ADAPTATION RESERVES OF MICROORGANISMS FROM EXTREAME ENVIRONMENTAL CONDITIONS.

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Abstract
The aim of the study was to find microorganisms resistant to radioactivity and chemicals for further developing a technology for utilization of industrial wastes. A unique collection of yeast from extreme environmental conditions of Kamchatca and Curil Island was collected. 448 strains were checked for their resistance to copper, cadmium, and cobalt heavy metal salts and to high temperature (37º C – 52º C). 30 strains resistant to these factors were investigated for their ability to grow on medium, with nonradioactive strontium and cesium. 4 resistant strains were able to accumulate 40 – 80% of radioactive strontium from nutrition medium. Our data could be a foundation for developing new efficient technology for concentration of radionucleoides and heavy metals from nature and industrial wastes.

Introduction
Nowadays radioactive and heavy metal waste utilization is becoming more and more important. Microorganisms could have certain advantages in new technologies to purify environment from such dangerous pollution. But of cause such microorganisms should be resistant to chemicals and radiation (1,2). We presumed that such microbes could be found in extreme natural environment. It is well known that volcanic activity cause ecological crises and could make selective conditions for surviving microorganisms resistant to extreme temperature and chemical pollution. It was the main reason to make a collection of microorganisms from Kamchatka and Curil Islands, a unique region famous for it's volcanic activity.

Methods
Microorganisms were collected from soil, plants, insects, and water from regions with thermic or volcanic activity. Taxonomic identification was made using known methods (3,4). Temperature resistance was tested at 25, 30, 37, 45, 50 and 52º C . Chemical resistance to heavy metal salts was studied, using medium with 50-500 μM/l copper (Cu), 5-150 μM/l cobalt (Co) and cadmium (Cd) ions. Strains with different types of resistance were checked for their ability to survive in the presence of 100 mM/l ^{87}SrO and $^{133}CsCl$. Resistance γ-radiation caused by ^{60}Co with dosage capacity 33,7Gy/min was tested by "Issledovatel" (Russia) at 4º C. Accumulation efficiency by (^{90}Sr) up to 3 μCι/ml using Beckman LS 5801 counter (USA) with previously calibrated program. Accumulation efficiency was calculated as percent of radioactivity in centrifuged cells to the total probe radioactivity.

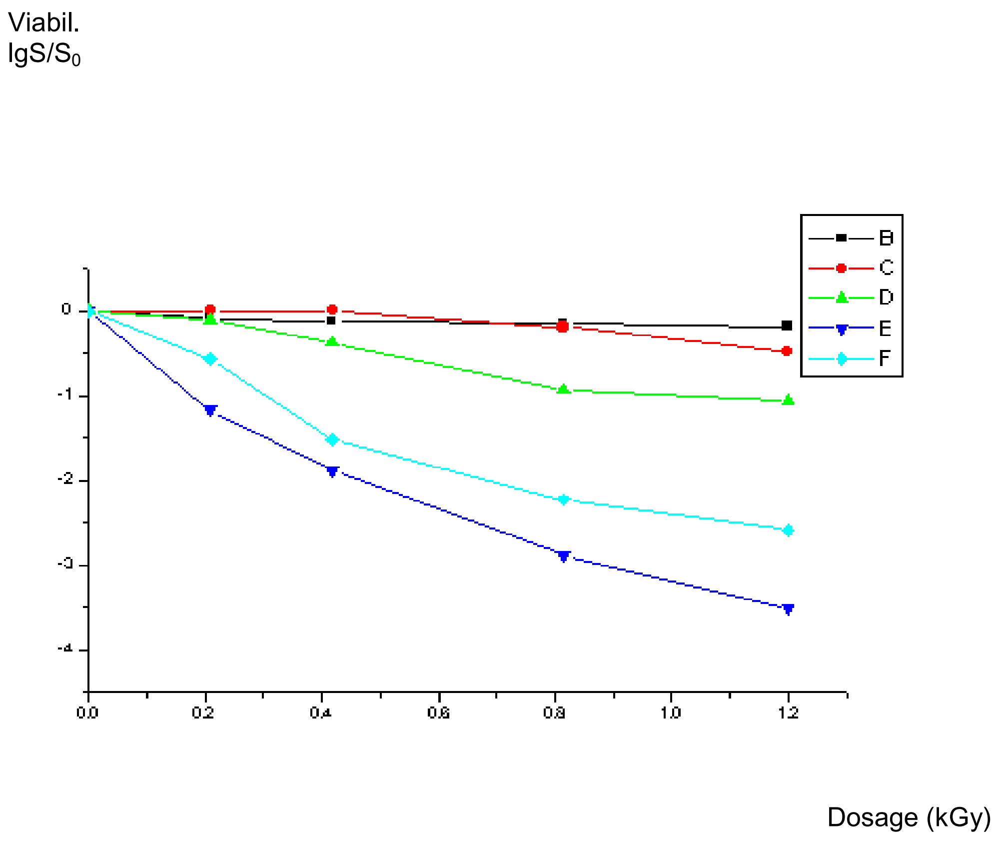
Results
A collection of 2107 new strains of microorganisms from Kamchatka and Curil Islands, a region with volcanic activity was created. Microorganisms from this region are living in conditions of "natural ecological crises", caused by volcanic pollution, and are constantly influenced by extreme temperature, chemical and radioactive treatment. Thus, it seems promising to find microbes selected to become resistant to stress environment.

448 strains from our collection were checked for their resistance to temperature t and to Cd, Cu or Co heavy metal salts. 36% were tCdCuCo resistant and 26% were CdCuCo resistant. In addition to this two phenotypic groups we found 12 other different phenotypic combinations among the strains. Thus, multiple resistance is typical for such strains. General mechanisms of such resistance could be interesting for biological science.

30 strains were studied for their ability to survive in the presence of 100mM/l ^{87}SrO and ^{133}CsCl. And 4 (N15, N46, N272, N273) most resistant were investigated in model experiments. N15 and N46 (*Pichia farinosa*) were more resistant to radiation then *Debaryomyces hansenei* strains (Figure 1).

Figure 1: Viability of microorganisms treated by γ-radiation

B - *Pichia farinosa* N46
C - *Pichia farinosa* N15
D - *Saccharomyces cerevisiae* XII$_7$
E - *Debaryomyces hansenei* N272
F - *Debaryomyces hansenei* N273



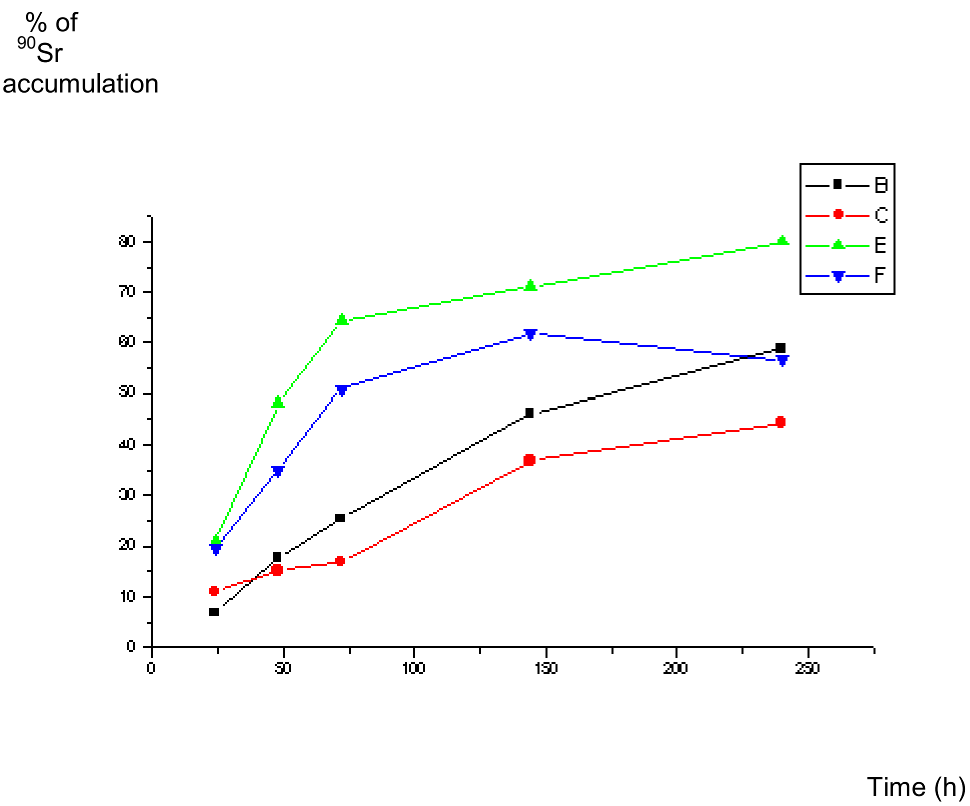
^{90}Sr accumulation by these strains was studied. Surprisingly less radio resistant strains N272 and N273 *(Debaryomyces hansenei)* accumulated 80% and 60% of ^{90}Sr from medium more then N15 and N46 (*Pichia farinosa*) (Figure 2).

Our data show that these 4 natural isolates from extreme environmental conditions belong to two yeast species *Pichia farinosa* and *Debaryomyces hansenei.* The strains are resistant to high concentration of nonradioactive ^{87}Sr and are able to grow in radioactive medium with 0.15 - 3 μCi/ml during at least 10 days. This ability to grow in radioactive medium seems very promising for using these strains in waste utilizing technology. And another unique capability of these microbes not only to grow but also to accumulate radioactive isotopes inside the cells is even more attractive.

If the living biomass could accumulate the isotopes thus lowering their concentration in radioactive waste it would be much easier to isolate the biomass and further more to reduce the biomass volume by simple drying the cell pellet.

The other important problem is how the resistant strains could survive in radioactive waste side by side with other microbes. Since yeast are very widely spread in nature they should be quite competitive and promising selection.

Figure 2: ^{90}Sr accumulation during microorganism's growth.
B - *Pichia farinosa* N46
C - *Pichia farinosa* N15
E - *Debaryomyces hansenei* N272
F - *Debaryomyces hansenei* N273



We would like to stress that cells pallet with accumulated isotope represented only 3% of the total volume and one could reduce this 3% volume to 25 times my drying the radioactive pallet. This is very important factor for waste utilization technology.

Discussion
The noble purpose of cleaning environment first of all from radioactive wastes is becoming more and more important. Our approach was to find microorganisms that should be able to survive at high radiation and to accumulate the radioactive isotopes and to be competitive with other microbes present in waste.

We presume that such microbes could be found in some unique isolated regions on the planet, with special climate, geology and volcanic activity. Since the volcans cause "ecological crises" by polluting the environment by chemicals and creating high temperatures. As a result of constant influence of all these factors during ages microorganisms could have been selected that were resistant to chemicals and high temperature. That is why we organized 3 expeditions to Kamchatka and Kuril Islands to create a unique collection of microorganisms.

It is known that the requirements for the industrial strains to be used in utilization of the radioactive waste are rather high. Such strains should be able to survive at at least 10μCi/ml (1). We investigated the chemical and radioactive resistance of the strains from our collection and among 448 isolates we found two major phenotypic groups with mutual resistance to temperature, Cd, Cu and Co (36%) and resistant to Cd, Cu and Co (26%) and 12 minor phenotypic groups.

This great variability of phenotypes could reflect the existence of at least several mechanisms of resistance in the strains. Some of such mechanisms are already known. Resistance could be caused by metallotionin proteins (5,6,7), by plasma membrane transporters (8,9,10,11), by increase of the transcriptional activity of the cell caused by mutation in termoshock transcription factor (HSF) (12) and by pdr system (pleiotropic drug resistance) (13). We are looking forward to investigating multiple resistance in our strains: the mechanisms and improvement that could be achieved to get more resistant strains to be used further in technology.

Conclusion

Presented data could be a foundation for developing new efficient technology for concentration of radionuclide**s** and heavy metals from nature and industrial wastes side by side with chemical and physical methods. A big number of different strains from our collection could give us opportunity to solve particular problems in particular situations. We are looking forward paying more attention to ecological microbiology.

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