METHODS

A Method for Biological Control of a Complex Phytoadaptogen

O. A. Bocharova, M. A. Lyzhenkova, O. N. Kurennaya*, and V. A. Knyazhev**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 12, pp. 694-696, December, 2003
Original article submitted July 10, 2003

We propose a method for standardization of complex adaptogen-containing preparations. The method is based on acceleration of baking yeast strain growth on energy-depleted medium in the presence of the test agent. This method allows simple quantitative biological control of phytoadaptogens and comparison of adaptogenic activity of mono- and complex preparations.

Key Words: *phytoadaptogen; biological standardization; yeast cultures*

Creation of new preparations of plant origin is a perspective trend in medicine. Due to a wide spectrum of regulatory effects and low toxicity, adaptogens find more and more extensive applications as means for prevention and treatment of many diseases [2,4,5].

Phytomix-40 (PM-40), a complex preparation proposed as an immunomodulator for preventive oncology, was tested along with 7 other phytocompositions and selected along with the reference variant in the *in vitro* selection system. This system included evaluation of the efficiency of suppression of tumor cell proliferation and immunomodulating and interferon-producing effects of the test preparation.

Clinical use of adaptogenic complexes is associated with the problem of biological standardization relative to known bioactivities, which impedes their correct dosage in clinical practice. The effects of these agents on physical endurance of experimental animals (weight-loaded forced swimming or rope climbing) were studied. Other methods for standardization of plant preparations were based on gonadotropic, antidiuretic, and stress-protective effects of adaptogens. However, these methods of standardization are labor consuming and inaccurate.

Paramecium caudatum, used for tentative evaluation of adaptogenic, antioxidant, and membrane-stabilizing effects of the drugs [3], are proposed for testing new drugs, including those of plant origin.

The method for evaluation of biological activity of some adaptogens using the *Sacharomyces cerevisiae* yeast cell culture allows qualitative and quantitative standardization of adaptogenic agents and can be used for testing phytocomplexes [1].

MATERIALS AND METHODS

Sacharomyces cerevisiae strain selected in preliminary screening was used; cell concentration of this yeast strain doubles during culturing in complete medium with ethanol within no more than 5 h.

S. cerevisiae cell culture was grown for 24 h at 30°C in complete agarized medium and then transferred into liquid nutrient medium. The concentration was adjusted to 1×10^6 cells/ml. Glucose (2%) was added to the incubation media as the source of carbon, and thus complete glucose medium was prepared which served as positive control; complete ethanol (2%) me-

N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow; *Institute of Gene Biology, Russian Academy of Sciences Moscow; **I. M. Setchenov Moscow Medical Academy. **Address for correspondence:** lmari@yandex.ru. Lyzhenkova M. A.

0007-4888/03/1366-0615$25.00

dium served as negative control; phytomixtures were added in different concentrations.

Culturing was carried out in conical flasks (250 ml) with 50 ml medium on a shaker (260 rpm) for 4 h at 30°C. Proliferation index (i_p) equal to the ratio of the final to inoculation concentration served as the indicator of yeast culture growth. Cell concentrations in the medium were counted in Goryaev chamber 3 times. The relationship between cell harvest in energy-depleted medium and phytopreparation concentration in incubation medium after 4-h culturing and the slope of the curve describing this relationship were evaluated.

Preliminary studies using various ingredients of the medium showed that the most active growth was observed during yeast cell culturing in complete glucose medium, when carbon was assimilated by most effective aerobic glycolysis pathway. The relationship between yeast proliferation index and glucose concentration in the medium can be presented as saturation curve, which is described by the following equation:

$$i_p = \frac{K_1 \times C}{K_2 + C}, \quad \textbf{(1)}$$

where K_1=2.62, K_2=0.05.

Replacement of glucose with ethanol as less preferable nutritive source led to deceleration of cell growth. Addition of various doses of adaptogens to the incubation medium stimulated cell proliferation. Presumably, this was caused by alteration of the metabolic pathway from glycolysis to gluconeogenesis and adaptogen-aided activation of ethanol assimilation as the only source of carbon. It was shown that the relationship between the yeast cell proliferation index and the content of bioactive adaptogens (*Rhodeola rosea*, ginseng) in complete medium with ethanol after 4-h culturing was individual characteristic of plant adaptogen and could be presented as an inverse parabola described by the following equation:

$$i_p = 1.86 + b\sqrt{C}, \quad \textbf{(2)}$$

where i_p is *S. cerevisiae* cell proliferation index in the adaptogen test, C concentration of the preparation in culture medium (ml), 1.86 the mean index of proliferation in the medium with ethanol (negative control -K), b parameter describing the relationship between yeast cell proliferation index and concentration of the preparation in incubation medium and characterizing the curve.

Biological activity of monopreparations and phytocomplexes was characterized and compared using relative adaptogenic activity (A_{adapt}). If glucose medium was taken as favorable culturing conditions and ethanol medium as unfavorable conditions, the relative adaptogenic activity of the preparation was defined as a measure of adaptogen-induced increase of cell proliferation index in ethanol medium. Mathematically it was expressed by the following equation:

$$A_{adapt} = \frac{Ip_{adapt} - Ip_{et}}{Ip_{glu} - Ip_{et}} \times 100\%, \quad \textbf{(3)}$$

where Ip_{adapt} is cell proliferation index in the medium with adaptogens, Ip_{et} in the medium with ethanol, and Ip_{gl} in the medium with glucose.

RESULTS

Functional relationship between yeast cell proliferation index and the content of reference phytomixture in the medium after4th hour of culturing in the studied range of doses can be presented as an inverse parabola (Fig. 1, *a*) which is described by the following equation:

$$i_p = 1.86 + 1.0\sqrt{C}, \quad \textbf{(4)}$$

where 1.0 is the parameter describing the relationship between yeast cell proliferation index and the preparation concentration in incubation medium and characterizing the optimized curve.

A similar relationship for PM-40 is also an inverse parabola (Fig. 1, *b*), described by equation with coefficient b=1.1:

$$i_p = 1.86 + 1.1\sqrt{C}, \quad \textbf{(5)}$$

The patterns of the resultant curves are identical to those of previously studied individual adaptogens, which suggests adaptogenic effects of the studied phytocompositions.

Comparing the results of experiments for two phytocompositions, let us note that lower coefficient b of the reference phytomixture curve indicates less expressed effect on yeast cell proliferation. The proliferation index values reached by inverse parabola at maximum concentrations of PM-40 are higher than the respective values for the reference composition curve.

It should be emphasized that coefficient b is individual, while the curves are characteristic of each composition. It is known that coefficient b differs for different adaptogens and coincides for homologous adaptogens derived from natural raw material and biomass. Hence, in our case phytocomposition can be identified by the pattern of the respective functional relationship and coefficient b.

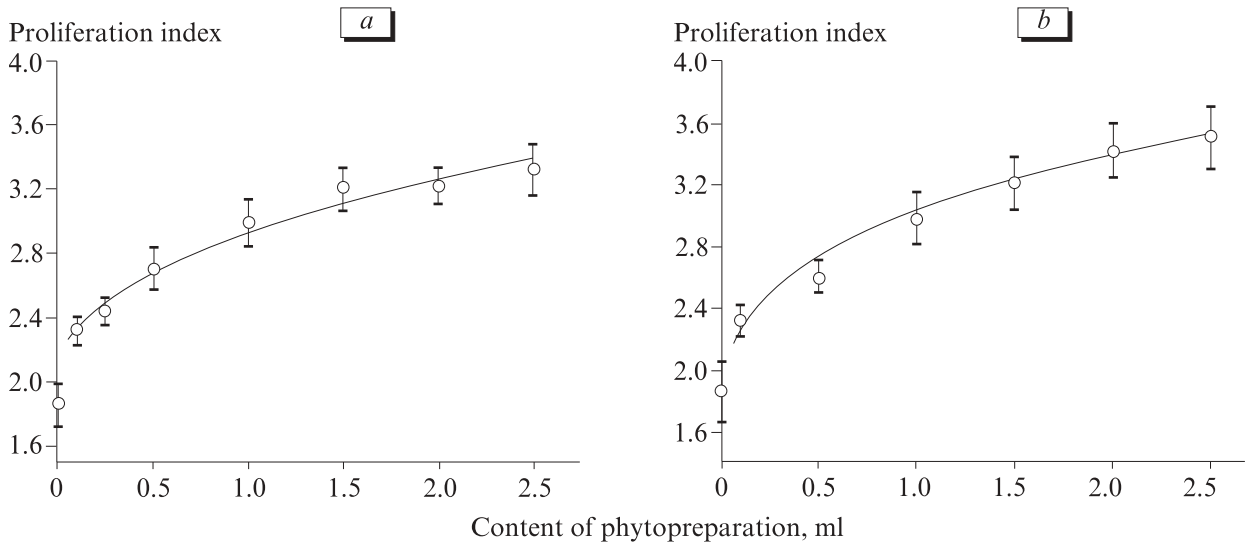

Fig. 1. Functional relationship between *S. cerevisiae* cell proliferation and content of reference phytomixture (*a*) and content of Phytomix-40 (*b*) in culture medium.

In addition, the relative adaptogenic activity is also a characteristic of adaptogen-containing preparations. It is noteworthy that activity of phytocompositions surpasses activity of monopreparations. Adaptogenic activity of PM-40 is the highest: A_{adapt}=51.6%; reference phytomixture 45.3%; natural jinseng (summary glycoside fraction 0.07%) 21.6%; biojinseng (summary glycoside fraction 0.12%) 30.6%; natural *Rhodeola* (phenyl compounds 0.07%) 10.8%; biorhodeola (phenyl compounds 0.17%) 29.8%.

Our results proved the adaptogenic effects of two compositions and the advantages of PM-40 phytoadaptogen over the reference preparation, which is in line with experimental findings, indicating better prospects of PM-40 as regards suppression of tumor cell proliferation and demonstrating a normalizing effect of this composition on the immunological phenotype and interferonogenesis by lymphocytes of cancer patients *in vitro*.

This paper offers a simple quantitative method for standardization of PM-40 phytocomplex, which will promote its correct dosing in further *in vivo* and clinical studies.

REFERENCES

1. O. A. Bocharova, O N. Kurennaya, R. V. Serebryakova, and N. B. Bodrova, *Biotekhnologiya*, No. 8, 28-34 (1993).
2. E. D. Gol'dberg and E. P. Zueva, *Plant Preparations in Combined Therapy of Malignant Tumors* [in Russian], Tomsk (2000).
3. A. N. Kudrin, V. V. Ananin, V. Yu. Balab'yan, *et al.*, *Ros. Khim. Zh.*, **41**, No. 5, 114-123 (1997).
4. K V. Yaremenko, *Adaptogens as Means of Preventive Medicine* [in Russian], Tomsk (1990).
5. K. V. Yaremenko and V. G. Pashinskii, *Malignant Tumors: Therapy and Drug Prevention* [in Russian], St. Petersburg (2003).