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Enzymatic profile of *Kluyvera* species

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Summary. *Kluyvera*, a proposed genus formerly known as Enteric group 8, was found to have similar enzyme profiles among the present 3 groups. *Kluyvera* species group 3 showed the most heterogeneous enzyme reactions.

Kluyvera is a proposed genus of a group of organisms formerly known as Enteric group 8². The organisms are gram-negative, oxidase-negative fermentative bacteria which were previously separated into 2 species³ but have recently been divided into 3 groups. *K. ascorbata* is the first species and may be isolated from clinical specimens such as sputum. *K. cryocrescens* is the second species which also has been isolated from clinical specimens but may be commonly isolated from the environment. The third group, referred to as *Kluyvera* species group 3, is a heterogeneous group that is rarely isolated². In an attempt to increase the available knowledge about these organisms, the present investigation determined their enzyme profiles to characterize their potential invasiveness and to determine whether the species could be further differentiated biochemically.

Methods. Because limited clinical isolates are available, only 20 strains of *Kluyvera* kindly provided by George Morris, Centers for Disease Control, Atlanta, GA. were used in this study, including 9 strains of *K. ascorbata*, 6 strains of *K. cryocrescens* and 5 strains of *Kluyvera* species group 3. The strains were maintained on MacConkey Agar (BBL Microbiology Systems,

Cockeysville, MD.) with incubation at 37°C for 24 h before testing. After the addition of 3 ml of sterile saline to the cultures, heavy cell suspensions were removed and adjusted to a turbidity approximately equal to that of a McFarland No. 5 standard.

Enzyme assays were performed on each strain by using API ZYM System (Analytab Products, Plainview, NY.). Enzyme strips were used for the enzyme assays as previously described for other organisms⁴.

Results and discussion. It was observed that enzyme reactions occurred uniformly in all strains for alkaline phosphatase, esterase lipase (C8), leucine aminopeptidase, valine aminopeptidase, acid phosphatase, phosphoamidase, β -galactosidase, α -glucosidase and β -glucosidase. Two variations were noted in group 3 for esterase (C4) and α -galactosidase.

All strains showed activity for an esterase lipase (C8) and phosphatases which may be involved in the virulence of *Kluyvera*. However, Farmer et al.² reported no activity for gelatinase and stated that these organisms are probably infrequent opportunistic pathogens and most common isolates from sputum are probably not clinically significant.

Another aspect shown in this study is the inability to differentiate the 3 *Kluyvera* groups by these 19 enzyme assays. A slight variation was found in group 3, further pointing out the heterogeneous reactions in this group. In addition, the colony growth characteristics of the members of the genus *Kluyvera* resemble that of *Escherichia coli*. The absence of β -glucuronidase activity among the *Kluyvera* strains could be used to differentiate them from *E. coli*. *E. coli* usually demonstrates β -glucuronidase activity, although several other biochemical assays can also be used to separate these genera including malonate utilization and growth in KCN².

Enzymatic reactions of 20 strains of *Kluyvera*

Enzyme ^a	<i>K. ascorbata</i>	<i>K. cryocrescens</i>	<i>K. Group 3</i>
Alkaline phosphatase	+	+	+
Esterase lipase (C8)	+	+	+
Leucine AP	+	+	+
Valine AP	+	+	+
Acid phosphatase	+	+	+
Phosphoamidase	+	+	+
β -Galactosidase	+	+	+
α -Glucosidase	+	+	+
β -Glucosidase	+	+	+
Lipase (C14)	—	—	—
Cystine AP	—	—	—
Chymotrypsin	—	—	—
Trypsin	—	—	—
N-Acetyl-			
β -glucosaminidase	—	—	—
α -Mannosidase	—	—	—
α -Fucosidase	—	—	—
β -Glucuronidase	—	—	—
Esterase (C4)	—	—	— ^b
α -Galactosidase	—	—	— ^c

^a AP, Aminopeptidase; ^b 1 strain of Group 3 showed positive results for Esterase (C4); ^c 2 strains of Group 3 showed positive results for α -Galactosidase.

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