the genetic structures of coincident *Aeromonas* populations.

Introduction

Aeromonads are implicated in self-limiting gastrointestinal infections [3,4,24] and extraintestinal infections, with wounds serving as the usual portal of entry [20]. Both environmental and clinical aeromonad isolates demonstrate resistance to antimicrobial agents, making the treatment of infections more difficult [23,55]. These resistances can be transferred by plasmids [22,41] or through the process of natural transformation. Evolutionarily recent high frequency of horizontal gene transfer among aeromonads has been reported [42]. The mechanism of these gene transfer events is unknown. Approximately 90 prokaryotic species have been characterized as naturally transformable [14] occurring in diverse ecological groups, such as photolithotrophs, chemolithotrophs, heterotrophs, methylotrophs, clinical pathogens, and members of *Archaea* [25].

Natural transformation in *Aeromonas* is not widely known or studied. Sakai [39,40] reported that a single strain of *A. salmonicida* was capable of competence and transformation but gave no detailed descriptions of the mechanisms involved or the conditions under which transformation occurred. No research has characterized this process in any aeromonad, nor have the evolutionary or clinical consequences of transformation in these ubiquitous aquatic organisms been considered. The goals of this study were to determine if *Aeromonas* species isolated from streams and lakes are generally competent for natural transformation, characterize optimal conditions for transformation in the laboratory, determine if the recognized species of aeromonads can be induced to competence under the same conditions, and assess the scope and evolutionary patterns of transformability within the genus.

Aeromonas isolates have been categorized into 30 validly published species names to date [5,6,15,19,29]. A limited number of biochemical tests can be used to place most strains into one of three phenotypic complexes [1]. The *A. hydrophila* complex is comprised of *A. hydrophila*, *A. bestiarum*, and *A. salmonicida*. The *A. caviae* complex is comprised of *A. caviae*, *A. media*, and *A. eucrenophila*. The *A.*

☆ The GenBank accession numbers for the sequences reported in this paper are GenBank: FJ159389 to FJ159424.

* Corresponding author. Tel.: +1 325 674 2010; fax: +1 325 674 2009.
E-mail addresses: jennifer.huddleston@acu.edu (J.R. Huddleston), josh.brokaw@acu.edu (J.M. Brokaw), john.zak@ttu.edu (J.C. Zak), randall.jeter@ttu.edu (R.M. Jeter).

0723-2020/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.syapm.2013.01.004

sobria complex is comprised of *A. veronii* biovar sobria, *A. jandaei*, *A. schubertii*, and *A. trota* [1].

Materials and methods

Bacterial strains and identifications with gyrB sequences

Aeromonas strains used in this investigation were isolated from rivers and playa lakes in west Texas and New Mexico [18]. DNA was extracted and purified from each strain using the method of Ulrich and Hughes [51]. The *gyrB* gene sequences, using the same primers and conditions as described by Yañez et al. [57], were amplified in order to obtain species identifications of the isolates and reconstruct a phylogenetic history. The 1100-bp *gyrB* PCR products were treated with ExoSAP-IT (USB Corporation, Cleveland, Oh.) to remove primers and extra dNTPs and sequenced at the Core Facility of the Texas Tech University Center for Biotechnology and Genomics. Sequences were analyzed using Vector NTI, 9.0.0 (Invitrogen, Carlsbad, Ca.), deposited into GenBank (accessions FJ159389 to FJ159424), and were compared against all microbe sequences deposited in NCBI using BLAST [7]. Two aeromonads from the American Type Culture Collection (ATCC), *A. caviae* ATCC 15468^T and *A. veronii* biovar sobria ATCC 9071, were used to confirm the accuracy of the sequences and identifications.

Mutagenesis and screening of aeromonads

Thirty-five wild-type environmental aeromonads and the two *Aeromonas* ATCC strains were mutagenized to auxotrophy with diethyl sulfate (DES) (Sigma–Aldrich, St. Louis, MO). Each aeromonad strain was inoculated into 2 ml of no citrate E (NCE) minimal medium [9] supplemented with 0.25% glucose (Sigma) and incubated with aeration overnight at 30 °C. Four drops of DES were added to 5 ml of each culture diluted 1:10 in NCE buffer, and the tubes incubated at room temperature for 1 h. Then, 0.1 ml of the DES-treated cells was inoculated into 2 ml of Difco tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD). The cells were incubated with aeration at 30 °C overnight, and 0.1 ml was inoculated into 2 ml of NCE glucose minimal medium and incubated for 2 h at 30 °C. Cefuroxime (Sigma) was added for a final concentration of 30 μg ml^{-1} to counter select for non-growing, auxotrophic cells. The cells were then incubated at 30 °C for 3 h. The cultures were diluted in NCE buffer, spread onto Difco tryptic soy agar (TSA) (Becton Dickinson), and incubated overnight. Colonies were patched to TSA, incubated overnight, and replica-plated to NCE glucose minimal agar. Auxotrophs that did not grow on minimal medium were subcultured and used in later experiments.

Quantitative transformation assays

Competent cells were prepared by inoculating a single colony into 20% nutrient broth (NB) (Becton Dickinson), pH 7.5, and incubating with aeration overnight at 30 °C. Each culture was diluted 1:100 in 20% NB and incubated again with aeration for 24 ± 2 h at 30 °C. The competency protocol was derived through trial and error in preliminary experiments that sought to obtain maximum response. Quantitative transformation assays were performed in 1.5-ml microcentrifuge tubes that contained 100 μl of standard transformation buffer consisting of 53.5 m*M* Tris (Sigma), pH 7.9, 20 m*M* $MgSO_4$ (Spectrum Chemical, Gardena, CA), and 50 m*M* NaCl (EM Science, Gibbstown, NJ). Competent cells were added in a volume of 40 μl [$(4.0 \pm 1.5) \times 10^7$ colony-forming units (CFU)]. Pure DNA from each wild-type aeromonad was prepared according to the method of Ulrich and Hughes [51]. One microgram of DNA was added in a volume of 10 μl (final concentration of 6.7 ng μl^{-1}). The assay mixture was incubated at 30 °C for 30 min. After the initial incubation, transformation was terminated by adding 10 μl of 200 μg ml^{-1} of deoxyribonuclease I (DNase I) (Sigma) solution and then incubating at 30 °C for 1 h to degrade free DNA. Controls were performed by adding DNase I to the assay mixture before the initial 30-min incubation to eliminate transformation. No-DNA and no-cell assays were also performed as negative controls; no transformants ever appeared on any of the control plates. Assay mixtures were diluted in 0.85% NaCl, spread onto NCE glucose minimal agar, and incubated 48 h at 30 °C. Colonies that grew on NCE glucose minimal agar were considered to be transformants. Transformants were counted, and transformation frequencies were determined by dividing the number of transformants by the initial number of CFUs added.

Determining optimal competence induction medium

One auxotroph (C-70, a histidine mutant derived from putative *A. salmonicida* subsp. *pectinolytica* strain 92 originally isolated from the North Fork of the Brazos River) was chosen for its high level of transformability to characterize optimal competence induction and transformation conditions. A single colony was inoculated into 20% NB and incubated with aeration overnight at 30 °C. The culture was diluted 1:100 in 20%, 40%, 60%, 80%, and 100% NB; 20% and 100% Luria broth (LB); 20% and 100% TSB; and NCE glucose minimal broth supplemented with 0.1 m*M* L-histidine (Sigma) and incubated with aeration overnight at 30 °C. Transformation assays were performed in triplicate in standard transformation buffer as described above. Transformation frequencies were calculated as "transformants per recipient" and used as an indicator of competence.

Effect of growth phase on competence

One colony of *Aeromonas* C-70 was inoculated into 2 ml of 20% NB, pH 7.5, and incubated with aeration overnight at 30 °C. The 2-ml culture was inoculated into 200 ml of 20% NB in a flask and incubated at 30 °C with aeration. Samples were taken hourly for 24 h and then at 36 h and 48 h. The absorbance of each sample was measured at a wavelength of 540 nm using a Spectronic Genesys 5 spectrophotometer (Spectronic Instruments, Rochester, NY). Samples were diluted in 0.85% NaCl and plated onto 20% Difco nutrient agar (Becton Dickinson), pH 7.5, and incubated at 30 °C overnight for viable counts. Samples were assayed for transformation as described above. This procedure was repeated with three different flasks and two replicates per flask at each sampling time.

Characterization of optimal transformation conditions

Assays for determining optimal transformation conditions were performed as described above for quantitative transformation assays except that each parameter was varied independently. Preliminary experiments indicated that NaCl and $MgSO_4$ were required for transformation to occur. Concentrations of NaCl and $MgSO_4$ were varied to determine optimal concentrations of each to maximize transformation. Varying concentrations of KCl (Fisher Scientific, Pittsburgh, PA), LiCl (Sigma), and NH_4Cl (Fisher Scientific) were tested in place of NaCl. Varying concentrations of $CaCl_2$ (Mallinckrodt Baker, Phillipsburg, NJ) were tested in place of $MgSO_4$. Other variations included Tris concentration, temperature, pH, DNA concentration, and DNA incubation time with cells before adding DNase I. Assays were performed on different days in triplicate or quadruplicate.