

Screening of Anticancer Materials from *Myxobacteria* in Different Natural Ecological Environment

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Abstract

By study on ecological diversity of *myxobacteria* in some areas of Hebei, Yunnan Province and the Qinghai-Tibet Plateau, more than 150 strains of 10 genera (*Archangium*, *Myxococcus*, *Cystobacter*, *Corallococcus*, *Melittangium*, *Sorangium*, *Polyangium*, *Chondromyces*, *Angiococcus* and *Stigmatella*) of *Myxococcales* were isolated from 42 samples, including some special strains that have never been described before. The isolated strains were preliminary identified at generic level by fruiting bodies, swarms, myxospores and vegetative cells. *Myxobacteria* strains that were preserved by the Key Lab of Microbial Diversity Research and Application of Hebei Province were used to screen anticancer drugs by MTT high throughput method, with tumor cell lines such as L1210, Hela and MRC-5 as screening model. The result demonstrated that most of the strains could inhibit growth of tumor cells. The inhibition rates were about 78.5% to L1210 cell line, about 59% to Hela cell line. Strain 910018 and 920036 showed higher bioactivity for growth inhibition of L1210 and Hela cell lines, while showed lower bioactivity to MRC-5 cell line. Therefore, both of strains are valued for in vivo tumor inhibition experiments.

Keywords: Myxobacteria, MTT, Tumor-inhibited rate, L1210, Hela

1. Introduction

Myxobacteria are a class of Gram-negative prokaryotes with complex multi-cellular behavior and morphology (Fang & Zhang, 2001). According to the phylogenetic analysis based on 16S rDNA sequences, *Myxobacteria* belong to δ - subclass of *Proteobacteria* (Iizuka *et al.*, 2003). In the second edition of "Bergey's Manual of Systematic Bacteriology", *Myxobacteria* are classified as *Myxococcales* of *Deltaproteobacteria* of *Proteobacteria* and include 3 orders, 6 families, 19 genus and 62 species (George *et al.*, 2004). In order to protect *Myxobacteria* biodiversity and exploit potential strains, regional distribution of diversity was investigated in this study. More than 150 strains of 10 genera (*Archangium*, *Myxococcus*, *Cystobacter*, *Corallococcus*, *Melittangium*, *Sorangium*, *Polyangium*, *Chondromyces*, *Angiococcus* and *Stigmatella*) of *Myxococcales* were isolated from 42 samples, which were collected from different areas in Hebei, Yunnan Province and the Qinghai-Tibet Plateau. The distribution information was demonstrated in Table 1.

2. Methods and Materials

2.1 Materials

2.1.1 Strains

All of the strains used in this study were isolated and stored by Key Lab of Microbial Diversity Research and

Application of Hebei Province.

2.1.2 Seed medium

CAS medium: 1% Casein, 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, adjust to pH 7.2.

2.1.3 Fermentation medium

VY/2 medium: 1% Yeast powder, 0.1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5mg/mL VB_{12} , adjust to pH 7.2.

2.1.4 Cell medium

10% bovine serum, 90% RPMI 1640

2.1.5 Cell lines

L1210, Hela and MRC-5 were murine leukemia cell lines, human cervical cancer cell lines and Normal diploid human embryonic lung fibroblasts, respectively. L1210 and MRC-5 were supplied by Animal Experimental Center of Hebei Medical University. Hela was supplied by Microbiology lab of Hebei Medical University.

2.1.6 Extraction of fermentation products

The *Myxobacteria* cultures were mixed with same volume of ethanol and incubated overnight. Then the leaching liquor was concentrated with rotary evaporator and the concentrated solution was lyophilized at -50°C .

2.2 Methods

2.2.1 Fermentation of *Myxobacteria*

Purified strains were remained in VY/2 medium and inoculated to CAS seed medium, shaking with 200r/min for 4 days at 28°C . Then the seed solution was inoculated to fermentation medium VY/2 with ratio of 10%, shaking with 200 rpm/min for 7 days at 28°C .

2.2.2 Screening of anticancer extract from *Myxobacteria* in vitro by MTT

2.2.2.1 Preparation of sample solution

Two hundred ml *Myxobacteria* cultures were extracted by ethanol, concentrated, lyophilized and weighted. Then 4 ml RPMI-1640 medium was used to dissolve the dry sample. The samples were marked with No.

2.2.2.2 MTT experiment

The experiment included 4 dose groups (3.33 mg/ml, 6.65 mg/ml, 13.3 mg/ml, 26.6 mg/ml) and the culture with same cell density was used as blank control. Solvent control was zero adjustment. 0.1 ml volumes of 10^5 cfu/ml cell cultures were added to each well of 96-well plate and the plate was incubated for 12 h. Then, 20 μl sample solution was added to each well with triplicate and incubated for 48 h at 37°C with 5% CO_2 saturation. 20 μl MTT was added to each well and incubated for 4 h, centrifuged for 10 min with 2000 rpm/min. Then the supernatant was discarded (should be careful, remain the Formazan particles). 150 μl DMSO was added to each well with shaking for 5-10 min until blue particles were soluble completely and the solution color became dark blue. The $\text{OD}_{570\text{nm}}$ was measured immediately and the inhibition rate (IR) of cancer cell proliferation was calculated (Li, 2004). Then half inhibitory concentration (IC_{50}) was calculated by modified Karber method (Mu, 2007).

$$\text{IR} (\%) = (1 - \text{OD}_{570\text{nm}} \text{ of experiment group} / \text{OD}_{570\text{nm}} \text{ of negative control}) \times 100 \%$$

$$\text{IC}_{50} = \lg^{-1} \left[X_m - I \times \left(P - \frac{3 - P_m - P_n}{4} \right) \right]$$

X_m : logarithm of maximum dose; I : difference of the two adjacent logarithmic dose; P : sum of positive reaction rate; P_m : maximum inhibition rate; P_n : minimum inhibition rate

2.2.2.3 Data statistical analysis

The experiment was carried out with six groups and 3 replicates. Data were analyzed by SPSS16.0, using ANOVA and t-test. Test level $\alpha=0.05$ and $P < 0.05$ means significant difference.

3. Results and discussion

3.1 MTT results

3.1.1 In vitro effect of extract on L1210 and Hela

Forty-eight *Myxobacteria* strains isolated and stored by Key Lab of Microbial Diversity Research and

Application of Hebei Province were fermented and extracted. Anticancer materials were screened by MTT method using 96-well plate, with L1210, Hela and MRC-5 cell lines as screening models. The results were showed in Table 2.

Most of the 48 samples could inhibit growth of L1210 and Hela cells. L1210 cells were more sensitive to the extracts than Hela cells. The inhibition activities at dose of 26.6 mg/mL of 7 strains to L1210 and Hela cells are more than IC_{50} of 85% strains (Table 3).

The results demonstrated that the secondary metabolite of the 48 *Myxobacteria* strains could widely inhibit growth of cancer cells. The IC_{50} of 78.5% of strains to L1210 amounts to the bioactive materials in 2~8 ml original fermentation broth. The IC_{50} of 59% of strains to Hela amounts to this value. The inhibition rates of most strain to L1210 were usually higher than that to Hela at the same doses. This demonstrated that L1210 was more sensitive and the inhibition activity of a sample was different to different cancer cells.

3.1.2 Effect of extracts with different concentration on relative survival of MRC-5

A good anticancer drug not only could kill cancer cell powerfully, but also minimize the damage to normal cells. Therefore, the sample should be screened by the Normal diploid cells. Fifteen out of 19 strains could inhibit growth of MRC-5 at dose of 20 μ l /well. No. 7, 20, 5, and 1 samples affected growth of MRC-5 least. The cells remain normal shape and transparent. In addition, No. 7 (extracted from strain 910018) and 20 (extracted from strain 920036) strains could inhibit growth of both L1210 and Hela cells powerfully.

4. Conclusion

The secondary metabolites of *Myxobacteria* have great potentials for application (Guo, 2007; Guo *et al.*, 2007). In the present study, 48 strains isolated and stored by Key Lab of Microbial Diversity Research and Application of Hebei Province were fermented, extracted, concentrated and lyophilized (Guo *et al.*, 2008). The samples were screened by MTT method with 3 cancer cell lines as models (Cheng *et al.*, 2009). Two strains (910018 and 920036), that could inhibit cancer cells but less or not inhibit normal diploid human embryonic lung fibroblasts, could be as candidates of anticancer drugs. However, the function mechanisms still remained to study.

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Table 1. Distribution of species in different sites

Sample source	No. of samples	Genus number isolated	Strain number isolated	Average genus number from each sample	Average strain number from each sample	Strain number of Myxococcus	Average strain number of Myxococcus each sample	Strain number of other genera	Average strain number of other genus each sample
Yunna	18	5	62	0.28	3.44	46	2.56	16	0.89
Hebei	15	10	50	0.67	3.33	19	1.27	31	2.07
<i>Qinghai-Tibet Plateau</i>	9	2	7	0.22	0.78	2	0.22	5	0.56

Table 2. In vitro inhibition effect of ethanol extract from 48 *Myxobacteria* strains on L1210 and Hela cell lines

Cell lines	doses	Number of strains with different inhibition rate		
		>30%	>60%	>90%
L1210	3.33 mg/mL	4	0	0
	6.65 mg/mL	13	0	0
	13.3 mg/mL	30	14	0
	26.6 mg/mL	43	36	6
Hela	3.33 mg/mL	0	0	0
	6.65 mg/mL	7	0	0
	13.3mg/mL	12	5	0
	26.6 mg/mL	33	13	4

Table 3. In vitro IC_{50} (mg/mL) to L1210 and Hela cell lines of extracts from 7 *Myxobacteria* strains

Cell lines	IC_{50} (mg/mL) of different strains						
	6#	7#	9#	16#	17#	18#	20#
L1210	10.98	9.46	6.10	6.42	7.88	6.83	7.99
Hela	11.35	9.47	16.99	7.34	8.34	11.60	8.41