A NEW METHOD USEFUL IN PREDICTIVE MICROBIOLOGY OF MEAT. THE POSSIBILITY OF QUANTITATIVE DIFFERENTIATION TWO BACTERIAL STRAINS GROWING TOGETHER IN A LIQUID MEDIUM DURING TURBIDIMETRIC GROWTH CURVES EXPERIMENTS.

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Background

In a predictive microbiology, a discipline aiming at elaboration truly working mathematical models describing behavior of microorganisms in food environment, little concern was paid so far to important role of concomitant microorganisms, acting as environmental factors, on microorganism or microorganisms of interest. In a meat environment growth of a single bacterial strain is being molded not only by simple environmental factors of chemical or physical nature such as NaCl, $NaNO_2$, pH, temperature, but also by metabolites produced by microorganisms, especially strange metabolites coming from other microorganisms present in the same environment. Automatic turbidimetry or conductometry are commode research tools (screening methods) to trace bacterial growth in a liquid medium. "In vitro" experiments are frequently used as screening methods serving a lot of useful digital data for subsequent mathematical modeling. Because of technical reasons (lack of possibility to distinguish individual microorganisms) above mentioned automatic methods are of no use if one has to trace the growth of two or more microorganisms growing together in the same medium.

Objective

Elaboration a method to distinguish quantitatively two bacterial strains *Escherichia coli* and *Weisella viridescens* growing together in the same liquid medium by only the turbidimetric measurements, lest not to deteriorate facility of automatic method.

Methods

Two bacterial strains from American Type Culture Collection were used:

- *Escherichia coli* ATCC 700599 (serotype: O 157:H7),
- *Weisella viridescens* ATCC 12706 (deposited in the collection as *Lactobacillus viridescens;* causative agent of green discoloration of meat products).

For cultivation *E.coli* in 25^0 C (tube cultures preparation; two consecutive passages from frozen stock) TSB liquid medium (Tryptic Soy Broth - Difco) was used;

For enumeration in 30^0 C (CFU/ml estimation, surface-plating), TSA (Tryptic Soy Agar –Difco) was used.

Weisella viridescens was cultivated in 25^0 C (tube cultures preparation; two consecutive passages from frozen stock) in MRS-broth (Man, Rogosa, Sharp broth - Merck), enumerated in 30^0 C (CFU/ml estimation, surface-plating) on MRS-agar (Man, Rogosa, Sharp agar - Merck).

Instrument "Spectramax 340 (Molecular Devices) was used as automatic turbidimetric analyzer. Spectramax is a specialized ELISA – reader designed for kinetic experiments with the possibility to preserve sterile conditions in microtiter plate (96 wells) during long lasting experiments. It is supplemented with incubator having the possibility to heat plate cover $+1^0$ C higher the bottom part of the plate containing liquids, to avoid dropping water on the inner surface of the cover. It is also supplemented with a shaker. All functions of the instrument can be U-controlled from a computer. In kinetic experiments simultaneous absorbance measurements with six free chosen wavelengths (from 350 nm to 750 nm with a step of 1 nm) can be performed.

The main concept in experiments planning was to chose six wavelengths most efficient in quantitative differentiation of the two above mentioned bacterial strains, and next to elaborate neural model, with the use of virtual neural network, explaining the relationship between six absorbance values and concentration (in CFU/ml) of two bacterial strains *Escherichia coli* and *Weisella viridescens*, appearing together in the same medium in various proportion. That model may be used in farther predictive microbiology experiments to transform absorbance vectors – dependent variables of growth curves functions - to bacterial count vectors.

All the calculations (elaboration of neural models) were performed with the use of Statistica Neural Networks Package.

Course of experiments

24 h tube cultures of *Escherichia coli* and *Weisella viridescens* in TSB and MRS broths, respectively, were prepared before the main experiments. To perform the main experiments, first the bacterial cultures kept in ice after completion cultivation were enumerated by surface-plating on the proper beds. Then serial dilutions of *E. coli* and *W. viridescens* were done in tubes in TSB-broth and MRS-broth and next mixed in various proportions in wells of microtiter plates. 792 combinations in general of the two strains were received including 24 repetitions of each and cases with 0 level (CFU/ml) of one of the strains. Individual wells contained two strains in different proportions and two liquid media mixed in different proportions too. MRS-broth and TSB-broth differed on eye in color. MRS-broth was apparently darker than TSB-broth.

Microtiter plates containing bacterial mixtures were subjected to spectrophotometric measurement with the use of automatic "Spectramax 340" scanning option. Spectrum in this instrument was obtained within a scope of 350 nm to 750 nm in a step of 10 nm. All the results were written to disk in internal Spectramax format, then exported as text format to Excel for farther preparation to a form that could be used in Statistica Neural Networks package.

Calculations

One record of 792 data set records representing one bacterial proportion of *E. coli* and *W. viridescens* consisted of 41 absorbance values measured with 41 different wave lenghts and two bacterial counts of the two strains. Bacterial counts expressed in CFU/mL were changed to logarithmic values to generate distribution of bacterial counts more close to normal. Every 12 absorbance values apearing in sequence in one row in microtiter plate were smoothed by approximation with various functions with the aid of TableCurve software (Jandel Scientific).

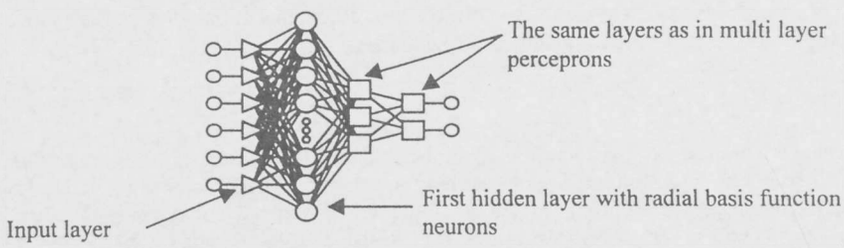
Neuro-genetic algorithm included to Statistica Neural Networks package as external software was applied to find a set of six wavelengths most instructive in explanation the relationship between absorbance values and bacterial counts in mixed populations. Penalty coefficient and smoothing factor were preset to 0.003 and 0.3 respectively. All other initial values were used as defaults.

Having an information on most instructive wavelengths, a farther step, it means elaboration of neural model for expression the relationship between absorbances and logarithm of CFU/ml, was done. Statistica Neural Networks package was used the second time in a version of automatic adviser. Automatic adviser tested various networks architectures including Linear Model, General Regression Neural Network, Radial Basis Function Network, Three and Four Layer Perceptrons. Among the architectures tested the most appropriate was chosen, then this kind of networks was trained with randomly separated data to training set (396 records) and validation set (198 records). Validation set of data was not directly given as input during network's learning, but was treating as a detector to avoid over-learning. The third randomly separated set of data - test data set - (198 records) was preserved for final quality valuation of the model.

Results and discussion

Neuro-genetic algorithm marked-out six the shortest wave lengths as more appropriate to subordinate logarithmic values of bacterial counts from six values of absorbance, it means: 350, 360, 370, 380, 390 and 400 nm.

These wavelengths used in searching final model gave the following results. The most proper architecture of neural networks appeared to be General Regression Neural Network (GRNN) of the following appearance:



Regression statistics of the model obtained

	E. coli			W. viridescens		
Statistics:	Training	Validation	Test	Training	Validation	Test
Data mean	1,8946	0,6573	0,8189	1,1792	2,0020	1,3575
Data S.D.	7,0352	7,4780	7,8424	7,6292	7,0891	7,4145
Error mean	-0,0557	0,0720	-0,1081	-0,0295	-0,0650	-0,0655
Error S.D.	0,9203	0,9379	1,0678	0,5992	1,1650	0,8631
Abs. Error mean	0,5670	0,5551	0,5511	0,3381	0,5998	0,4855
Ratio: predicton error S.D. / Training data S.D.	0,1308	0,1254	0,1362	0,0785	0,1643	0,1164
Correlation coefficient	0,9915	0,9921	0,9907	0,9969	0,9864	0,9932

Conclusions

1. In spite of different colors of two liquid media mixed in different proportion, artificial neural network is able to correctly distinguish two bacterial strains mixed in different proportion as well, and give the correct output values of log (CFU/ml) for each strain.
2. Turbidimetric method for growth curves analysis equipped with artificial neural network algorithm, applied to change absorbance values to Colony Forming Units, may be a useful tool in predictive microbiology.

References

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