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Rapid Plasmid Analysis for Identification of *Edwardsiella ictaluri* from Infected Channel Catfish (*Ictalurus punctatus*)

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Eighteen different strains of *Edwardsiella ictaluri* isolated from infected channel catfish (*Ictalurus punctatus*) were screened to determine whether plasmid DNA was present. Two plasmids of 5,700 and 4,900 base pairs were identified. Restriction enzyme analysis showed that each of the strains harbored these same two plasmids. Restriction maps of the separated plasmids indicated that these plasmids were not closely related to each other. A rapid screening technique was developed that would allow the presence of these plasmids from either broth cultures or single colonies of *E. ictaluri* to be determined within 2 to 3 h by agarose gel electrophoresis. These results suggest that plasmid fingerprinting of *E. ictaluri* should become a useful tool in the presumptive identification of this bacterium from infected channel catfish.

Edwardsiella ictaluri is the bacterial pathogen responsible for enteric septicemia of catfish (ESC). *E. ictaluri* was characterized and named by Hawke et al. (5) in 1981 and can be distinguished biochemically from the more common member of the genus *Edwardsiella tarda*. Since the first description of ESC (4) in channel catfish (*Ictalurus punctatus*), there have been subsequent isolations of *E. ictaluri* from nonictalurid fish (12; M. L. Kent and J. M. Lyons, Report, Fish Health News, 2:ii, 1982). ESC has been viewed as an acute septicemia which can progress rapidly in apparently healthy, fast-growing catfish and can result in significant mortality. ESC has become a significant problem in the aquaculture of channel catfish in the southeastern United States. The incidence of disease occurs within the optimum in vitro growth temperature of the bacteria, e.g., 22 to 28°C. When isolated from kidney, brain, blood, etc., the bacterium requires 48 h at 30°C to form typical colonies that are 2 mm in diameter (5). This relatively slow rate of growth makes subsequent biochemical identification an extended process which may require up to 5 days for confirmation.

Plasmid DNA has been shown in a variety of different bacterial species to code for enzymes in specific biochemical pathways; some plasmid-encoded functions are responsible for some of the virulence characteristics of a particular species (2). In this study various isolates of *E. ictaluri* were examined to determine if plasmid DNA was present. These studies led to this report, the results of which show that 18 different isolates of *E. itcaluri* each harbor the same two plasmids. In addition, a rapid technique for plasmid identification is described which can presumptively identify *E. ictaluri* strains isolated from infected channel catfish. This technique can reduce the 3 to 5 days presently required for biochemical identification to 2 to 3 h.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains of *E. ictaluri* were obtained as follows and are summarized in Table 1. The 12 Mississippi isolates were obtained from infected channel catfish grown in various commercial ponds in the Mississippi Delta during 1985. These isolates were identified and obtained from T. Santucci of the Cooperative Extension Service, Stoneville, Miss. The five strains of *E. ictaluri* isolated from channel catfish from Alabama were obtained from Y. Brady of the Department of Fisheries, Auburn University, Auburn, Ala. The type strain ATCC 33202 was obtained from the American Type Culture Collection, Rockville, Md. All of the cultures described above were grown at 30°C in brain heart infusion medium (Difco Laboratories, Detroit, Mich.).

Additional strains of bacteria were obtained as follows: *E. tarda* ATCC 15947 was obtained from the American Type Culture Collection. *Escherichia coli* J53 bearing the pJB4JI plasmid (6) was obtained from B. Rowe Byers, Department of Microbiology, University of Mississippi Medical Center, Jackson. These cultures were grown at 37°C in brain heart infusion medium.

Plasmid detection. Bacteria were grown in 5 ml of brain heart infusion broth overnight. A 0.3-ml fraction of these cultures was centrifuged in a 1.5-ml microcentrifuge tube in a microfuge (Eppendorf) for 2 min. Three plasmid DNA isolation methods were used. With the first method (7) the cell pellet was suspended in 100 μl of lysing solution (3% sodium dodecyl sulfate [SDS] and 50 mM Tris adjusted to pH 12.6 with NaOH) and incubated at 65°C for 30 min. An equal volume of freshly prepared phenol-chloroform (1:1; vol/vol) was added, and the emulsion was mixed and centrifuged briefly to break the emulsion. The upper aqueous phase was carefully removed to avoid the precipitate at the interface and was saved until analysis.

With the second method (3) the cell pellet was suspended in 0.5 ml of lysing solution (50 mM Tris [pH 8.5], 50 mM EDTA, 15% sucrose, supplemented just before use with 1 mg of lysozyme per ml). After incubation at room temperature for 10 min, 10 μl of 10% SDS was added to the cell suspension. The tubes were incubated at 65°C for 30 min, and 50 μl of 5 M potassium acetate was added. After 30 min on ice the tubes were centrifuged and the supernatant liquid was removed to another 1.5-ml microcentrifuge tube. The supernatant liquid was mixed with 2 volumes of 100% ethanol and incubated on ice for 10 min. The tube was centrifuged for 10 min in microfuge (Eppendorf), and the pellet was allowed to air dry. The precipitated DNA was allowed to dissolve in 50 μl of TE (10 mM Tris [pH 8.0] and 1 mM EDTA) for 30 min prior to agarose electrophoresis.

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TABLE 1. Identification numbers and geographic origin of *E. ictaluri* strains isolated from infected channel catfish

Identification no.	Geographic origin
S85-762	Mississippi
S85-765	Mississippi
S85-1354	Mississippi
S85-1362	Mississippi
S85-1377	Mississippi
S85-1381	Mississippi
S85-1397	Mississippi
S85-1398	Mississippi
S85-1399	Mississippi
S85-1400	Mississippi
S85-1401	Mississippi
S85-1404	Mississippi
AL78-119	Alabama
AL83-189	Alabama
AL84-122	Alabama
AL84-142	Alabama
AL84-187	Alabama
ATCC 33202[a]	Georgia

[a] Source and geographic origin reported by Hawke et al. (5).

The third method used for the identification of plasmids in *E. ictaluri* was the rapid identification methods described by Barnes (1) and Maniatis et al. (8) with slight modifications. Bacterial colonies (diameter, 1 to 2 mm) were transferred from the plates with a sterile toothpick. A small quantity of the colony was used to inoculate a reference plate of brain heart infusion agar, and the remainder was transferred to a 1.5-ml microcentrifuge tube containing 50 μl of 50 mM NaOH–0.5% SDS–5 mM EDTA (cracking buffer). The contents were mixed and incubated at room temperature for 5 to 10 min prior to electrophoresis on agarose gels. Plasmid analyses were also done with the cells obtained from a 50-μl fraction of an overnight broth culture of *E. ictaluri*. The NaOH used in the cracking buffer should be freshly prepared. This can be most conveniently accomplished by adding freshly prepared 1 M NaOH to buffer stocks lacking NaOH.

Gel electrophoresis. DNA samples (25 μl) obtained by the methods described above were electrophoresed through 0.7% agarose (type 1; Sigma Chemical Co., St. Louis, Mo.) in TAE buffer (8) (40 mM Tris acetate, 1 mM EDTA [pH 8.0]) at 2.5 or 5 V/cm for 2.5 or 1 h, respectively. Electrophoresis was performed on a horizontal apparatus, and bromophenol blue tracking dye (0.025%) was added to follow the rate of migration. The gels were stained with ethidium bromide (0.5 μg/ml) for 45 to 60 min, briefly rinsed, and then photographed (type 55 or 667 film; Polaroid) under a shortwave UV light source (Ultraviolet Products, Inc., San Gabriel, Calif.) with an orange filter (23 A; Wratten). To identify plasmid DNA within 2 h from the beginning of the experiment, ethidium bromide was included in the running buffers. Size estimations of the supercoiled plasmid DNA of *E. ictaluri* was made by comparison with a commercially prepared plasmid ladder (Bethesda Research Laboratories, Inc., Gathersburg, Md.) in 0.9% agarose gels according to the instructions of the manufacturer.

Restriction digests. Restriction enzymes were obtained from Bethesda Research Laboratories and New England BioLabs, Inc. (Beverly, Mass.) and were used according to the instructions of the manufacturers to digest either lambda DNA for molecular weight standards or the plasmids from *E. ictaluri* prepared by the method described by Davis et al. (3). The plasmids from each of the 18 different strains of *E. ictaluri* were analyzed with each of the following restriction enzymes: *Bam*HI, *Bgl*I, *Eco*RI, *Hin*dIII, *Pst*I, *Sal*I, *Sst*II, and *Xho*I. Restriction fragments were separated by agarose gel electrophoresis in 1.0% gels as described above. In restriction mapping experiments the plasmids obtained from *E. ictaluri* S85-1377 were used. In these experiments the following restriction enzymes were also used: *Apa*I, *Bgl*II, *Bst*EII, *Eco*RV, *Pvu*II, and *Sac*I. Restriction mapping experiments were done by first separating the two *E. ictaluri* plasmids by preparative agarose electrophoresis. The plasmids were excised separately from the agarose and recovered by NACS chromatography following the directions of the manufacturer (Bethesda Research Laboratories). Alternative separation procedures were also used. In these experiments the two plasmids were restricted with enzymes determined to digest only one of the plasmids. After digestion the intact supercoiled (nondigested) plasmid was separated by hydroxylapatite chromatography (10). Restriction sites on the separated plasmids were mapped by single and multiple digests.

RESULTS

Identification of plasmids in *E. ictaluri*. Eighteen different strains of *E. ictaluri* were analyzed to determine if plasmid DNA was present. All isolates were initially recovered from infected channel catfish (Table 1). Twelve isolates were from Mississippi, whereas five were from Alabama. These isolates were used in conjunction with the type strain for the species ATCC 33202; this strain was initially isolated from infected catfish from Georgia (5). Two plasmid preparation methods were used. The first method (7) used alkaline hydrolysis, SDS, and heat followed by phenol-chloroform extraction prior to agarose electrophoresis. The second method (3) was a lysate method in which lysozyme, SDS, and heat were used for cell lysis, followed by ethanol precipitation for plasmid enrichment prior to agarose electrophoresis. A representative comparison of the plasmids obtained by the second isolation method from the various strains next to a reference plasmid ladder is shown in Fig. 1. Each of the 18 strains was found to harbor two plasmids. These two plasmids were seemingly identical in all the strains based on their relative mobility in agarose gels. By interpolation from the reference plasmid ladder, the estimated size of the two plasmids in each of these strains was 5,700 and 4,900 base pairs (bp). There were no apparent differences in the relative mobilities of the plasmids isolated by these two different methods. There was no indication by either of these methods that plasmids of other sizes were present. In some of the preparations other forms of these same plasmids were found. These other forms did not represent additional plasmids, as determined by restriction enzyme analysis (see below).

The plasmid isolation method described by Kado and Liu (7) has been shown to be effective in detecting the presence of plasmids ranging in size from 2.6 to 350 megadaltons in a variety of other bacterial species (7). Likewise, the lysozyme extraction procedure described by Davis et al. (3) has also been found to be effective in isolating plasmids of greater than 15 kilobases pairs (kbp) in both *Escherichia* and *Aeromonas* strains (unpublished observations). This suggests that if higher molecular mass plasmids were present in these various isolates of *E. ictaluri*, then these methods should have been appropriate for their detection. As an additional control, a strain of *E. tarda* (ATCC 15947) was analyzed for the presence of plasmids to determine if the 5,700- and 4,900-bp plasmids identified in the various strains of *E.*

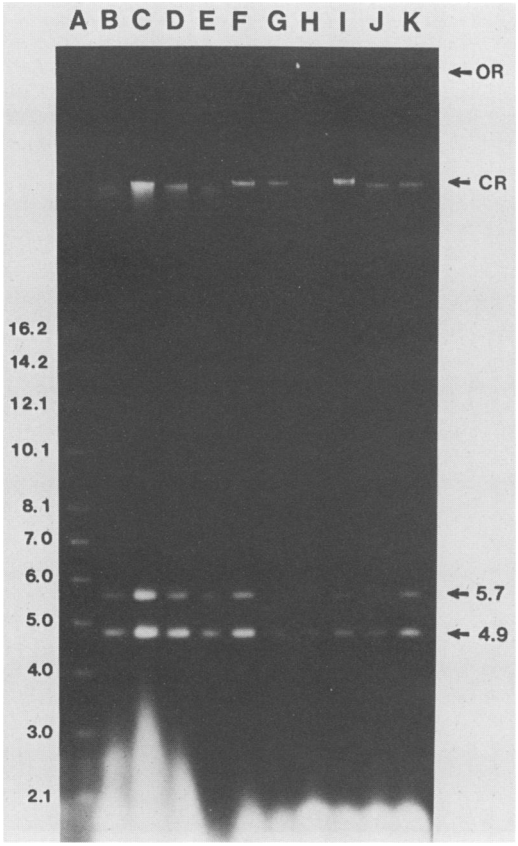

FIG. 1. Agarose gel electrophoresis of plasmid DNA isolated by the method described by Davis et al. (3) in various strains of *E. ictaluri*. Lane A, a plasmid ladder; the sizes of these reference plasmids (in kilobase pairs) are indicated to the left of the gels; lanes B to G, plasmids detected in strains S85-762, S85-1354, S85-1362, S85-1377, S85-1381, and S85-1397, respectively; lanes H to J, plasmids detected in strains AL78-119, AL83-189, and AL84-187, respectively; lane K, plasmids detected in strain ATCC 33202. The origin (OR) of electrophoresis, the position of chromosomal (CR) DNA, and the position of the 5.7- and 4.9-kbp plasmids are indicated.

ictaluri were also represented in this closely related species. This strain did not carry plasmids with sizes similar to those found in the various *E. ictaluri* isolates.

Restriction analysis shows the 5,700- and 4,900-bp plasmids are conserved in the various strains of *E. ictaluri*. The two plasmids present in each of the strains of *E. ictaluri* were restricted with different enzymes. The two plasmids were observed to have different restriction patterns. Representative results obtained with eight restriction enzymes are shown in Fig. 2. The larger plasmid, with an estimated size of 5,700 bp, as determined with the reference covalently closed circular plasmids, was cleaved by *Bam*HI, *Bgl*I, *Eco*RI, *Pst*I, and *Xho*I. There were also cleavage sites for *Apa*I, *Bgl*II, *Bst*EII, *Eco*RV, *Pvu*II, and *Sac*I (not shown). There were no restriction sites for *Hin*dIII, *Sal*I, or *Sst*II. The estimated size of the linear form of this plasmid following digestion with *Eco*RI was 5,700 bp when compared with *Hin*dIII and *Pst*I digests of lambda DNA.

The 4,900-bp plasmid was restricted by *Bgl*I, *Eco*RI, and *Pst*I. This plasmid also was restricted by *Bgl*II, *Eco*RV, and *Pvu*II (not shown). There were no restriction sites for *Bam*HI, *Hin*dIII, *Sal*I, *Sst*II, or *Xho*I. In addition, there were no restriction sites for *Apa*I, *Bst*EII, or *Sac*I (not shown). The estimated size of the linear form of this plasmid was 4,900 bp when restricted with *Eco*RI, which was in agreement with the estimated size of the covalently closed circular form. These analyses show that these same two plasmids are apparently conserved in all of the various strains of *E. ictaluri* studied because the restriction patterns for each individual plasmid were identical.

To further define the relationships of these two plasmids, restriction maps of the individual plasmids were constructed. These plasmids were separated either by preparative electrophoresis or by hydroxylapatite chromatography following digestion of the plasmid pool with enzymes designed to cleave only one of the plasmids. The isolated individual plasmids were subsequently analyzed by single and multiple digestions to construct the maps.

The 5,700-bp plasmid, designated pCL1, was found to have single sites for *Apa*I, *Bam*HI, *Bgl*II, *Bst*EII, *Eco*RI, *Eco*RV, *Pst*I, *Pvu*II, *Sac*I, and *Xho*I (Fig. 3). The *Eco*RI site was arbitrarily chosen as the zero-degree reference point. The *Bst*EII, *Eco*RV, and *Pvu*II restriction sites mapped in the same location. Likewise, the *Bgl*II and *Pst*I sites mapped in the same relative position. Digestion with *Bgl*I yielded fragments of 2,550, 1,600, and 850 bp that were mapped without difficulty. Based on the locations of these fragments, the existence of at least two additional, small (<400 bp) *Bgl*I fragments was inferred (Fig. 3).

The 4,900-bp plasmid, designated pCL2, was found to have single restriction sites for *Bgl*II, *Eco*RI, and *Pvu*II (Fig. 3). The *Eco*RI site likewise was arbitrarily chosen as the

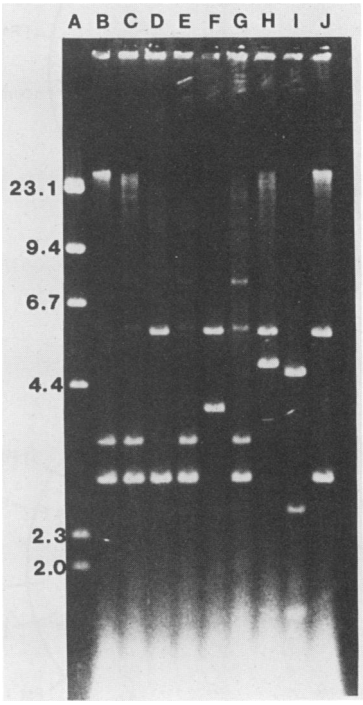

FIG. 2. Restriction analysis of the 5,700- and 4,900-bp plasmids found in *E. ictaluri*. Lane A, a *Hin*dIII digest of lambda DNA; the sizes of the standard fragments are indicated to the left of the gel; lane B, the two undigested plasmids found in *E. ictaluri*; lanes C to J, analysis of the two plasmids following digestion with the following restriction endonucleases; respectively: *Hin*dIII, *Bam*HI, *Sst*II, *Pst*I, *Sal*I, *Eco*RI, *Bgl*I, and *Xho*I.

reference point for this plasmid. Plasmid pCL2 had two *Bgl*I sites and three *Pst*I sites. The two *Bgl*I sites were located within ~300 bp of each other; likewise, two of the *Pst*I sites were located close to each other (~200 bp). Digestion with *Eco*RV resulted in three fragments. The *Eco*RV sites mapped in the regions defined by *Pst*I, *Bgl*I, or *Bgl*II. Thus, comparison of the restriction maps of pCL1 and pCL2 indicated that there is little homology between these two plasmids. It should also be stated that if there was partial homology between these two plasmids, then observation of cointegrate structures might be expected when plasmid pools are analyzed by agarose electrophoresis. None of the plasmid analyses done with the various strains indicated the presence of possible cointegrate structures as judged by the comparison of plasmid mobilities.

Individual colonies of *E. ictaluri* can be rapidly screened to detect the presence of the two plasmids. With the results presented above showing that the restriction sites in plasmids pCL1 and pCL2 are conserved in the host strains that were analyzed, efforts were undertaken to determine if a rapid method could be found to quickly define the presence of these plasmids in individual bacterial colonies. Such a method could become an important test to aid in the quick identification of clinical isolates of *E. ictaluri* from channel catfish. A suitable method was subsequently identified based on a rapid protocol developed by Barnes (1) and reported by Maniatis et al. (8). The only modification of this method was the elimination of the 45- to 60-min incubation of the cell lysate at 68°C prior to agarose gel electrophoresis; this step was found to be nonessential. A 5- to 10-min incubation of the lysate at room temperature gave similar resolution of the plasmids on agarose gels when compared with the plasmids obtained from heated lysates. A plasmid screen of individual colonies from the various strains of *E. ictaluri* grown on solid media is shown in Fig. 4. In the far right lane in Fig. 4 is a phenol-extracted standard prepared by the plasmid isolation method described by Davis et al. (3). These results show that the migration of the two plasmids from the individual colonies is identical to that of the standard, although there was a relative change in the position of the chromosomal DNA. As with the other lysate methods described above, there was no indication that other plasmids besides these two were present in these various strains. *Escherichia coli* J53 bearing the 97.2-kbp pJB4JI plasmid was analyzed by this method to determine if a relatively large plasmid could be detected. This plasmid was identified when plasmid preparations were analyzed by agarose gel electrophoresis (data not shown).

Plasmid fingerprinting of individual colonies by this technique was found to be extremely rapid. Results can be obtained in 2 to 3 h from the beginning of the experiment. In

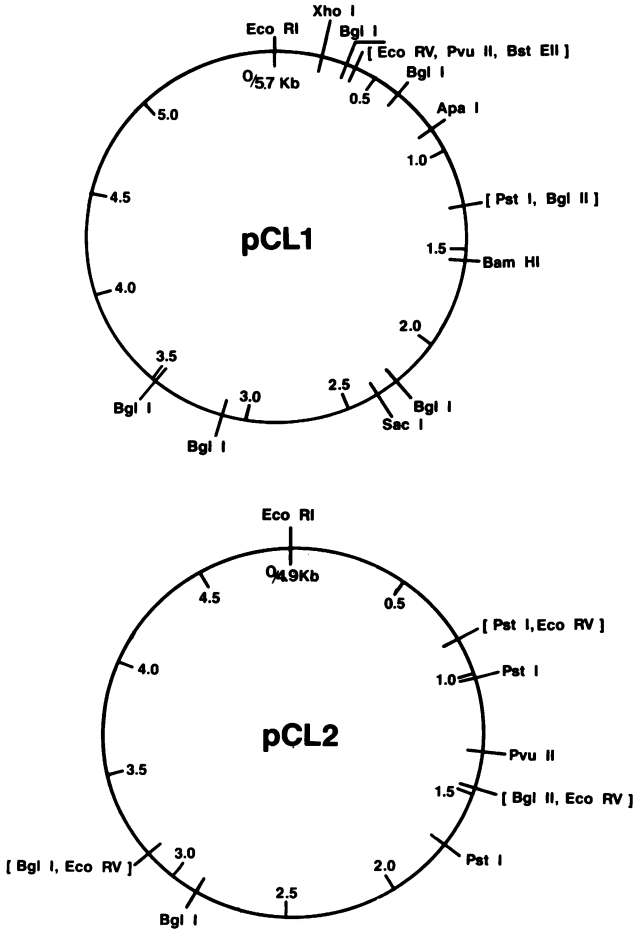

FIG. 3. Restriction maps of *E. ictaluri* plasmids. The *Eco*RI site on pCL1 and pCL2 was arbitrarily chosen as the zero-degree reference point. The relative orientation of the two maps was arbitrarily chosen to maximize possible similarities.

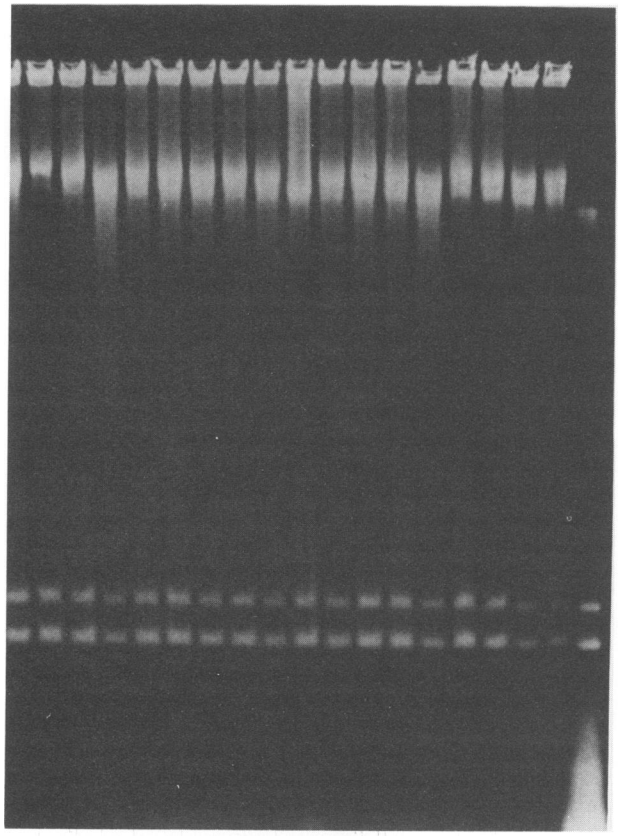

FIG. 4. Agarose gel electrophoresis of plasmid DNA from individual colonies of 18 strains of *E. ictaluri* following alkaline-SDS lysis (1). The last lane contains a preparation of ATCC 33202 prepared by the method described by Davis et al. (3) to indicate the mobilities of the 5,700- and 4,900-bp plasmids.

fact, within 1 h adequate resolution of the plasmids can be obtained by agarose gel electrophoresis to confirm the presence of the two plasmids. Furthermore, if desired, a small portion of the isolated colony to be analyzed can be transferred to a master plate containing appropriate growth media prior to alkaline hydrolysis. Thus, a continued stock of the isolate can be maintained for further biochemical analysis. This technique was equally effective for analyzing plasmids from broth cultures of *E. ictaluri*. The cells from a 50-μl fraction of an overnight culture could be lysed and likewise analyzed for the presence of these two plasmids.

DISCUSSION

Two plasmids were identified in isolates of *E. ictaluri* obtained from infected channel catfish. These two plasmids had sizes of 5,700 and 4,900 bp and were identified as pCL1 and pCL2, respectively. One of the most intriguing aspects of this study was the finding that all of the isolates of *E. ictaluri* analyzed (18 of 18) harbored both of these plasmids. These different strains of *E. ictaluri* were isolated from infected catfish in various years from 1978 to 1985 from various locations from three states in the southeastern United States. The isolates from Mississippi were isolated in 1985 from catfish from various commercial ponds in the Mississippi Delta. The relative amount of each plasmid in these various strains was similar, as judged by the ethidium bromide staining intensities of the plasmids extracted from similar cell numbers. Although the function(s) of these plasmids remains to be determined, the uniformity of these results suggests that plasmid function may be either metabolically important to the host bacteria, that these plasmids may code for factor(s) important to the virulence of the organism during infection of channel catfish, or both.

These plasmids apparently are very stable in the various isolates. This conclusion is attested to, first, by the consistent presence of these plasmids in the strains studied. Second, ongoing experiments to cure the plasmids by incubation at elevated temperatures have, as yet, not been successful. For example, S85-1377 was in continuous culture at 35°C for over 2 months and still retained both plasmids. Other methods are currently being explored to cure these plasmids from *E. ictaluri* to determine if biochemical properties attributable to the organism are lost and to determine if the loss of these plasmids alters the virulence of the organism when used in challenge experiments with susceptible catfish.

Results of this study show that the various isolates of *E. ictaluri* are very homogeneous with regard to the presence of the same plasmid DNA. In earlier studies various isolates of *E. ictaluri* were compared to determine if biochemical differences exist between various strains. In the work of Hawke et al. (5), in which this species was identified and characterized, it was found that 13 strains were almost identical in a battery of biochemical tests. In an earlier report (4) the biochemical homogeneity of 16 different isolates was also shown. In an expanded biochemical study of 119 isolates it was also concluded that these strains are essentially homogeneous (13). Thus, the conclusion from these various studies is that different strains of *E. ictaluri* are bichemically homogeneous. The general conclusion has been that the species lacks the enzymes that are required for the fermentation of most carbohydrates, and few extracellular enzymes have as yet been identified (13).

The optimum growth temperature of *E. ictaluri* is between 25 and 30°C, which closely parallels the incidence of ESC in commercial catfish farms (4). On isolation from clinical specimens, the bacteria can require up to 5 days for biochemical identification due to this comparatively slow rate of growth. Equally difficult can be the problem discussed by Hawke (4), which is that the clinical signs of disease are not consistent. In some cases external signs of disease include petechial hemorrhages of the skin, exophthalmia, and an open lesion in the fontanelles of the skull. This open skull lesion has resulted in this disease being referred to as "hole in the head" disease of catfish. This spectrum of signs has lead to the finding that the bacterium may gain entrance through the olfactory tract, resulting in a generalized systemic infection which can involve the brain, liver, kidneys, and spleen (9). In addition, there is evidence that pathogenesis may proceed through infection of the gastrointestinal tract (11). However, gross external signs of disease can also be absent. Thus, the combination of the growth rate required for biochemical definition coupled with the lack of clear diagnostic signs in infected fish can impede the rapid identification of this bacterium.

The finding that individual colonies of *E. ictaluri* can be rapidly screened for the presence of both pCL1 and pCL2 should become a useful tool to aid in the rapid identification of clinical isolates from channel catfish. The modified alkaline-SDS procedure described by Barnes (1) was found to be easy, reliable, and quick. Results can be routinely obtained within 2 to 3 h from the beginning of the experiment, depending on the rapidity required for plasmid detection. Because all isolates harbored both plasmids, the potential problem of confusing other bacteria harboring plasmids of similar relative mobility is decreased. Other bacteria are not likely to have two other plasmids with identical mobilities similar to those found in *E. ictaluri*. It should also be mentioned that in an ongoing study to characterize the plasmids in *Aeromonas hydrophila* and *A. sobria*, other important pathogens of channel catfish, no isolate has been identified that bears both a 5,700- and 4,900-bp plasmid, even though over 50 isolates have been examined (C. J. Lobb and M. Rhoades, manuscript in preparation). In addition, isolates of *Aeromonas* have not been found to be effectively lysed by the rapid alkaline-SDS hydrolysis method of Barnes (1), a feature which might be useful in the quick differentiation of these important pathogens of catfish.

At this point it seems premature to conclude that all *E. ictaluri* strains harbor these two plasmids. However, the potential to rapidly identify *E. ictaluri* by plasmid screening has immediate benefit to circumvent the time presently required for biochemical identification. As other strains of *E. ictaluri* are analyzed for the presence of these plasmids, further conclusions may be drawn. It does appear, however, that endemic strains of *E. ictaluri* isolated from infected catfish in the southeastern United States contain these plasmids. This statement is based on the following probability arguments. If an equal frequency is assumed for either observing or not observing one of these plasmids in *E. ictaluri* isolates, then only 50% of the isolates would be expected to contain a plasmid. Because 18 of 18 isolates of *E. ictaluri* contained a plasmid, the probability of sequentially observing by chance 18 plasmid-bearing strains is $(1/2)^{18}$ or 3.8×10^{-6}. By analogy, the chance of finding both plasmids strictly by chance in *E. ictaluri* is $(1/4)^{18}$ or 1.45×10^{-11}. Even if it is assumed that there is a 90% chance of observing each plasmid, the probability is still very small that 18 strains would sequentially be found to harbor both plasmids, i.e., $(81/100)^{18}$ or 2×10^{-2}. With this low probability it seems likely that pCL1 and pCL2 should prove to be

reliable markers for identification of *E. ictaluri* isolates obtained from channel catfish in the southeastern United States. Whether other strains such as those isolated from nonictalurid fish, such as the tropical danio (*Danio devario* [12]) or the green knife fish (*Eigemannia virescens* [Kent and Lyons, Report, Fish Health News, **2**:ii, 1982]), will bear these plasmids remains to be determined. It will also prove interesting to determine if strains isolated from nonictalurid fish will be virulent in channel catfish. The identification of plasmids in virulent strains of *E. ictaluri* from channel catfish opens the opportunity for relatively simple probes for these plasmids to be developed to follow the in vivo course of *E. ictaluri* infection. Such techniques may also be of special interest in defining the carrier state of this bacteria in catfish to help monitor and develop protocols for immunization to eliminate potential sites of colonization. The continuing efforts to identify these plasmids in other strains of *E. ictaluri* and to address the role of these plasmids in the virulence of this organism should prove to be rewarding.

ACKNOWLEDGMENTS

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