ISOLATION AND METABOLIC CHARACTERIZATION OF BACTERIA THAT HYDROLYZE THE UREA CONTAINED IN HUMAN URINE

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Introduction. Human urine containing high concentrations of urea, and other salts, urea is the main form of nitrogen in fresh urine (1). Urea, ammonium and phosphorus have value as fertilizer, may be another option compared to chemical fertilizers (2). Urease catalyzes hydrolysis of urea to yield two molecules of ammonia and carbonic acid, which establishes equilibrium between its protonated and deprotonated forms (3).The primary function of urease is to provide nitrogen as ammonia for organisms. Urease is found in seeds of plants, microorganisms and invertebrates. Regardless of the source of urease, it follows the same pattern of catalysis, primarily due to the similar amino acid sequence and the presence of two Ni^{2+} ions in the active site of the enzyme (4). The aim of this study was to isolate and characterize hydrolytic bacteria from different sources of inoculum.

Methods. Cow manure (E) and granular anaerobic sludge (L) were used as inoculum fresh. Three enrichments were performed for each inocula evaluating the urea hydrolysis. Positive cultures were isolated in Rustigian - Stuart medium (5), by mean of surface dilution technique. The pure and mixed cultures were evaluated based on the production of ammonium measured by the Nessler's method for 120 h of hydrolysis and 35°C at pH 7. Pure and mixed cultures were tested using C/N ratio of 1,3. Table 1 show the experimental design performed in this study.

Results. Tree strains were isolated from granular sludge enrichment (LO_2 10^{-1}, LO_2 10^{-2}, LO_2 10^{-3}) and two from cow manure enrichment (EO_2G 10^{-2}) under aerobic conditions. Table 2 show the production of ammonium as evidence of hydrolysis of urea in pure and mixes cultures. It was detected highest yields of ammonium were obtained with mixed cultures EO_2G and L which with 0,9 and 0,72 respectively, while for pure cultures low yields were obtained. The high yields of hydrolysis found in mixed cultures, may be related to the concentration of biomass present, or with syntrophic relationships present in the inocula.

Table 1. Experimental design

Mixed cultures	Treatment	
L	Granular anaerobic sludge in anoxic conditions without additional carbon source.	
LG	Granular sludge anaerobic and anoxic conditions with glucose as carbon source.	
LO_2	Anaerobic granular sludge in oxic conditions, without additional carbon source.	Pure culture: LO_2 10^{-1}, LO_2 10^{-2}, LO_2 10^{-3}
LO_2G	Granular sludge anaerobic and oxic conditions with glucose as carbon source.	
EO_2G	Fresh cow manure, in oxic conditions with glucose as carbon source.	Pure culture: EO_2G 10^{-2}
L= Granular sludge ; E= cow manure; G= glucose and O_2= Oxygen		

Table 2. Production of ammonium in mixed and pure cultures as response of hydrolysis urea.

mg NH_4^+/L				
Culture	Initial	Final	Produced	Yield
L	568,39	4874,08	4305,68	0,72
LG	582,42	4720,43	4138,01	0,69
LO_2	446,16	2454,94	2008,78	0,33
LO_2G	502,77	1706,91	1204,14	0,20
EO_2G	479,44	5849,70	5370,26	0,90
LO_2 10^{-1}	163,59	199,63	36,04	0,006
LO_2 10^{-2}	208,86	240,53	31,67	0,005
LO_2 10^{-3}	170,44	204,94	34,50	0,006
EO_2G 10^{-2}	159,66	184,84	25,18	0,004

Conclusions. Mixed cultures from two sources of inoculum had a high yield in the production of ammonia in response to hydrolysis of urea.

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