Technological characterization of strain *Azotobacter vinelandii* using API 20NE test

1. The studied strain is cultivated on a suitable nutrient medium for 24 hours on a shaker apparatus at optimal conditions. The obtained culture liquid is centrifuged in order to separate the accumulated biomass from the liquid phase. The resulting residue (biomass) is washed twice and resuspended in API AUX suspension medium.

2. Examination of the enzyme activities profile

   API 20NE (BioMericux, France) system for determining the assimilation profile and the conversion of various substrates is used, which is suitable for identification of gram-negative, non-sporulating, non-enteric bacteria. The manufacturer's instructions are observed during the analysis. The API 20NE strip is put in the incubation box and the microtubes are inoculated with the so prepared cell suspension. The samples are incubated at 30^0C for 48h and recorded at the 24^{th} and 48^{th} hour.

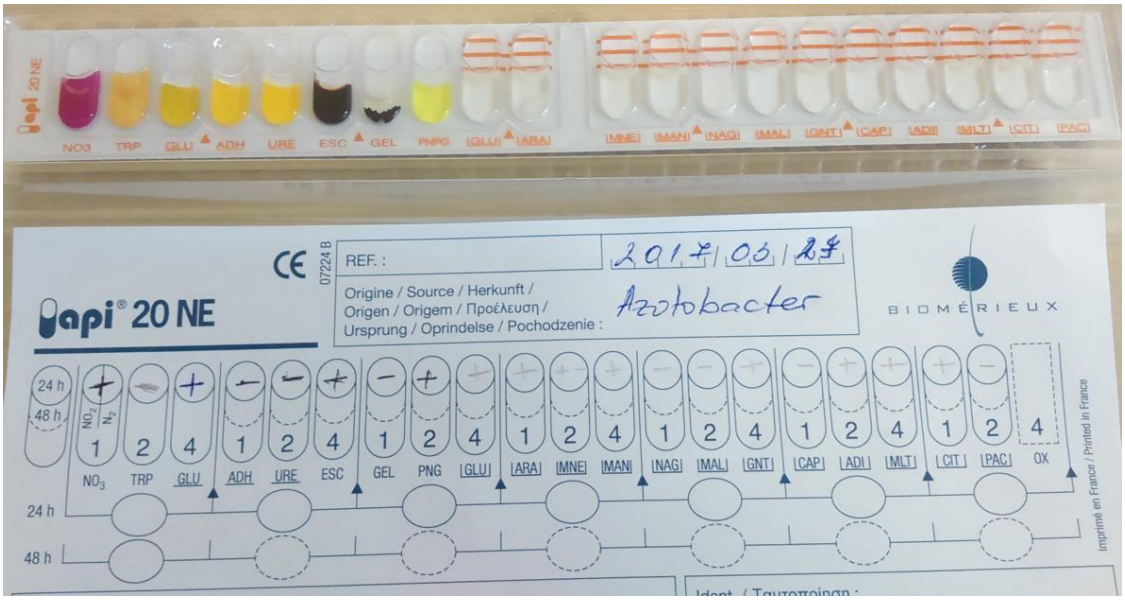
   The coloring change in positions GLU, ADH, URE, ESC, GEL and PNPG is recorded at the 24^{th} hour. One drop of NIT 1 and NIT 2 reagents is added in positions NO_3 and TRP (NO_3 test) and after 5 minutes the lack or presence of change in coloring is observed. If there are bubbles, reactive Zn is added and the lack or presence of change in coloring is observed.

   The Trp test is recorded in positions from ADH to PAC by adding James reactive at the 48^{th} hour. The recording is done by „+" и „-„ depending on the presence or lack of the corresponding coloring.

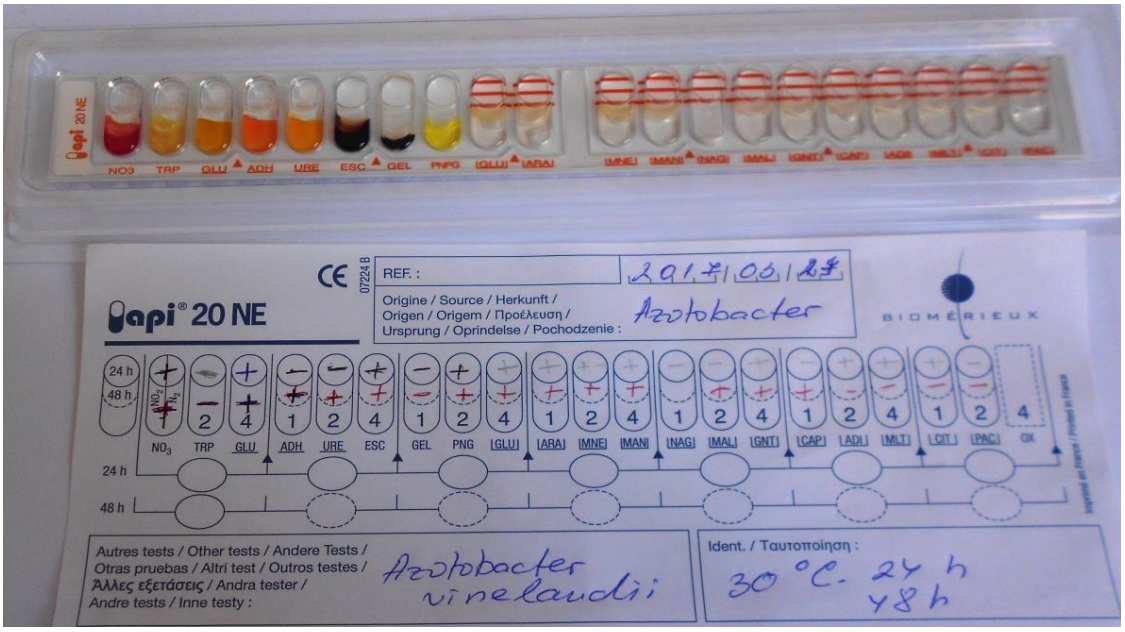
   3. Results

hour	NO_3	Trp	D-glucose (converts)	L-Arg	Urea	Esculic acid	Gelatin	4- nitrophenyl-D-galactopyranoside	D-glucose (assimilates)	L-arabinose	D-mannose	D-mannitol	N-aacetyl glucosamine	D-maltose	Na gluconate	Caprinic acid	Adipic acid	Maleic acid	Triisodium citrate	Phenylacetic acid
24	+	-	+	-	-	+	-	+	+	+	-	+	-	-	+	-	-	-	-	-
48	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	-	-

27.03.2017 Chief assistant Dr. Y. Evstatieva, PhD student T. Georgieva, Assoc. prof. Dr. D. Nikolova



24th hour



48th hour

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