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RESERCH CONCERNING THE ESTIMATE OF QUANTITATIVE AND QUALITATIVE PHYSIOLOGICAL GROUP BACTERIA IN PEATS SAMPLE

CERCETARI PRIVIND APRECIEREA CANTITATIVĂ ŞI CALITATIVĂ A GRUPELOR FIZIOLOGICE DE BACTERII ÎN PROBE DE TURBĂ

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The total aerobe micro flora can be determined on solid mediums for the aerobe bacteria and this relive quantity of micro organisms from the peat samples. The quantitative evaluation was done using solid nutritive mediums which allows the estimation of nr CFU/g as well observing the morphology of the colonies and their utility through their emplacement and morphological and biochemical characterization of isolated strains.. The evaluations where done through the method of dilution, using selective liquid mediums. Every day the characteristic reaction of the respective group was observed, either through the metabolising of the substrate, or through the appearance of a catabolic product in the medium.
Key words: peat, microbiological flora, biochemical characterisation

Introduction

Evaluation of the physiological groups of micro organisms represents their activity respectively the rapport between a few of the physiological groups of bacteria. The identification of these conditions may facilitate the biological characterization of the analyzed samples, allowing information about the important transformation processes to be obtained: the nitration and denitrification process etc

Material and Methods

Liquid mediums have been used for the microbiologic analysis of the peat samples, to give a qualitative estimate of the physiological group of bacteria and solid mediums for the quantitative estimate of bacteria. In order to accomplish the

seeding decimal dilutions had to be done. From them, 1 ml was taken and used for the seeding on the special mediums. Out of each dilution 3 plates were placed on.

Determining the total aerobe microflora The total aerobe microflora was determined on solid mediums for the aerobe bacteria. The estimate of the total microflora of aerobe bacteria was made from the plates in which 30 to 300 colonies were grown. The medial number of the three plates was made and then corrected with the dilution factor, obtaining the number of CFU/g peat.

Evaluation of the physiological groups of micro organisms More important than the total quantity of microorganisms from the peat samples is their activity respectively the rapport between a few of the physiological groups of bacteria. The identification of these special conditions may facilitate the biological characterization of the analysed samples, allowing information about the important transformation processes to be obtained (the nitration and denitrification process etc). The qualitative evaluation of the physiological groups of microorganisms is done on nutritive liquid medium, having as a principle the emphasis of some metabolism products resulted after the bacterial development.

The quantitative evaluation was done using solid nutritive mediums which allows the estimation of CFU/g as well observing the morphology of the colonies and their utility through their emplacement and morphological and biochemical characterization of isolated strains.

Qualitative evaluation of the physiological groups of microorganisms The evaluations where done through the method of dilution, using selective liquid mediums. Every day the characteristic reaction of the respective group was observed, either through the metabolising of the substrate, or through the appearance of a catabolic product in the medium.

- **Determining the atmospheric nitrogen fixating microflora**

Determining of the number of nitrogen fixating aerobe microorganisms was done through seeding with peat dilutions of a liquid medium which doesn't contain combined nitrogen.

- **Determining the ammonification microflora**

The ammonifying microflora achieves the mineralization of the aminoacids, peptides and of other organic substances containing nitrogen all the way to NH_3, CO_2 and H_2O. This is how the appearance of ammonia with a Nessler reactive is researched. It is seeded with ground dilutions suspension (10^{-1} – 10^{-18}) a salty medium to which asparagines was added as unique source of C and N,

- **Determining the nitrating microflora**

The presence of nitrate and nitrite bacteria was noted separately. Two elective liquid mediums where seeded with suspension dilutions, 1 ml for each tube, using 5 tubes for each dilution (10^{-1} – 10^{-6}). For the research of nitrates bacteria the nitrogen is supplied in the form of ammonium sulphate $(NH_4)_2SO_4$. For the nitric bacteria the nitrogen is supplied in the form of NO_2Na. The presence of nitrites is tested with a sulphuric diphenylamine reactive, and in the case of nitrates in the presence of CH_4ON_2(urea) in a sulphuric medium.

- **Determining the denitrating microflora**

The number of micro organisms capable of producing denitrification in the soil is quantified through the disappearance of nitrates over time, with a sulphuric diphenylamine reactive. It is seeded soil dilutions suspension (10^{-1} – 10^{-12}) in a liquid medium in which the nitrogen is under the shape of nitrates (NO_3K). They where all incubated at $+30^0$C. The results where reported comparing with control probes that weren't seeded with soil dilutions suspension. Interpretation of the results was made depending on the researched group of microorganisms. The total microflora was cited after 3, 5 and 7 days of incubation. To estimate the number of positive tubes for bacterial growth, the readings were done after 5, 7 and 10 days. The ammonifying and denitrificating microflora were read after 3, 5, 7, 10, 12, 15 days of incubation. The nitric and nitrate bacteria were analysed after 20 days of incubation.

Quantitative evaluation of the physiological groups of micro organisms

In order to estimate the total number of microorganisms belonging to each physiological group, the same culture medium was used, with addition of 12% agar. Before inoculation, decimal dilutions were made, according to the previously mentioned method. From each dilution 3 plates with appropriate medium where inoculated with 1 ml suspension. The total aerobe microflora was quantified in plates containing 30 to 300 colonies. The mean number of the three plates was calculated and corrected with the dilution factor obtaining the number of CFU/ gr peat. All the samples where incubated at 30^0C. Interpretation of the results was made depending on the researched group of microorganisms. The total microflora was quantified after 3, 5 and 7 days of incubation of the samples. The quantification of bacterial colonies, mushrooms, actinomycetes was done after 5, 7 and 10 days. The ammonifying and denitrificating microflora was quantified after 3, 5, 7, 10, 12, 15 days of incubation. The nitric and nitrate bacteria where determined after 20 days of incubation.

Results and Discussions

Qualitative evaluation of the physiologic group of microorganisms. After the microbiologic analysis of the peat samples received from CHIMGRUP , the qualitative presence of embryos of each physiological group of microorganisms.

Determining the atmospheric nitrogen fixating microflora. The development of nitrogen fixating microflora is estimated by the forming of brown gauze, typical for the development of these bacteria especially of the *Azobacter* kind. The specific cultural characters where observed in the 10^{-1}, 10^{-2} and 10^{-3} dilutions.

Determining the ammonifying microflora. The decomposition of the organic compounds containing nitrogen, as a result of the metabolization of atmospheric nitrogen by free or symbiotic microorganism with the nitrogen

fixating plants, respectively the process of transforming organic nitrogen into a mineral as an ammoniac or ammonium is called ammonification.

A considerable number of bacteria participate in the ammonification process as well as other specific microorganisms. The process is influenced by a series of factors such as: pH, oxygen, temperature, humidity, concentration of the substrate, the presence of certain ions etc. The presence of ammonifying bacteria is highlighted through the appearance of ammoniac in the culture medium with the help of the Nessler reactive.

The positive reaction was identified in every tube with dilutions ranging from 10^{-1} to 10^{-8}.

Determining the nitrating microflora. The ammonium salts resulting from the ammonification process dissociate into ammonium ions of the respective acids (Cl^-, HCO_3^-, SO_4^{2-}, $2HPO_4^{2-}$) which in turn are put through exchange processes with ions attached by the colloids and oxidation processes. The steps through which the oxidation process of the NH_4^+ ion and of the NH_3 ammoniac go through are called denitrification.

The nitrification process plays an important role in the nitrogen cycle in nature. The organisms that oxidises NH_4^+ to HNO_2 (nitrate acid) belong to the group of nitric bacteria, and those microorganisms who oxidises NO_2 to NO_3 belong to a group of nitratic bacteria. The nitrifiying bacteria are split into too large groups: autotrophic and heterotrophic. The factors that influence nitrification are humidity, temperature and especially the substrate of NH_3 and NH_4^+.

The presence of nitrifying bacteria is highlighted through the presence of nitrites and nitrates with a sulphuric diphenylamine reactive, and in the case of nitrates in the presence of urea in a sulphuric medium. The positive reaction was identified in all tubes with dilutions from 10^{-1} to 10^{-6}

Determining the denitrating microflora. Denitrification is the last link in the chain of the nitrogen cycle in nature. It's a process of reduction of the nitrates to nitrites, a process which can be achieved in a biological way either directly or indirectly. The presence of microorganisms capable of producing denitrification is researched by observing the disappearance of nitrates according to time, with a sulphuric diphenylamine reactive. The positive reaction has been identified in the tubes with the 10^{-1}, 10^{-2}, 10^{-3} dilutions.

Quantitative evaluation of the physiological group of micro organisms. After the quantitative sampling, which was made in triplicates, of the peat samples recieved from CHIMGRUP, the total microflora was determined and also the total gauze number of each physiological group of microorganisms implicated in the circuit of carbon and nitrogen elements.

For an appropriate analysis the sampling has been made in triplicates. The obtained results are displayed in table 1 and graphically represented in figure 1, reflecting the absolute value and the log number of microorganisms/g peat.

Table 1

Quantitative evaluation of the physiological groups of micro organisms from the peat samples

Microbiological Indicator	Determination I	Determination II	Determination III	Average	Log10
Total micro flora	280 $x10^5$	226 $x10^6$	118 $x10^6$	124 x 10^6	8,093
Aerobe nitrogen fixation bacteria	$350x10^2$	$247x10^3$	$141x10^3$	141 x 10^3	5,149
Ammonifyers	160x 10^7	$79x10^8$	$67x10^8$	54 x 10^8	9,732
Nitrite bacteria	$250x10^5$	$54x10^6$	$47x10^6$	42 x 10^6	7,623
Nitrate bacteria	$280x10^5$	$86x10^6$	$60x10^6$	58 x 10^6	7,763
Denitrificators	240 $x10^2$	123 $x10^3$	87 $x10^3$	78 x 10^3	4,892

For better representation of the presence of different physiological groups of bacteria in the analysed peat samples the logarithmic values have been graphically represented.

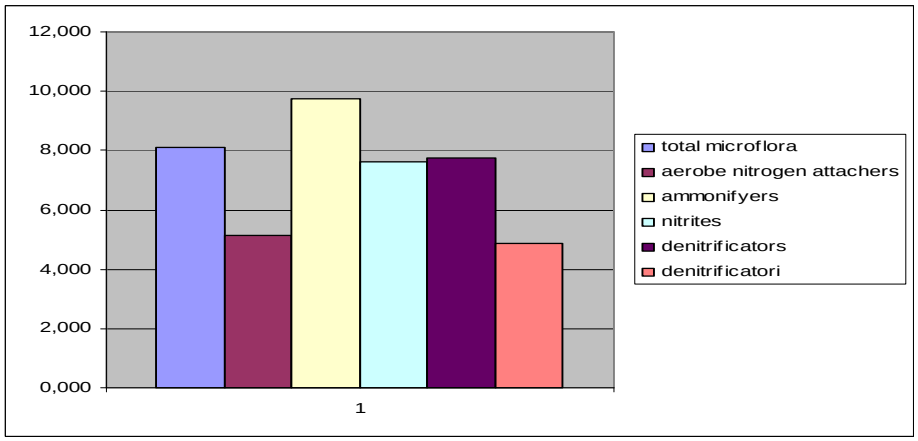

Fig.1 The physiological groups of micro organisms in studied peat samples

In figure 1 shows that the dominant physiological group is represented by ammoniating bacteria (54 x 10^8CFU) which represent the majority of the microflora in peat samples followed by nitrating bacteria represented by the nitrite (42 x 10^6CFU) and nitrate bacteria (58 x 10^6CFU). The denitrating bacteria and the nitrogen fixating bacteria have been found in the smaller number.

The microbiological analysis of the peat samples showing the categories of microorganisms presented in the analysed samples has been followed by isolation of pure cultures in order to obtain certain strains of bacteria, which will subsequently tested for use on different substrates represented by hydrocarbons.

Conclusions

The dominant physiological group is represented by ammoniating bacteria which represents the majority of the microflora in peat samples followed by the nitrating bacteria represented by the nitrite and nitrate bacteria. The denitrating bacteria and the nitrogen fixating bacteria have been found in smaller number.
The microbiologic analysis of the peat samples which show the categories of microorganisms found in analysed samples has been followed by isolation of pure cultures in order to obtain certain strains of bacteria, which will then be tested for use on different substrates represented by hydrocarbons.

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