International Scholars Journals

African Journal of Ecology and Ecosystems ISSN: 9428-167X Vol. 6 (5), pp. 001-008, May, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Cytotoxic and topoisomerase I inhibitory activities from extracts of endophytic fungi isolated from mangrove plants in Zhuhai, China

Cai Xiaoling[1]*, Liang Xiaoli[1], Zhou Shining[2], Gao Junping[1], Wu Shuiping[1], Lai Xiaoming[3], She Zhigang[1] and Lin Yongcheng[1]

[1]School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou, People's Republic of China.
[2]School of Life Science, Sun Yat-Sen University, Guangzhou, People's Republic of China.
[3]Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, People's Republic of China.

Accepted 21 January, 2019

To isolate endophytic fungi from mangrove plants in Zhuhai, China and examine their DNA topoisomerase I inhibitory and antitumor activity. Endophytic fungi were isolated using surface-sterilization methods and were identified by morphological or molecular evidence. DNA topoisomerase I inhibitory activities were measured by topoisomerase I-mediated DNA plasmid relaxation assay. Antitumor activity *in vitro* was measured by MTT (3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide) method. A total of 209 endophytic fungal strains were isolated from surface sterilized leaves and branches of eight mangrove plants collected in Qi'ao island of Zhuhai, China. Crude ethyl acetate extracts of fermentation broths from 54 fungal cultures were tested for antitumor activities. Extracts of 19 isolates inhibited the activity of DNA topoisomerase I and extracts of 16 isolates displayed strong cytotoxicity against KB and KBv200 cells (IC_{50} < 11 µg ml^{-1}). Among them, nine isolates of fungi showed both cytotoxic and topoisomerase I inhibitory activities, which are potential producers of antitumor compounds targeting at topoisomerase I. Endophytic fungal strains were successfully isolated and identified and nine strains showed both cytotoxic and topoisomerase I inhibitory activities. Endophytic fungal strains displaying both topoisomerase I inhibitory and antitumor activity together may indicate their potential ability of producing antitumor compounds targeting at topoisomerase I.

Key words: Mangrove endophytic fungi, antitumor activity, molecular characteristic.

INTRODUCTION

Located at the marine environments, marine mangrove endophytic fungi are important resources of new and bioactive compounds (Pan et al., 2008). A number of new bioactive compounds from marine microorganisms have been identified with promising a potential source for industrial, agricultural, environmental, pharmaceutical and medical uses (Blunt et al., 2008). However, marine microorganisms remain a largely untapped resource of clinically useful anti- cancer agents (Banerjee et al., 2008; Gordaliza, 2007).To shorten potential drug-lead marine natural products discovery time lines, we screened crude ethyl acetate extracts of fermentation broths from 54 representative fungal cultures to identify potential producers of natural compounds inhibiting DNA topoisomerase activity and tumor growth.

*Correspondence author. E-mail: cescxl@mail.sysu.edu.cn. Tel/Fax: (86)-20-84039623.

MATERIALS AND METHODS

Source of endophytic fungi

Different parts of *Sonneratia caseolaris*, *Brine fern*, *Pluchea indica*, *Bruguiera gymnorrhiza, Sonneratia apetala, Kandelia candel, Angier's corniculatum* and *Heritiera littoralis* were collected from apparently healthy plants in the Mangrove Reserve in Qi'ao island of Zhuhai in Guangdong province in the south of China in July,

2007. All plant samples were kept in plastic bags and brought back to the Insititute of Marine Nature Product in Sun Yat-sen University for fungal isolation on the same day. The specimens of plant samples were compared with the voucher specimens at Sun Yat-sen University Herbarium. The identification of the eight species of plant was confirmed by Dr Shi Xianggang, Department of Botany, School of Life Science, Sun Yat-Sen University.

Isolation and identification of endophytic fungi

The isolation of endophytic fungi from the mangrove plant material interior was performed as described by Cao et al. (2004) and Schulz et al. (1993). A total of 209 fungi were isolated and 54 isolates were selected for antitumor assay based on their morphotypes (Table 1).

For identification, 54 isolates from slants were transferred to potato dextrose agar (PDA) medium covered with a sterile cover-glass on a sterile microscope slide, incubated at 26°C and morphological characteristics were observed periodically by light microscope. Molecular identification was conducted by analyzing the DNA sequences of the ITS1–5.8S–ITS2, ITS regions of their rRNA gene (Hamelin et al., 1996). Genomic DNA isolation, amplification and sequencing of the internal transcribed spacer (ITS) region and partial 28S rDNA gene (LSU) were done following the protocol described by White et al. (1990). Analyses of sequences were performed with the basic sequence alignment BLAST program run against the database (National Center for Biotechnology Information website [http://www.ncbi.nlm.nih.gov]). The ITS sequences obtained in this study were submitted to the GenBank (see accession numbers in Table 2).

Extraction and isolation of crude ethyl acetate extracts from fungus fermentation broths

Each of the isolated fungus was grown on PDA at 28°C for 5 days. Three pieces (0.5 × 0.5 cm^2) of mycelial agar plugs were inoculated into 500 ml Erlenmeyer flasks containing 300 ml potato dextrose broth (PDB) and incubated at room temperature for four weeks under stationary conditions. The broth culture was filtered through cheesecloth to separate the filtrate and mycelia. The filtrate (1,000 ml) was extracted three times by shaking with an equal volume of ethyl acetate (EtOAc). Collection and evaporation of the extracts under reduced pressure were used for enzyme inhibitor and cytotoxic activities test.

Topoisomerase I inhibition activities assay

Camptothecin (CPT) (Sigma) 10 mM and crude ethyl acetate extracts 10 mg l^{-1} was dissolved in dimethyl sulfoxide (DMSO) at 10 mM concentration and stored at -20°C. In a total volume of 20 µl hTopoI reaction system[containing 35 Mm Tris–HCl (pH 7.5), 50 mM KCl, 5 mM MgCl2, 1 mM DTT, 2 mMspermidine, 0.1 mM EDTA, 50 mg l^{-1} BSA], 4 µl hTopoI reaction buffer (5×) [175 mM Tris–HCl (pH 7.5), 250 mM KCl, 25 mM MgCl2, 5 mM DTT, 10 mM spermidine, 0.5 mM EDTA, 250 mg l^{-1} BSA], 0.5 µg supercoiled pBR322, 1 U of the recombinant enzyme and appropriate concentrations of CPT or crude ethyl acetate extracts were included. The reaction was carried out at 37°C for 30 min and then terminated by adding 4 µl hTopoI stop buffer (6×)[3% SDS, 60 mM EDTA, 50% glycerol, 0.25% bromphenol blue]. Samples were electrophoresed in 1% agarose gel in TBE (0.5×) at 1.5 - 4 Vcm^{-1} for 2 - 8 h at room temperature or 4°C. The gels were stained with ethidium bromide (5 mg l^{-1}) for 30 min, destained in distilled water (dH2O) for 30 min and then photographed under UV illumination with Fluor-S multimager (Bio-Rad) (Yang et al., 2004).

Cytotoxicity assay

Cytotoxicity assay were maintained in RPMI 1640 medium containing 100 U ml^{-1} penicillin, 100 g ml^{-1} streptomycin and 10% fetal bovine serum (FBS). All cells were grown in a humidified atmosphere incubator of 5% CO2 and 95% air at 37°C (Abe et al., 1998; Mosmann, 1983).

Cells were harvested and seeded in 96-well plates at 3.0 × 103 $well^{-1}$ for KB and KBv200 in a final volume of 190 µl. After 24 h incubation, 10 µl crude ethyl acetate extracts from different concentration of fermentation broths of fungi were added to each well. After 68 h, 10 µl MTT solution was added to each well followed by DMSO (100 µl) 4 h later. The concentrations of crude ethyl acetate extracts required to inhibit KB or KBv200 cells growth by 50% (IC50) were calculated from the cytotoxicity curves using Bliss's software.

RESULTS

Isolation and identification of endophytic fungi

In total, 54 out of 209 endophytic fungi were evaluated due to their unique morphological characteristics. Among the 54 isolated, 14 were isolated from *S. caseolaris* , six from *B. fern* , two from *P. indica*, eight from *B. gymnorrhiza*, two from *S. apetala*, four from *K. candel*, twelve from *Aegiceras corniculatum* and six from *H. littoralis* (Table 1). Morphological and molecular evidence showed 54 isolates were from 20 taxa (Table 2), nine isolates displayed both strong enzyme inhibitory activity and strong cytotoxicity against KB and KBv200 cells belong to seven genera, *Curvularia*, *Fusarium, Cladosporium, Alternaria*, *Gloeosporium, Diaporthe, Talaromyces.* It maybe indicates the diversity of endophytic fungi associated with mangrove plants.

Screening of endophytic fungi for topoisomerase I inhibitory activities

Ethyl acetate extracts from the fermentation broths of the 54 isolates of fungi were tested for topoisomerase I inhibitory activity. The results were showed in Figure 1 (a - h) and Table 1 showed the number and percentage of the active isolates. Nineteen extracts displayed strong topoisomerase I inhibitory activity, thirteen extracts showed weak topoisomerase I inhibitory activity and the other 22 showed very little or no inhibition (Table 1).

Determination of the IC50 of fungal crude extract against KB and KBv200 cells

KB and KBv200 are human epidermoid carcinoma cell lines. KBv200 cells, a classic multidrug resistant cell line expressing high levels of P- gp, were cloned from drug-sensitive parental KB cells by stepwise exposure to increasing doses of vincristine (VCR) and ethylmethane sulfonate (EMS) mutagenesis. Compared to KB cell line,

Table 1. Symbiotic fungi isolated from each mangrove plant.

	Host plant								
	Sonneratia caseolaris	***Brine fern***	***Bruguiera gymnorrhiza***	***Pluchea indica***	***Sonneratia apetala***	***Kandelia candel***	***Aegiceras corniculatum***	***Heritiera littoralis***	**Total**
No. of fungi isolated.	71	20	9	27	6	16	37	23	209
No. of fungi selected for screening.	14	6	2	8	2	4	12	6	54
No. of fungi which extract with enzyme inhibition activity.	2	5		5		1	1	5	19
Percentage of fungi which extract with enzyme inhibition activity.	14.3	83.3	0	62.5	0	25.0	8.3	83.3	35.2

Table 2. *In vitro* antitumor activity of fementation extract of fungi on KB and KBv200 cells.

Host plant	**Strain no.**	**IC50 (μg ml^{-1})**		**GenBank accession no. of ITS sequence**	**Identification result (species)**
		KB	**KBv200**		
Sonneratia caseolaris	ZH116	<<11	<<11	EU330614	*Fusarium* sp
	ZH117	18	24		*Trichoderma* sp
	ZH173	6	15		*Cephalosporium* sp
	ZH61	53	61		*Fusarium* sp
	ZH87	21	32		*Fusarium moniliform*
	ZH88	<<11	<<11		*Phomopsis* sp
	ZH91	11	19		*Penicillium* sp
	ZH178	35	49		*Aureobasidium sp*
	ZH57	11	23	EU330620	*Diaporthe* sp
	ZH4	36	55		*Penicillium sp*
	ZH62	24	31	EU330624	*Pleosporaceae* sp
	ZH111	176	205		*Ascomycota* sp
	ZH222	42	56		*Aureobasidium* sp
	ZH75	<<11	<<11	EU330625	*Chaetomium* sp
Brine fern	ZH71	<<11	<<11		*Fusarium* sp
	ZH55	32	38		*Cladosporium* sp
	ZH30	<<11	<<11		*Gloeosporium* sp
	ZH218	31	48		*Phomopsis* sp
	ZH154	<<11	<<11		*Talaromyces* sp
	ZH11	14	27		*Alternaria* sp
Bruguiera gymnorrhiza	ZH45	81	98		*Hypocreales* sp
	ZH15	<<11	<<11		*Fusarium* sp

Table 2. Contd.

	ZH50	39	52		*Ascomycota* sp
	ZH51	46	61		*Stemphylium* sp
	ZH68	8	12	EU330615	*Phomopsis* sp
	ZH164	22	25		*Phomopsis* sp
	ZH58	<<11	<<11		*Alternaria* sp
	ZH44	<<11	<<11		*Diaporthe* sp
Pluchea indica	ZH79	88	100	EU330617	*Phomopsis* sp
	ZH177	<<11	<<11	EU330636	*Cytospora* sp
Sonneratia apetala	ZH80	19	22	EU330629	*Fusarium* sp
	ZH131	15	23	EU330634	*Diaporthe* sp
Kandelia candel	ZH9	38	33		*Phomopsis* sp
	ZH82	<<11	<<11		*Trichoderma* sp
	ZH83	112	133		*Penicillium* sp
	ZH151	<<11	<<11		*Cladosporiu* sp
Aegiceras corniculatum	ZH63	52	34	EU330622	*Ascomycete* sp
	ZH64	<<11	<<11	EU330633	*Ascomycete* sp
	ZH93	49	31		*Alternaria* sp
	ZH22	63	51	EU330626	*Diaporthe* sp
	ZH65	33	23	EU330628	*Botryosphaeria* sp
	ZH97	<<11	<<11		*Cephalosporium* sp
	ZH81	42	42		*Diaporthe* sp
	ZH135	<<11	<<11		*Aspergillus* sp
	ZH208	222	205		*Cytospora* sp
	ZH209	201	178		*Trichoderm* sp
	ZH10	65	52		*Aspergillus* sp
	ZH14	35	27		*Penicillium* sp
Heritiera littoralis	ZH73	456	401		*Phomopsis* sp
	ZH74	22	14	EU330623	*Fusarium* sp
	ZH228	<<11	<<11		*Curvularia* sp
	ZH34	27	17	EU330616	*Phomopsis* sp
	ZH72	80	31	EU330621	*Pestalotiopsis* sp
	ZH182	79	66		*Colletotrichum* sp

KBv200 cell line was resistant to VCR about 100-fold. KB and KBv200 cells, obtained from Chinese Academy of Medical Sciences (Beijing, China). Antitumor activities against KB and KBv200 cells of the 54 ethyl acetate extracts were determined (Table 2). Extracts from 16 isolates (ZH116, ZH64, ZH71, ZH75, ZH82, ZH15, ZH30, ZH44, ZH58, ZH88, ZH97, ZH135, ZH151, ZH154, ZH177 and ZH228) showed significant cytotoxicities (IC50 < 11 μg ml^{-1}, crude extract concentration is 20 mg ml^{-1}). Extracts from nine isolates (ZH228, ZH116, ZH151, ZH58, ZH30, ZH44, ZH71, ZH15 and ZH154) exhibited both good topoisomerase I inhibitory activity and antitumor activity against KB cells, suggesting that they may be potential producers of antitumor compounds targeting at human DNA topoisomerase I (Yang et al., 2004). Extracts from ZH64, ZH75, ZH82, ZH88, ZH97, ZH135 and ZH177 displayed weak enzyme inhibitory activity, however, with strong cytotoxicity against KB and KBv200 cells, indicating that their antitumor activity is associated with other different site-specific targets. The isolation of bioactive

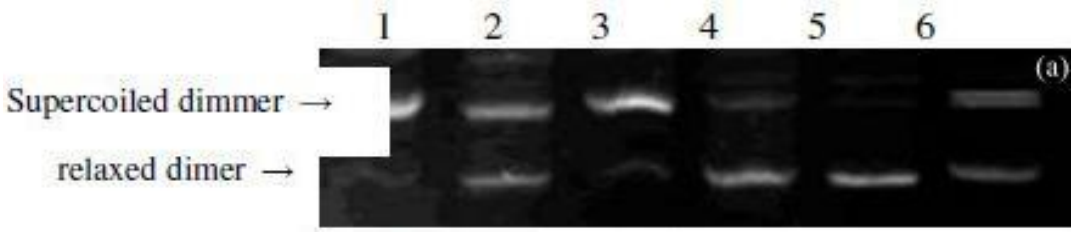
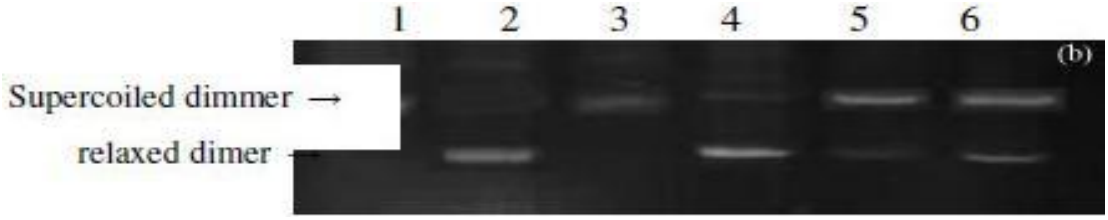
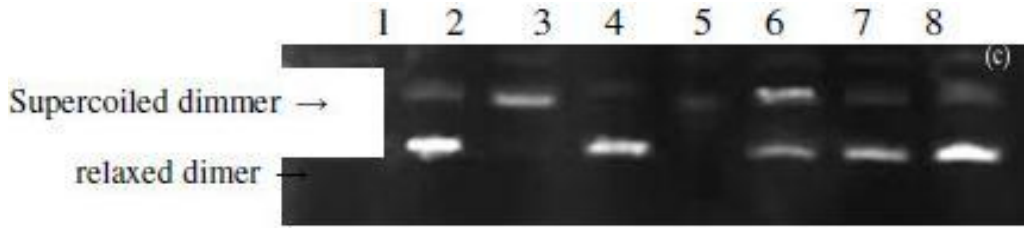


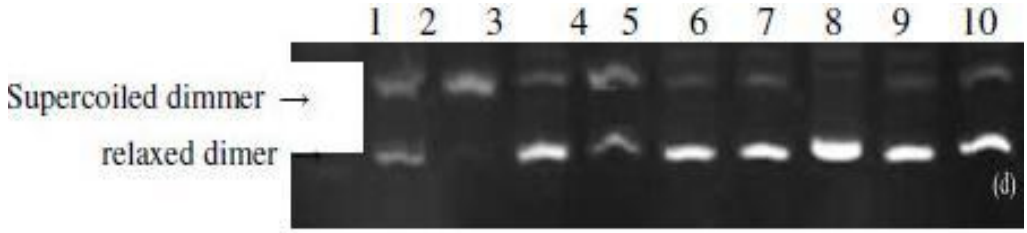
Figure1 (a-h). Topoisomerase I-mediated DNA plasmid relaxation in presence of fermentation crude extract of fungi isolated from mangrove plant. **(a)** Topoisomerase I-mediated DNA plasmid relaxation in presence of fermentation crude extract of fungi isolated from *Pluchea indica* and camptothecin. Lane 4, blank control (20 L hTopo I reaction system only with 1.5 g of supercoiled pBR322); lane 1, negative control (same as lane 4, but with hTopo I); Lane 2, positive control (same as lane 4, but with camptothecin; lane 3, solvent control (same as lane 4, with DMSO); Lane 5 and 6, same as lane 4, but with fermentation crude extract of ZH79 and ZH177, respectively.



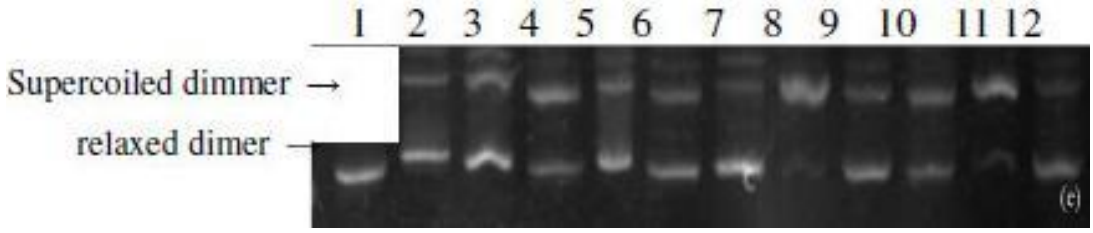
(b) Topoisomerase I-mediated DNA plasmid relaxation in presence of fermentation crude extract of fungi isolated from *Sonneratia apetala* and camptothecin. Lane 4, blank control; Lane 1, negative control; lane 3, solvent control; Lane 5 and 6, same as lane 4, but with fermentation crude extract of ZH80 and ZH131 respectively.



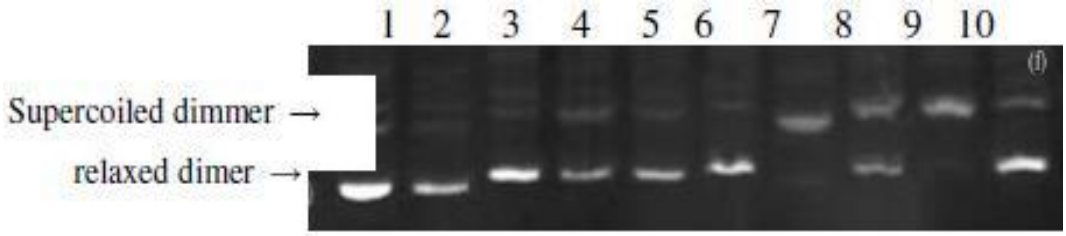
(c) Topoisomerase I-mediated DNA plasmid relaxation in presence of fementation crude extract of fungi isolated from *Kandelia candel* and camptothecin. lane 4, blank control; Lane 1, negative control; Lane 2,positive control; lane 3, solvent control; Lane 5 and 8, same as lane 4, but with fementation crude extract of ZH9, ZH82, ZH83 and ZH151, respectively.



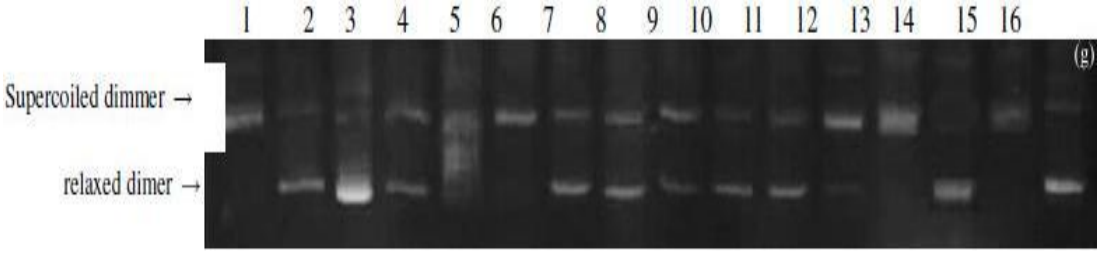
(d) Topoisomerase I-mediated DNA plasmid relaxation in presence of fermentation crude extract of fungi isolated from *Heritiera littoralis* and camptothecin. Lane 4, blank control; Lane 1, negative control; Lane 2,positive control; lane 3, solvent control; Lane 5 and 10, same as lane 4, but with fermentation crude extract of ZH73, ZH74, ZH228, ZH34, ZH72 and ZH182, respectively.



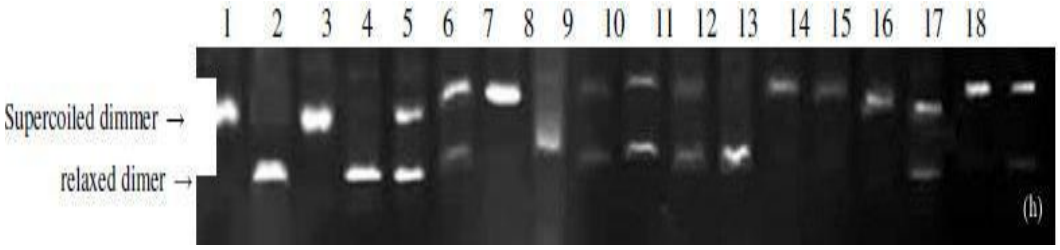
(e) Topoisomerase I-mediated DNA plasmid relaxation in presence of fermentation crude extract of fungi isolated from *Bruguiera gymnorrhiza* and camptothecin. Lane 12, blank control; Lane 8, negative control; Lane 10, positive control; lane 11, solvent control; Lane 1to 7 and 9, same as lane 12, but with fementation crude extract of ZH45, ZH15, ZH50, ZH51, ZH68, ZH164, ZH58 and ZH44, respectively.



(f) Topoisomerase I-mediated DNA plasmid relaxation in presence of fementation crude extract of fungi isolated from *Brine fern* and camptothecin. Lane 10, blank control; Lane 7, negative control; Lane 8, positive control; lane 9, solvent control; Lane 1to 6, same as lane 10, but with fementation crude extract of ZH71, ZH55, ZH30, ZH218, ZH154 and ZH11, respectively.



(g) Topoisomerase I-mediated DNA plasmid relaxation in presence of fermentation crude extract of fungi isolated from *Aegiceras corniculatua* and camptothecin. Lane 16, blank control; .Lane 13, negative control; Lane 14, positive control; lane 15, solvent control; Lane 1 to 12, same as lane 16, but with fementation crude extract of ZH63, ZH64, ZH93, ZH2, ZH65, ZH97, ZH81, ZH135, ZH208, ZH209, ZH10 and ZH14, respectively.



(h) Topoisomerase I-mediated DNA plasmid relaxation in presence of fermentation crude extract of fungi isolated from *Sonneratia caseolaris* and camptothecin. Lane 4, blank control; .Lane 1, negative control; Lane 2, positive control; lane 3, solvent control; Lane 5 to 18, same as lane 4, but with fementation crude extract of ZH116, ZH117, ZH173, ZH61, ZH87, ZH88, ZH91, ZH178, ZH57, ZH4, ZH62, ZH111, ZH222 and ZH75, respectively.

compounds from those promising fungal isolates will be assessed.

DISCUSSION

54 isolates derived from nine mangrove plants were identified from 20 taxa according to morphological and molecular evidence and top nine biologically active isolates belong to seven genera, may be indicate the diversity of fungi in mangrove plants. Nineteen extracts (35.2%) displayed strong topoisomerase I inhibitory activity, five from *B. fern* (83.3%), five from *B. gymnorrhiza* (62.5%) and five from *H. littoralis* (83.3%) (Table 1), indicating that fungi isolated from these three mangrove plants are a good source of natural antitumor compounds. It is speculated that biological activity of the fungi extracts is associated with the endogenous environment of the plant (Engel et al., 2002).

It is observed that the existing anticancer drugs have a limited selectivity and are highly toxic. Researchers in the molecular and cellular biology are regularly identifying novel potential targets, which are specific or selective for cancer cell (Kohn, 1996). DNA is the molecular target for many of the drugs that are used in cancer therapeutics (Hurley, 2002). DNA topoisomerases play important roles in cellular processes such as DNA replication, transcription, recombination, chromosome assembly and segregation by regulating the topological state of DNA (Nitiss, 1998; Wang, 1996). The enzymes can be divided into two types, type I and type II. Eukaryotic DNA topoisomerase I and II have been identified to be the targets of many effective antitumor drugs. For example, camptothecin and its derivative topotecan and irinotecan target at the eukaryotic DNA topoisomerase I, while m-AMSA, etoposide (VP-16), VM-26 and Adriamycin display strong antineoplastic activity mediated by mammalian DNA topoisomerase II (Liu, 1989; Pommier, 1998). Therefore, the present study evaluated the effect of the extracts of a diversity of endophytic fungal extracts isolated from mangrove plants on the cancer cells as well as their effects on topoisomerases to know their mechanism of action of cytotoxicity.

In this study, extracts from nine isolates showed strong inhibition of topo I enzyme and cytotoxicity indicating their potentials of producing natural antitumor compounds targeting at the eukaryotic DNA topoisomerase I. A new hTopo I isomerase inhibitor, (+)-3,3',7,7',8,8'-hexahydroxy-5,5'- dimethylbianthraquinone (2240A), was recently isolated by our research group from the mangrove endophytic fungus No. 2240 (Tan N et al., 2008). Six active compounds, vermistatin and methoxvermistatin, tenellic acid A and alternin, lichexanthone and bikaverin, were isolated from the culture of ZH44, ZH15 and ZH151 separately. These compounds had also been isolated from other fungus strains in our group and published (Xia et al., 2007; Cai et al., 2008). Likewise, further studies of the bioactive compounds of these promising active isolates will be performed in the future. Extracts of seven isolates displayed weak enzyme inhibitory activity but strong cytotoxicity against KB and KBv200 cells suggesting that they might be good candidates for producers of antitumor compounds.

ACKNOWLEDGEMENTS

This work was supported by the 863 Fund of China (2006AA09Z422, 2006AA09Z419), the National Natural Science Fund of China (No. 20572136) and the Natural Science Fund of Guangdong Province (No. 05003268).

REFERENCES

Abe K, Saito H (1998). Amyloid β protein inhibits cellular MTT reduction not by suppression of mitochondrial succinate dehydrogenase but by acceleration of MTT formazan exocytosis in cultured rat cortical astrocytes. Neurosci. Res. 31: 295-305.

Banerjee S, Wang Z, Mohammad M, Sarkar F, Mohammad RM (2008). Efficacy of selected natural products as therapeutic agents against cancer. J. Nat. Prod. 71: 492-496.

Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR (2008). Marine natural products. Nat. Prod. Rep. 25: 35-94.

Cao L, Qiu Z, You J, Tan H, Zhou S (2004). Isolation and characterization of endophytic Streptomyces strains from surface-sterilized tomato (*Lycopersicon esculentum*) roots. Lett. Appl. Microbiol. 39: 425-430.

Cai XL, Gao JP, Li Q, Wen L, She ZG, Lin YC (2008). Cytotoxicity of the Secondary Metabolites of Marine Mangrove Fungus *Paecilomyces* sp. Tree 1-7 on Human Hepatoma Cell Line HepG2. J. Chin. Med. Mater. 31(6): 864-865.

Engel S, Jensen PR, Fenical W (2002). Chemical ecology of marine microbial defense. J. Chem. Ecol. 28: 1971-1985.

Gordaliza M (2007). Natural products as leads to anticancer drugs. Clin. Transl. Oncol. 9: 767-776.

Hamelin RC, Berube P, Gignac M, Bourassa M (1996). Identification of root rot fungi in nursery seedlings by nested multiplex PCR. Appl. Environ. Microbiol. 62: 4026-4031.

Hurley LH (2002). DNA and associated processes as targets for cancer therapy. Nat. Rev. Cancer. 2: 188-200.

Kohn KW (1996). DNA filter elution: A window on DNA damage in mammalian cells. Bioessays. 18: 505-513.

Liu LF (1989). DNA topoisomerase poisons as antitumor drugs. Annu. Rev. Biochem. 58: 351–375.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65: 55 – 63.

Nitiss JL (1998). Investigating the biological functions of DNA topoisomerases in eukaryotic cells. Biochim. Biophys. Acta. 1400: 63–81.

Pan JH, Jones EBG, She ZG, Pang JY, Lin YC (2008). Review of bioactive compounds from fungi in the South China Sea. Bot. Mar. 51: 179-190.

Pommier Y (1998). Diversity of DNA topoisomerases I and inhibitors. Biochimie 80: 255–270.

Schulz B, Wanke V, Draeger S, Aust HJ (1993). Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycolo. Res. 97: 1447-1450.

Tan N, Cai XL, Wang SY, Pan JH, Tao YW, She ZG, Zhou SN, Lin YC, Vrijmoed LLP (2008). A new hTopo I isomerase inhibitor produced by a mangrove endophytic fungus no. 2240. J. Asian Nat. Prod. Res. 10: 607-710.

Wang JC (1996). DNA topoisomerases. Annu. Rev. Biochem. 65: 635–692.

White TF, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocol: A Guide to Methods and Applications (Innis MA, Gelfand DH, Sninsky FS and White TT, eds). Academic Press, San Diego pp. 315–322.

Xia XK, Huang HR, She ZG, Cai JW, Liu L, Zhang JY, Fu LW, Vrijmoed LLP, Lin YC (2007). Structural and Biological properties of Vermistatin and Two New Vermistatin Derivatives Isolated from the Marine Mangrove Endophytic fungus *Guignardia* sp. NO. 4382. Helvetica Chimica Acta 90: 1925-1931.

Yang GW, Zhou HQ, Lu YJ, Lin YC, Zhou SN (2004). Comparing expression of different forms of human DNA topoisomerase I in *Pichia pastoris*. Enzyme Microb. Tech. 34: 139–146.