BIOCHEMICAL CHARACTERIZATION OF THE AEROMONAS SPECIES ISOLATED FROM FOOD AND ENVIRONMENT

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Background: Very few studies have been carried out to comprehensively characterize Aeromonas isolates. This study reports biochemical and enzymatic characterization of 60 isolates belonging to seven Aeromonas species. **Methods:** Seven types of API (Analytab Production Incorporation, France) strips (API 20 E, API 20 NE, Rapid ID 32 E, API 50 CH, ID 32 GN, ID 32 C, Rapid ID 32 A) were used for the biochemical characterization of the isolates. **Results & Conclusions:** These strips provided an extensive biochemical profile of the isolates. AI 20 E gave reliable results. The H_2S tubule of this strip was added with gelatin-cysteine-thiosulfate medium and got excellent results for H_2S production from cysteine. Esculin formulation in ID 32 C was proved most reliable as no false positive or as many false positive and false negative results were given by it. All other strips provided test profile and some of the characteristics in them appeared as significant in differentiation of the various species.

Keywords: Aeromonas, Phenotype, virulence

INTRODUCTION

The genus Aeromonas is a phenotypically and genetically heterogeneous taxon. By now 18 DNA hybridization groups (HGs) have been known for this genus.[1] Many of the genospecies have been named but still there are many which are not named yet due to lack of phenotypic markers.[2] Phenotypic indentification of aeromonas is necessary for the diagnostic laboratories because there are differences in epidemiology, antibiotic susceptibility and possibly clinical significance between species.[3] Also, speciation of the aeromonas is necessary to understand the virulence mechanism of different species.[4]

Biochemical reactions are very important for biotyping or speciating the Aeromonas isolates. Castro-Escarpulli et al.[5] while employing 14 biochemical tests described by Altwegg[6] for identification of their isolates could get a correct identification of only 28.5% of his strains as compared to the genetic methods. In contrast, some other authors have successfully used conventional biochemical tests to separate at least the major phenospecies. One of such schemes is proposed by Carnahan et al[7], the Aerokey II. These authors were able to correctly identify 97% of their isolates and 100% reference cultures. It is a dichotomous key which in combination with API 20 E strip and three conventional tests can yield accurate identification of all the 7 major clinical species. Merino et al.[8] and Longa et al.[9] have also suggested that the Aerokey II, is the best identification method. Using Aerokey II and other tests proposed by the authors, it was possible to identify all of the isolates in the present study.

The identification and characterization of isolates require large number of test media. To reduce the work burden, various rapid test systems have commercially been developed to aid microbiologists in identifying their isolates. The API system is one of these. This system has four types of strips i.e. API 20 E, API 20 NE, ID 32 GN and Rapid ID 32 E and has a database of biochemical reactions for Aeromonas that can be called upon for identifying the isolates. However, none of these strips encompasses the appropriate battery of tests to distinguish even the three mahor species (A. hydrophila, A. Sobria and A. Caviae) without additional tests but these strips, alongwith others, can be used to characterize and compare Aeromonas isolates for their biochemical and enzymatic profiles. Thus, even types of API strips, namely API 20 E, API 20 NE, API 50 CH, Rapid ID32 E, ID32 GN, ID32 C and Rapid ID 32 A were used in the present work.

In the past, very few studies based on only a few species have been made, to comprehensively characterize Aeromonas isolates. This study reports biochemical and enzymatic characterization of 60 isolates belonging to seven Aeromonas species.

MATERIAL AND METHODS

Seven API strips, namely API 20 E, API 20 NE, API 50 CH, Rapid ID32 E, ID32 GN, ID32 C and Rapid ID32 A were used for comparing biochemical and enzymativ profiles of the Aeromonas strains. The isolates were grown on trypticase soy agar plates at 37ºC overnight for the purpose of preparation inocula, adjustment of the turbidity and the inoculation of various strips were carried out according to the manugacturer's instructions with the exception that the H_2S tubule in the API 20 E strip

was filled with gelatin-cysteine-thiosulfate (GCF) medium[10] and inoculated directly with the strain using a needle. The strips were incubated at strip specific temperature and time. The incubation time was extended to 24 hours or more in case of weak reactions. The reading of the strips was carried out according to the prescribed instructions. The results were entered in the computer using API 20 E, API 20 NE and API 50 CH identification programs for the corresponding strips. The readings for Rapid ID32 E, ID32 GN, ID32 C and Rapid ID32 A were performed automatically using ATB reader. A printout of the test report was obtained for each isolate. For ID32 C, the esculing hydrolysis results were entered in the report manually.

RESULTS

Table 1 compares the results for API 20E, API 20 NE and ID32 GN with those of Aerokey II. It is evident that these strips could not differentiate *A. hydrophila* and *A.* caviae. Also, in most of the cases the species identified by the API system did not match the results of the Aerokey II. If at all it matched, it was always with lower confidence limits. Since the aforementioned API strips lack a database for motile species of Aeromonas except for *A.hydrophila, A. Caviae* and *A. Sobria*. Therefore, the results accrued on the basis of these strips could not be relied upon and are not comparable with those of the Aerokey II.

API 20 E is designed to identify members of the family enterobacteriaceae but a number of tests that it contains are also useful for the biochemical characterization and identification of other gram-bacteria including the aeromonads. Therefore this strip was used in the present study to biochemically characterize the Aeromonas isolates. Table 2 shows the API 20 E results for all Aeromonas species and isolates. Six tests out of 21 were positive for all the isolates. All isolates were negative for 4 tests. The remaining 11 of the 21 tests showed variable results.

The API 20 NE strip consists of 20 tubules and cupules containing dehydrated media and substrates for eight conventional and twelve assimilation tests. This strip is designed to identify non-enterobacteriaceae gram negative bacteria. Table 3 shows the API 20 NE results for Aeromo nas isolates. The isolates were positive for most of tests in this strip. All were negative for urea hydrolysis and assimilation of adipate and phenyl acetate. Only six tests showed variable results for the various isolates.

The API 50 CH provides 49 carbohydrate fermentation tests for a given isolate. The biochemical profiles of the aeromonads are shown in Table 4. Out of the 49 tests, only seven carbohydrates were fermented by all isolates. None of the isolates produced acid from the twentyone carbohydrate substrates. Of the remaining tests, five were positive for only a few isolates of either *A. veronii bv. Sobria* or *A. caviae*. These inclused inositol for *A. caviae* (3.8%), melibiose for *A. veronii bv. Sobria (6.7%),* gentiobiose for *A. caviae (34.6%),* D-arabitol for *A.caviae (7.7%)* and raffinose for *A. veronii bv. Sobria (6.7%).* Sorbitol waspositive for only a few of *A. hydrophila* and *A. caviae* strains. Thirteen tests were variable for *A. hydrophila, A. veronii bv. Sobria* and *A. caviae.* For some, there was no significant difference among the isolates of *A. hydrophila, A. veronii bv. Sobria* and *A. caviae.* Glycerol, N-acetyl glucosamine, maltose, trehalose and gluconate exemplify this. Of the other 7 tests, few could be used to separate the species. For example, L-arabinose was 96.2% positive for the *A. caviae* isolates and 27.3% and 13.3% for *A. hydrophila* and *A. veronii bv. Sobria* isolates respectively. Mannose was fermented by 100% isolates of *A. hydrophila* and *A. veronii bv. Sobria* but only 26.9% of the *A. caviae* isolates. ? -methyl-D-glucoside was fermented by 81.8% of *A. hydrophila* isolates as compared to 26.7% of *A. veronii bv. Sobria* and 11.5% of the *A. caviae* isolates. Arbutin could not be fermented by any of the *A. veronii bv. Sobria* isolates whereas a high percentage (90.1% and 84.6% respectively) of *A. hydrophila* and *A. caviae* were positive in fermenting it. Esculin turned out to be negative for *A. veronii bv. Sobria* but was 100% positive for the other two species. Salicin is another carbohydrate which failed to be utilized by *A. veronii bv. Sobria*, whereas 90.1% of *A. hydrophila* and 80.8% of *A. caviae* were aboe to ferment it. Cellobiose was fermented by all three species in a range of 27.3%-76.9%, *A. caviae* fermenting the most. Lactose could not be metabolised by any of the *A. hydrophila* isolates but a small percentage of *A. veronii bv. Sobria* and *A. caviae* were able to produce acid from lactose. The lesser-isolated species (i.e. *A. trota, A. schubertii* and *A. jandaei*) matched the most isolated species in most of the reactions included in this strip.

Rapid ID32 E, is used for rapid identification of enterobacteriaceae bacteria. The isolates were tested for various tests contained in this strip and the data are shown in Table 5. Out of 31 parameters tested, only two weere positive for all of the isolates. These were coumarate fermentation and splitting of p-nitrophenyl-ß-D-galactopyranoside. Other tests which were positive for the majority of the isolates of all species were: fermentation of mannitol, maltose, trehalose, saccharose and production of indole. Only urea hydrolysis was negative for all of the isolates. Some tests were positive for only a few isolates of one or two species. On the whole, *A. hydrophila* isolates showed high positivity for the available tests in this API strip.

Table 1: Aeromonads Identification on the Basis of Aerokey-II and API System

Isolates	Methods			
	Aerokey-II	API 20 E	API 20 NE	ID 32 GN
CH-1	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CH-2	A. hydrophila	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
CH-3	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CH-4	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CH-5	A. Veronii bv. sobria	A. sobria	A. sobria	A. hydrophila/caviae
CH-6	A. hydrophila	A. sobria	A. sobria	A. hydrophila/caviae
CH-7	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CH-8	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CH-9	A. Trota	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CH-10	A. Veronii bv. Sobria	A. sobria	A. sobria	A. sobria
CH-11	A. schubertii	A. sobria	A. sobria	A. sobria
CH-12	A. hydrophila	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
CH-13	A. hydrophila	–	A. hydrophila/caviae	–
CH-14	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
CH-15	A. Caviae	A. sobria	A. hydrophila/caviae	A. sobria
CH-16	A. Trota	A. sobria	A. sobria	A. sobria
CH-17	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CH-18	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CH-19	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
CH-20	A. Trota	A. sobria	A. sobria	A. sobria
CH-21	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
CH-22	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
CH-23	A. veronii bv. sobria	A. sobria	A. hydrophila/caviae	A. sobria
CHF-1	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CHF-2	A. Caviae	A. sobria	A. sobria	A. hydrophila/caviae
CHF-3	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
CHF-4	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
WB-1	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
WB-2	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
WB-3	A. hydrophila	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
FV-1	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
FV-2	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
MK-1	A. hydrophila	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
MK-2	A. veronii bv. sobria	A. sobria	A. sobria	A. hydrophila/caviae
MK-3	A. veronii bv. sobria	A. sobria	A. sobria	A. hydrophila/caviae
MK-4	A. hydrophila	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
MK-5	A. hydrophila	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
BF-1	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
BK-1	A. hydrophila	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
BK-2	A. Caviae	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
BK-3	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
FS-1	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
FS-2	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
FS-3	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
FS-4	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
FS-5	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
FS-6	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
FS-7	A. hydrophila	A. hydrophila/caviae	A. hydrophila/caviae	–
FZ-1	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
WA-1	A. jandaei	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
WA-2	A. hydrophila	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
WA-3	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
WA-4	A. Caviae	A. hydrophila/caviae	A. sobria	A. hydrophila/caviae
WA-5	A. schubertii	A. hydrophila/caviae	A. hydrophila/caviae	–
WA-6	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
WA-7	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae
WA-8	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
WA-9	A. veronii bv. sobria	A. sobria	A. sobria	A. sobria
WA-10	A. Caviae	A. sobria	A. hydrophila/caviae	A. hydrophila/caviae
WA-11	A. Caviae	A. hydrophila/caviae	A. hydrophila/caviae	A. hydrophila/caviae

Table 2: Biochemical characteristics of the isolates using API 20 E

Test	Cumulative % positive					
	A.hydrophila N=11	A. veronii bv. sobria N= 16	A. Caviae N = 26	A Trota N = 3	A.Schubertii N = 2	A. Jandaei N = 1
o-Nitro phenyl Galactoside	100.0	100.0	100.0	100.0	100.0	100.0
Arginine dihdrolase	100.0	100.0	100.0	100.0	100.0	100.0
Lysine decarboxylase	90.9	100.0	15.4	33.7	50.0	100.0
Ornithine decarboxylase	0	0	0	0	0	0
Citrate utilization	45.5	68.8	38.5	33.3	0	0
Hydrogen sulfide	81.8	62.5	0	66.7	50.0	100.0
Urea	0	0	0	0	0	0
Troptophane deaminase	0	0	0	0	0	0
Indole	90.9	100.0	100.0	100.0	0	100.0
Acetoin production	100.0	93.8	19.2	0	0	100.0
Gelatin hydrolysis	100.0	100.0	100.0	100.0	100.0	100.0
Glucose fermentation	100.0	100.0	100.0	100.0	100.0	100.0
Mannitol fermentation	100.0	100.0	100.0	100.0	100.0	100.0
Inositol fermentation	0	0	3.8	0	0	0
Sorbitol fermentation	18.2	0	0	0	0	0
Rhamnose fermentation	0	0	0	0	0	0
Sucrose fermentation	100.0	100	100.0	100.0	100.0	0
Melibiose fermentation	0	6.3	0	0	0	0
Amygdalin fermentation	90.9	37.5	80.8	0	0	100.0
Arabinose fermentation	36.4	06.3	92.3	33.3	50.0	100.0
Cytochrome oxidase	100.0	100.0	100.0	100.0	100.0	100.0

N= Number of isolates tested

Table 3: Biochemical characteristics of the isolates using API 20 NE

Test	Cumulative % positive					
	A.hydrophila N=11	A. veronii bv. sobria N= 16	A. Caviae N = 26	A Trota N = 3	A.Schubertii N = 2	A. Jandaei N = 1
Nitrate reduction	100.0	100.0	100.0	100.0	100.0	100.0
Indole production	90.9	100.0	100.0	100.0	0	100.0
Glucose acidification	100.0	100.0	100.0	100.0	100.0	100.0
Arginine dihydrolase	100.0	100.0	96.2	66.6	50.0	100.0
Urea hydrolysis	0	0	0	0	0	0
Esculin hydrolysis	90.9	0	92.3	0	50.0	100.0
Gelatin hydrolysis	100.0	100.0	100.0	100.0	100.0	100.0
p-Nitropyhenyl-ßD Galactopyranoside	100.0	100.0	100.0	100.0	100.0	100.0
Glucose assimilation	100.0	100.0	100.0	100.0	100.0	100.0
Arabinose assimilation	27.3	12.5	96.2	33.3	50.0	100.0
Mannose assimilation	100.0	100.0	34.6	66.6	50.0	100.0
Mannitol assimilation	100.0	100.0	100.0	100.0	100.0	100.0
N-Acetyl Glusomine	100.0	100.0	100.0	100.0	100.0	100.0
Maltose Glusomine	100.0	100.0	100.0	100.0	100.0	100.0
Gluconate Glusomine	100.0	100.0	100.0	100.0	100.0	100.0
Caprate Glusomine	100.0	100.0	100.0	100.0	100.0	100.0
Adipate Glusomine	0	0	0	0	0	0
Malate Glusomine	100.0	0	100.0	100.0	100.0	100.0
Citrate Glusomine	90.9	87.5	80.8	33.3	50.0	100.0
Phenyl acetate Glusomine	0	0	0	0	0	0
Cytochrome oxidase	100.0	100.0	100.0	100.0	100.0	100.0

N= Number of isolates tested

ID32 GN is used for the identification of gram negative bacteria and has 32 carbon substrates. Table 6 lists the results. All of the aeromonads were positive for nine substrates. None of the isolates of any species metabolized eight substrates. *A. hydrophila*, *A. veronii bv. Sobria* and *A. caviae* similarly utilized 10 substrates with 100% positivity and for nine substrates the results were entirely negative. The remaining 14 substrates were assimilated differenly by the various isolates.

IS32 C, designed for yeast identification and never used previously for bacterial isolates, allowed testing

of 31 substrates for the ability of the isolates to use them as carbon source and for growth. Of the 31 substrates (Table 7) only four were used by all isolates. These were glyverol, galactose, maltose and mannitol. Leaving aside the lesser species (*A. trota, A. schubertii* and *A. jandaei),* gluconate was utilized as another common carbon source. None of the isolates metabolized seven of the substrates, namely, D-xylose, rhamnose, erythritol, melezitose, levulinate, sorbose and 2-ketogluconate. Of the remaining 20 substrates, the most used by the aeromonads were ribose, glucose, actidione, sucrose, N-acetyl-glucosamine and trehalose. Some substrates were used by only a few isolates of one species.

Table 4: Biochemical characteristics of the isolates using API 50 CH

Test	Cumulative % positive					
	A.hydrophila N=11	**A. veronii bv. sobria** N= 15	**A. Caviae** N = 26	**A Trota** N = 3	**A.Schubertii** N = 2	**A. Jandaei** N = 1
Glycerol	100.0	100.0	88.5	100.0	50.0	100.0
Erythritol	0	0	0	0	0	0
D-Arabinose	0	0	0	0	0	0
L-Arbinose	27.3	13.3	96.2	33.3	50.0	100.0
Ribose	100.0	100.0	100.0	100.0	100.0	100.0
D-Xylose	0	0	0	0	0	0
L-Xylose	0	0	0	0	0	0
Adonitol	0	0	0	0	0	0
ß-Methyl – D-Xyloside	0	0	0	0	0	0
Galactose	100.0	100.0	100.0	100.0	100.0	100.0
Glucose	100.0	100.0	100.0	100.0	100.0	100.0
Fructose	100.0	100.0	100.0	100.0	100.0	100.0
Mannose	100.0	100.0	26.9	66.7	50.0	100.0
Sorbose	0	0	0	0	0	0
Rhamnose	0	0	0	0	0	0
Dulcitol	0	0	0	0	0	0
Inositol	0	0	0	0	0	0
Mannitol	100.0	100.0	100.0	100.0	100.0	100.0
Sorbitol	18.2	0	7.7	0	0	0
a-Methyl-D-Mannoside	0	0	0	0	0	0
a-Methyl-D-Glucoside	81.8	26.7	11.5	0	0	100.0
N-Acetyl Glucosamine	100.0	93.3	100.0	100.0	100.0	100.0
Amygdalin	0	0	0	0	0	0
Arbutin	90.1	0	84.6	0	50.0	100.0
Esculin	100.0	0	100.0	33.3	50.0	100.0
Salicin	90.1	0	80.8	0	0	100.0
Cellobiose	27.3	40	76.9	0	0	0
Maltose	100.0	93.3	100.0	100.0	100.0	100.0
Lactose	0	6.7	15.4	0	50.0	0
Melibiose	0	6.7	0	0	0	0
Sucrose	100.0	100.0	100.0	100.0	100.0	0
Trehalose	100.0	100.0	96.2	100.0	100.0	100.0
Inulin	0	0	0	33.3	0	0
Melezitose	0	0	0	0	0	0
Raffinose	0	6.7	0	0	0	0
Starch	100.0	100.0	100.0	100.0	100.0	100.0
Glycogen	100.0	100.0	100.0	100.0	100.0	100.0
Xylitol	0	0	0	0	0	0
Gentiobiose	0	0	34.6	0	0	0
Turanose	0	0	0	0	0	0
D-Lyxose	0	0	0	0	0	0
D-Tagatose	0	0	0	0	0	0
D-Fucose	0	0	0	0	0	0
L-Fucose	0	0	0	0	0	0
D-Arabitol	0	0	7.7	0	0	0
L-Arabitol	0	0	0	0	0	0
Gluconate	81.8	100.0	100.0	100.0	100.0	100.0
2-Keto Gluconate	0	0	0	0	0	0
5-Keto Gluconate	0	0	0	0	0	0

N= Number of isolates tested

Table 5: Biochemical characteristics of the isolates using ID 32 E

Test	Cumulative % positive		
	A. hydrophila N = 6	A. veronii bv. sobria N = 12	A. Caviae N = 11
Urease	0	0	0
Lysine decarboxylase	50.0	83.3	36.4
Ornithine decarboxylase	66.7	91.7	27.3
Esculin	100.0	8.3	81.8
Fermentation	66.7	25.0	100.0
Arabinose	50.0	25.0	100.0
Adonitol	0	0	9.1
Rhamnose	16.7	8.3	27.3
Mannitol	100.0	100.0	90.9
Sorbitol	16.7	0	18.2
Cellbiose	16.7	25.0	81.8
Melibiose	0	8.3	9.1
Glucuronate	0	8.3	9.1
Mannose	88.3	83.3	18.2
Maltose	100.0	91.7	90.9
Trehalose	100.0	91.7	90.9
Indole	100.0	91.7	90.9
Malonate	83.3	66.7	90.9
Phenyl alamine deaminase	66.7	33.3	27.3
Saccharose	100.0	91.7	100.0
5-Ketogluconate	16.7	8.3	9.1
Platinose	100.0	25.0	9.1
Galacturonate	0	0	18.2
Colistin	100.0	33.3	27.3
Coumarat	100.0	100.0	100.0
Tetra Thionate Reductase	0	8.3	45.5
O-Nitrophenyl N-Acetyl ß-D-Glucosaminidase	33.3	41.7	27.3
p-Nitrophenyl ß-D-Galactopyranoside	100.0	100.0	100.0
? -Galactosidase	0	8.3	0
Inodxyl Phosphate	0	91.7	18.2
Raffnose	0	8.3	9.1

N= Number of isolates tested

Rapid ID32 A has 32 tests substrates and is used for identification of the anaerobic bacteria. The results are listed in Table 8. Only three tests were positive for all of the Aeromonas isolates. These were ß-galactosidase, nitrate reductase and arginine arylamidase. None of the isolates showed presence of enzymes such as urease, a-galactosidase, ß-glucuronidase and a-fucosidase. Arginine dihydrolase was present in almost all of the isolates except a few isolates of *A. veronii bv. Sobria,* Similarly, ß-N-acetyl glucosaminidase and glycine arylamidase were present in all isolates except a few isolates of *A. caviae.*

DISCUSSION

The API system has been used in the past for characterizing and identifying a variety of microorganisms. A range of strips is available, each having its own repertoire of tests and utility in differentiating particular groups and sub-groups of microorganisms. None of the strips in the API system in the present study has, however, been proved to be useful for identification of even the major Aeromonas species with confidence. In fact, in the past very little work has been done for comprehensice evaluation of the API system alone or in combination with other methods of identification of the Aeromonas species and strains. In the present work, seven types of API strips were used and three of these were compared with the data of the Aerokey II to assess the extent of their reliability in differentiating various aeromonads in given microbial isolates. Some of the strips used in the past exclusively for particular for particular tazonomic categories of microorganisms were used for the first time in this study. The observations presented show that one of the API strips, having database for the genus Aeromonas (API 20 E, API 20 NE, ID32 GN, Rapid ID32 E) have value in differentiating the Aeromonas species and strains. However, these strips turned out to be effective in identification to the genus level. These strips as well as other strips such as API 50CH, ID32 C have been shown here to be entirely reliable for biochemical characterization of the aeromonads. Their usefulness in combination with the Aerokey II has been clearly demonstrated by the results of the present investigation.

The results pertaining to the API system show that these strips cannot correctly identify the Aeromonas isolates but can provide help in identifying them when combined with other schemes like Aerokey II. A comparison of the API 20 NE, API 20 E, ID 32 GN and Aerokey II reveals that these strips cannot correctly differentiate even the major species of the Aeromonas. One reason for this is that they lack the database for the entire range of the known Aeromonas species.

The aeromonads fermented or utilized many of the substrates contained in the API 20 E strip. This strip was good for yielding information about 11 biochemical characteristics of the aeromonads. These characteristics fall under the general distinguishing features of the genus Aeromonas and have been mentioned in Bergey's Manual Determinative Bacteriology[11] and also reported by other workers.[3,12-15] For the remaining tests, highly variable results for the different isolates were obtained in the present study. While the results regarding some of the tests (such as LDC, VP, arabinose fermentation) are comparable with the observations of earlier workers,[13-15] those for the other parametes turned out to be entirely contradictory. It seems that this strip

has tendency to correctly differentiate the seven major species if the database is reviewed by incorporation of a few extra tests such as esculin hydrolysis, gas from glucose and cephalothin resistance. The biochemical profile of *A. trota* in this study was comparable to that reported by Carnahan et al.[16], except that one of our isolates was citrate positive. Similarly, the API 20E results for the *A. schubertii* isolates are comp[arable to those reported by Carnahan et al,[17] excepting the ones for citrate utilization and sucrose fermentation. Also, one of the two isolates was positive for arabinose fermentation. In contrast, Carnahan et al.[17] have reported negative reaction for their *A. schubertii* isolates. *A. janadaei* isolates also differ in arabinose fermentation and citrate utilization from those reported by Carnahan et al.[18]

The API 20 NE strip is designed to identify non-enterobacteriaceae gram-negative bacteria such as the vibrios, the pseudomonads and the aeromonads. In the present study it identified the Aeromonas isolates as either *A. Sobria or A. hydrophila/caviae* (Table 1). This identification by API 20 NE did not match the identification obtained through the Aerokey II (Table 1). This is due to the fact that the API 20 NE does not have the database for the other species. Therefore, identification of the aeromonads through the API 20 NE database was invalid. However, it provided some useful tests (such as esculing hydrolysis, indole production, arabinose assimilation) that can be used for separating the various species with were similar for all the isolates in respect to positivity or negativity. The remaining six tests gave highly variable results. Similar results have been reported by Krovacek et al.[19] for their isolates. Although this strip contains tests for *A.jandaei* and *A. schubertii* as well as *A. trota*, the insufficient number of their isolates in the present investigation precents valid comparisons with the past observations on these species. Some of the characteristics of these species studied, however, are comparable in terms of positivity and negativity with the reports by Carnahan et al.[16-18]

Table 6. Biochemical characteristics of the isolates using ID 32 GN

Test	Cumulative % positive					
	A.hydrophila	**A. veronii bv. sobria**	**A. Caviae**	**A Trota**	**A.Schubertii**	**A. Jandaei**
	N=11	**N= 15**	**N = 26**	**N = 3**	**N = 2**	**N = 1**
Rhanose	0	0	0	0	0	0
N-Acetyl Glucosamine	100.0	100.0	100.0	100.0	100.0	100.0
D-Ribose	100.0	100.0	100.0	100.0	100.0	100.0
Inositol	0	0	3.8	0	0	0
D-Saccharose	100.0	100.0	100.0	100.0	100.0	0
Maltose	100.0	100.0	100.0	100.0	100.0	100.0
Itaconate	0	0	0	0	0	0
Suberate	0	0	0	0	0	0
Malonate	0	0	0	0	0	0
Acetale	100.0	100.0	80.7	66.7	50.0	100.0
DL-Lactate	63.6	6.7	88.5	33.3	50.0	100.0
L-Alanine	81.8	53.3	84.6	66.7	50.0	100.0
Mannitol	100.0	100.0	100.0	100.0	100.0	100.0
D-Glucose	100.0	100.0	100.0	100.0	100.0	100.0
Salicin	90.9	0	88.5	0	0	100.0
D-Melibiose	0	6.7	0	0	0	0
L-Fucose	0	0	0	0	0	0
D-Sorbitol	18.2	0	0	0	0	0
L-Arabinose	27.3	13.3	96.2	33.3	50.0	100.0
Propionate	9.1	0	7.7	0	0	0
Caprate	100.0	100.0	100.0	100.0	100.0	100.0
Valerate	45.5	40.0	3.8	33.3	50.0	0
Citrate	45.5	86.7	57.7	33.3	50.0	-
Histidine	100.0	60.0	100.0	100.0	100.0	0
5-Keto-Gluconate	0	0	0	0	0	0
Glycogen	100.0	100.0	100.0	100.0	100.0	100.0
3-Hydroxy Benzoate	0	0	0	0	0	0
2-Keto Gluconate	0	0	0	0	0	0
3-Hydroxy Butyrate	0	0	7.7	0	0	0
4-Hydroxy Benzoate	0	0	7.7	0	0	0
L-Serine	100.0	100.0	100.0	100.0	100.0	100.0
L-Proline	100.0	100.0	100.0	100.0	100.0	100.0

N = Number of isolates tested

Table 7. Biochemical characteristics of the isolates using ID 32 C

Test	Cumulative % positive					
	A.hydrophila	A. veronii bv. sobria	A. Caviae	A Trota	A.Schubertii	A. Jandaei
	N=11	N= 15	N = 26	N = 3	N = 2	N = 1
Sorbitol	18.2	0	0	0	0	0
D-Xylose	0	0	0	0	0	0
Ribose	100.0	100.0	96.0	100.0	100.0	100.0
Glycerol	100.0	100.0	100.0	100.0	100.0	100.0
Rhamnose	0	0	0	0	0	0
Palatinose	90.9	33.3	8.0	0	0	100.0
Erythritol	0	0	0	0	0	0
Melibiose	0	6.7	0	0	0	0
Gluconate	100.0	100.0	100.0	66.7	100.0	100.0
Levulinate	0	0	0	0	0	0
Glucose	100.0	100.0	92.0	66.7	100.0	100.0
Sorbose	0	0	0	0	0	0
Glucosamine	9.1	6.7	40.0	0	100.0	100.0
Esculin	100.0	0	100.0	0	0	0
Galactose	100.0	100.0	100.0	100.0	100.0	100.0
Actidione	100.0	100.0	88.0	66.7	100.0	100.0
Sucrose	90.9	100.0	96.0	66.7	100.0	0
N-Acetyl-Glucosamine	100.0	100.0	92.0	100.0	100.0	100.0
DL-Lactate	63.6	6.7	88.0	33.3	50.0	100.0
L-Arabinose	27.3	13.3	92.0	33.3	50.0	100.0
Cellobiose	9.1	20.0	80.0	0	0	0
Raffinose	0	6.7	0	0	0	0
Maltose	100.0	100.0	100.0	100.0	100.0	100.0
Trehalose	100.0	100.0	92.0	66.7	100.0	100.0
2-Keto-Gluconate	0	0	0	0	0	0
? -Methyl-D-Glucoside	0	26.7	0	0	0	0
Mannitol	100.0	100.0	100.0	100.0	100.0	100.0
Lactose	0	6.7	8.0	0	50.0	0
Inositol	0	0	4.0	0	0	0

N= Number of isolates tested.

The test for indole production proved to be a good parameter to be a good parameter for separating *A. schubertii* from the rest of the species. The percent positivity data reported presently and by past workers[13] are very similar. ADH was positive for all the isolates in the present study except one strain each of *A. caviae, A. trota* and *A. schubertii*. Altwegg et al.[13] have also found some *A. caviae* and *A. hydrophilas* strains to be ADH negative. Esculin hydrolysis is an important parameter in the differentiation of the Aeromonas species. The present results on this test with API 20 NE, also lend support to earlier observations.[7,13,15] A small percentage of *A. hydrophila* and *A. veronii bv. Sobria* was positive for arabinose assimilation, whereas all *A. Caviae* isolates were positive for it with one exception. Altwegg et al.[13] have reported similar result for their *A. caviae* and *A. veronii bv. Sobria* isolates but much higher percentage for *A. hydrophila* as compared to the results presented here. Mannose assimilation was 100% positive for *A. hydrophila* and *A. veronii bv. Sobria* but 34.6% for the *A. caviae* isolates. No comparable data are available in the literature. Citrate assimilation was positive for *A. hydrophila, A.veronii bv. Sobria* and *A. caviae* isolates, giving an almost equal percentage. Altwegg et al.[13] have reported growth with citrate as the only carbon source for *A. hydrophila* isolates (32%), *A. veronii bv. Sobria (68%)* and *A. caviae (56%)*.

The API 50 CH strip provided data on metabolization of various carbohydrates by the Aeromonas isolates. Of the 49 substrates contained in this strip, only seven could be metabolized by all isolates of the Aeromonas studied presently. Although some of the parameters gave results generally comparable to those given by Altwegg et al.[13] in terms of reactivity, these differ in regard to percentage utilization by the various isolates of the Aeromonas examined. These differences seem to be largely owing to differences in the technique, media and the isolates. The data of the above workers are based on conventional biochemical methods used for examination of 13 genogroups. Also, the literature does not contain any information on *A. trota, A. schubertii* and *A.Jandaei* isolates. Thus no valid comparisons of the present results for these species are possible. However, some of the characteristics have been reported by Camahan et al.[16-18] for these species. The results of the present study are in agreement with their observations. In general, the API 50 CH strip has provided enough

information about the ability of the aeromonads to ferment carbohydrates, some of which can be used for species differentiation. Altwegg et al.[13] have reported biochemical profiles based on API 50 CH for *A. hydrophila, A. veronii bv. Sobria* and *A. caviae* but the percentages shown by these workers are different from those obtained in the present investigation.

Table 8. Biochemical characteristics of the isolates using RAPID ID 32 A

Test	Cumulative % positive					
	A.hydrophila	A. veronii bv. Sobria	A. Caviae	A Trota	A.Schubertii	A. Jandaei
	N=11	N= 16	N = 25	N = 3	N = 2	N = 1
Urease	0	0	0	0	0	0
Arginine dihydrolase	100.0	93.8	100.0	100.0	100.0	100.0
a-Galactosidase	0	0	0	0	0	0
ß-Galactosidase	100.0	100.0	100.0	100.0	100.0	100.0
ß-Galactosidase-6-P	0	56.3	44.0	50.0	50.0	0
a-Glucosidase	60.0	43.8	28.0	50.0	0	0
ß-Glucosidase	90.0	6.3	76.0	0	50.0	100.0
a-Arabinosidase	0	0	4.0	0	0	0
ß-Glucuronidase	0	0	0	0	0	0
ß-N-Acetyl-Glucosaminidase	100.0	100.0	96.0	100.0	100.0	100.0
Mannose fermentation	100.0	87.6	44.0	100.0	50.0	100.0
Raffinose fermentation	0	6.3	0	0	0	0
Glutamic acid decarboxylase	0	12.5	4.0	50.0	0	0
a-Fucosidase	0	0	0	0	0	0
Nitrate reduction	100.0	100.0	100.0	100.0	100.0	100.0
Indole production	100.0	93.8	100.0	100.0	0	100.0
Alkaline phosphatase	90.0	81.3	84.100	100.0	50.0	100.0
Arginine arylamidase	100.0	100.0	100.0	100.0	100.0	100.0
Proline arylamidase	90.0	100.0	100.0	100.0	100.0	100.0
Leucyl Glycine arylamidase	60.0	43.8	64.0	100.0	50.0	0
Phenylalanine arylamidase	50.0	50.0	68.0	100.0	50.0	0
Leucine arylamidase	70.0	62.5	76.0	100.0	50.0	0
Pyroglutamic acid arylamidase	0	0	12.0	0	50.0	0
Tyrosine arylamidase	70.0	43.8	60.0	50.0	50.0	0
Alanine arylamidase	90.0	100.0	92.0	100.0	100.0	100.0
Glycine arylamidase	100.0	100.0	92.0	100.0	100.0	100.0
Histidine arylamidase	10.0	25.0	32.0	0	0	0
Glutamyl Glutamic acid arylamidase	0	12.5	8.0	0	0	0
Serine arylamidase	80.0	93.8	92.0	100.0	100.0	100.0

N= Number of isolates tested

Rapid ID32 E, which uses standardized enzymatic tests for identification of members of enterobacteriaceae family, also contains database for biochemical profile of the Aeromonas. However, this strip failed to provide a reliable basis for separating the various isolates even upto the genus level. A comparison of the test results for the parameters examined with those of the other strips revealed that Rapid ID32 E give many false positive decarboxylase in API 20 E, which is a reliable strip, but with Rapid ID32 E a fair number of the isolates were positive for it. Similarly, rehamnose fermentation was negative for all isolates in the other strips such as API 50 CH. Rapid ID32 E gave positive reactions for it for some isolates of all of the three species tested. Esculin hydrolysis was positive for 100% *A. caviae* with API 50 CH but Rapid ID32 E yielded a positive percentage of 81.8%. Also lysine decarboxylase activity was positive for most of the *A. hydrophila* and all *A. veronii bv. Sobria* tested on API 20 E but Rapid ID32 E showed positive percentages of 50 and 83.3 for the respective isolates. Thus this strip turned out to be unreliable for the biochemical testing of the aeromonads in view of the false results yielded by it.

According to Carnahan et al.[20] several carbon substrates (such as cellobiose, Lecturer-arabinose, Lactose) are valuable for species identification of the aeromonads. ID32 GN consists of 32 cupules, each with a different carbon substrate and is used for the identification of gram-negative bacteria. This strip was thus used for testing the ability of the Aeromonas isolates to use them as a carbon source in the present work. All of the isolates of the Aeromonas tested here gave positive reaction with nine substrates. *A. hydrophila, A. veronii bv. Sobria* and *A. caviae* shared 10 substrates with 100% positivity and 8 with 100% negativity. The remaining 14 were metabolized differently in a species-specific manner. The present results, thus show good agreement (90%). Pasquale et al.[21] have also reported the biochemical profiles of *A.*

hydrophila strains using ID 32 GN. The results of the present study for *A. hydrophila* agree with their observations for the majority of the tests except saccharose, D-sorbitol, propionate, and citrate utilization which have been demonstrated to be negative by Pasquale et al..[21] This contrasts with the present results where some of the *A. hydrophila* isolates were positive. Altwegg et al. (1990) have also used ID32 GN for testing *A. hydrophila, A. veronii bv. Sobria* and *A. caviae.* The pattern of 90% of the present results is comparable to their observations. Only arabinose for *A. hydrophila*, valerate for *A. caviae* and propionate for *A. sobria* differed significantly (6-10 times). Some of these tests were also carried out by Carnahan et al.[20] but positive percentages reported by them are much lower than those presented here. It appears from the data that this strip has a tendency to separate the various Aeromonas species if its database is reviewed following incorporation of results of some manual tests.

The ID32 C strip has been used in the past for identification of yeast but never for bacterial isolates. It was thus tested for the first time for bacterial isolates in the present work. Very few of the 31 substrates contained in the strip were utilized by all of the isolates of the aeromonads studied here. Only a low percentage of the various isolates of each species used the majority of the substrates. The esculin hydrolysis test turned out to be the most reliable test with this strip as it did not give any false positive or false negative result and this test has differentiated the *A. hydrophila* and *A. caviae* isolates from other isolates with a high degree of confidence. The data shows that this strip has also a few other tests (such as assimilation of palatinose, glucosamine, DL-lactate, Lecturer-arabinose and cellobiose) which could be useful in species differentiation.

Rapid ID32 A contains standardized enzymatic tests for the identification of anaerobic bacteria and was used in this study for characterizing the Aeromonas isolates for the first time. This strip provided data on the presence of various enzymes in the Aeromonas isolates. In general, most of the substrates were utilized by a low percentage of the various isolates and the results were variable in respect to the isolates and the species.

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