ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 8 (4): 405-415, 2009
ISSN 1682-296X
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Evaluation for the Production of Antialgal Substances from *Streptomyces neyagawaensis*

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Abstract: Optimization of *S. neyagawaensis* N_{60} (Egyptian isolate) for the production of natural antialgal substance was carried out. The Clear Inhibition Zone (CIZ) in the different algal species indicated the maximum biological activity of metabolite was attained at 6 g L^{-1} maltose 1.05 g L^{-1} NH_4Cl and 1 g L^{-1} K_2HPO_4 under pH 6.5, temperature 28°C and incubation period 7 days. For microanalysis, xylene was the most efficient solvent for extraction of the lytic substance which has one spot under UV lamps at RF 0.65 using TLC. Identification of the antialgal substance produced by *S. neyagawaensis* was carried out on the basis of elementary analysis, IR, mass and NMR spectra. The earlier analysis emphasized that the molecular weight equal 369.45 kDa with chemical formula $C_{19}H_{21}NO_6$ (Anthracidin A). Different concentrations of Anthracidin A were tested against *Anacystis nidulans* revealed that chlorophyll a, nucleic acids were reduced with increasing the concentration of Anthracidin to 40 μg mL^{-1}.

Key words: Anthracidin A, antialgal, *Streptomyces*, *Anabaena*, *Nostoc*, algicidal

INTRODUCTION

Streptomycetes especially the genus *Streptomyces* were very potent producers of secondary metabolites including antibacterial enzymes and toxins (Chater and Bibb, 1997; Habib *et al*., 2001; Kokare *et al*., 2004; Choi *et al*., 2005; El-Shirbiny *et al*., 2007; Mu *et al*., 2007; Kang *et al*., 2008; Ren *et al*., 2009). About of 10.000 known antibiotics, 45-55% was produced by *Streptomyces* (Demain, 1999; Lazzarini *et al*., 2000).

Various actinomycetes were found to possess strong algicidal properties. Such forms have been found extensively in cultures isolated from muds and other natural products (Safferman and Morris, 1962, 1963; Amaro *et al*., 2005; Su *et al*., 2007; Roth *et al*., 2008). It has been reported that chloramphenicol, produced by *Streptomyces venzuelae* inhibits both chloroplast and protein synthesis in Euglena (Jacobson *et al*., 1964; Pogo and Pogo, 1965). Rifamycin, produced by *Streptomyces mediterranei* has been reported to inhibit the *Euglena*, the chloroplast target of rifamycin in *Euglena* chloroplasts is the DNA dependent RNA-polymerase that blocks its activity and inhibits RNA synthesis (Rodrigues *et al*., 1974). A *Streptomyces* species capable of lysing both *Anabaena cylindrica* and *Tolypothrix tenuis* and this lysis was attributed to interference of the antibiotic with cell wall function and/or other structural components (Whyte *et al*., 1985). Streptomyces cellulose enzyme system releasing glucose from walls of *Chlamydomonas reinhardtii* and *Ulorhrix fimrata* and this preparation also converted the algal cells to spheroplasts and this lytic appearance was due to streptomycetes lysozymes (Gunnison and Alexander, 1975).

Yamamoto *et al*. (1998) showed that *Streptomyces phaeofaciens* produced compound causing extensive lysis of mycrocystis cells.

Blooms of cyanobacteria (as *Microcystis*, *Anabaena*, *Aphanizomenon*, *Nidularia*) can cause significant public health problems, such poisonings of farm animals, wildlife and adverse health effects in humans (Oliver, 1994; Zingone and Enevoldsen, 2000; Jeong *et al*., 2005; Kim *et al*., 2009). Natural blooms of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* produce hepatotoxins, neurotoxins and other potential inhibitors (Sasner *et al*., 1994). *Microcystis aeruginosa* is widespread in eutrophic lakes and reservoirs throughout the world (Carmichael, 1992; Han *et al*., 2002; Hong *et al*., 2002; Ahn *et al*., 2003; Choi *et al*., 2005; Mu *et al*., 2007) and may lead to the production of microcystin, a hepatotoxins that affects fish, birds, wild animals, livestock and humans. Also it is associated with allergies, irritation reactions, gastroenteritis, liver diseases and tumors (An and Carmichael, 1994; Bell and Codd, 1994; Harada, 1995; Dawson, 1998; Nagayama *et al*., 2003). *Oscillatoria rubescens* and *Oscillatoria agardhi* complex produce hepatotoxins (Carpenter and Carmichael, 1995) and may be responsible for dermatitis or skin irritation

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when people come in contact with polluted water. Also, algal blooms on our lakes and water resources are mostly from cyanobacterial groups that caused other problems, such as nuisance foul odors, decreased aesthetic value, taste and odor in water supply and depletion of dissolved oxygen of water (American Public Health Association, 1992; Carmichael, 1997; Sigee *et al*., 1999; Kim *et al*., 2009).

The present study aims to evaluate the biological activity some actinomycetes and their metabolites to overcome the trouble shouting produced from the growth of different algal species.

MATERIALS AND METHODS

Strains and media: Isolate No. 60 was isolated on starch-nitrate medium from cultivated soil according to Waksman (1959). Following the diagnostic key of Bergey's Manual (William and Felscher, 1989) and surveying the literatures on the description of Streptomyces species and isolate No. 60 was identified belonging to *Streptomyces neyagawaensis* (Pridham *et al*., 1958).

Detection of algal lytic actinomycetes: The experimental organisms was streaked on agar plates and incubated for 8 days at 28°C. After complete growth of *S. neyagawaensis* No. 60, the solid medium was cut into discs (5 mm) using cork borer and placed on watanabe medium preinculated with cyanobacteria (*Anabaena* sp. *Anabaena flos-aqueae* and *Nostoc* sp.) according to Yamamoto (1978). After the elapse of incubation period, the Clear Inhibition Zones (CIZ) induced by *S. neyagawaensis* No. 60 were measured. All experiments were carried out in phycology and bacteriology labs of botany department, faculty of science, Zagazig University during 2005.

Biological control for *Anacystis nidulans*: With respect to the growth conditions of *Anacystis nidulans*, 0.1 mL of *S. neyagawaensis* No. 60 suspension was seeded to the nutritive liquid medium of BGII, the initial optical density of algal medium at zero time was calculated at 665 nm. Then all the flasks were incubated for 2 weeks under normal growth conditions. The optical density (growth rate) was calculated every 2 days (Uchida *et al*., 1998).

Determination of chlorophyll "a": The technique applied was described by Richards and Thompson (1952).

Nucleic acid analysis: The quantitative determination of RNA was carried out according to the method of Ashwell (1957) while DNA determination was carried on according to Burton (1968).

Extraction and purification of antialgal substance: The optimum medium for the maximum production of the antialgal substance that contained the best carbon, nitrogen, phosphorus, microelement, vitamin sources with optimum pH value was prepared, autoclaved and inoculated with the experimental organism and incubated on a rotary shaker (200 rmp) for 7 days (optimum incubation period) at 37°C. the active ingredient substances for the best medium were of (g L^{-1}) maltose, 6 g, NH_4Cl, 1.05 g, K_2HPO_4 1.0 g; $MgSO_4$. $7H_2O$, 0.5 g; NaCl, 0.5; $CaCO_3$, 3 g; $FeSO_4$. $5H_2O$, 0.01 g; vitamin B_{10}, 10 µg and distilled water up to 1000 mL at the end of incubation period, the cultured broth (5 L) was centrifuge and the resulting supernatant was dialyzed against hypertonic sugar solution (cellulose lag method). Subsequently, the resulting residue was mixed with xylene (3 times). The organic layers were combined and concentrated under vacuum to about 5 mL using a rotary evaporated. To the concentrated layer, a non-polar organic solvent (petroleum either 40-60) was added drop by drop until reddish crystalline substance appeared. The solid fraction was washed with ether and then dried in air. Continuous purification of substance using TLC (silica gel G54) was carried out. The major spots appeared at $R_f = 0.65$ was gathered and eluted with petroleum ether (40-60). The partially purified fractions were microanalyzed using IR, Mass and NMR spectra and elementary analysis for complete identification of the antialgal substance.

RESULTS

Antimicrobial activity of *S. neyagawaensis* No. 60 against tested cyanobacteria: The production of antibiotic substances by streeptomycetes was found to be largely influenced both qualitatively and quantitatively by the type of test organism (Waksman, 1961; Hussien *et al*., 1998; Salama *et al*., 1980; Mohamadin, 1987). The data recorded in Table 1 and Fig. 1a-c showed that the highly sensitive algal species to the block agar born streptomyces was detected against *Anabaena* sp. which gave a clear inhibition zone (30 mm) followed by *Sphaeronostoc microscopica* (25 mm inhibition zone) and *Nostoc* sp. (24 mm). In a similar manner results in Table 2 showed chlorosis and complete lysis of *Syncoccus* sp. and *Anacystis nidulans* cells when treated with 1 mL spore suspension of *Streptomyces neyagawaensis* No. 60. The maximum drop in algal growth is indicated after 10 days, with percentage of inhibition for both tested alga 52.5 and 82.4%, respectively. These results are in accordance with the results obtained by El-Sherbiny *et al.* (2007).

(a) (b) (c)

Fig. 1: Inhibition zone (mm) of *Streptomyces neyagawaensis* N40 against tested cyanobacteria, (a) *Nostoc* sp. (b), *Anahaena* sp. and (c) *Anabaena flos-aqueae*

Table 1: Clear inhibition zones of *S. neyagawaensis* No. 60 against tested Cyanobacteria

Tested algae	Inhibition zone (mm) (Diameters of disk = 5 mm)
Nostoc pisanale	22
Anabaena sp.	30
Anabaena flos-aquae	22
Sphaeronostoc microscopica	25
Nostoc sp.	25

Table 2: Antialgal activity induced by *S. neyagawaensis* No. 60 against tested cyanobacteria in liquid media (inhibition expressed as a reduction in optical density at 665 nm)

	Absorbance reading at 665					
Samples	Zero time	2nd day	4th day	6th day	8th day	10th day
Untreated alga*	0.11	0.13	0.15	0.17	0.19	0.40
Treated alga**	0.11	0.14	0.15	0.16	0.23	0.19
Untreated alga***	0.12	0.13	0.17	0.34	0.37	0.85
Treated alga****	0.12	0.13	0.14	0.19	0.16	0.15

*Untreated alga: *Syncoccus* sp., **Treated alga: Syncoccus + *S. neyagawaensis* No. 60. ***Untreated alga : *Anacystis nidulans*, ****Treated alga: *Anacystis nidulans* + *S. neyagawaensis* No. 60

Biosynthesis requirements for production of antialgal substance by *S. neyagawaensis* No. 60:

Earlier investigators have shown that the production of antibiotics is not a fixed property of organism as it is variably affected by environmental and nutritional factors (Ammar *et al*., 2003; Choi *et al*., 2005; Volka and Furkert, 2006). The data presented in Table 3-5 revealed that maximum production of the antialgal substance was attained at a growth pH 6.5 and 28°C for 7 days incubation on a rotary shaker operating at 200 rpm. The results in Table 6-10 showed that the highest production of lytic agent by *S. neyagawaensis* No. 60 was achieved using different carbon, nitrogen, phosphorous, microelement and vitamin sources. In conclusion the optimum medium was as follows (g L^{-1}): maltose, 6 g; NH_4Cl, 1.05 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; NaCl, 0.5 g; $CaCO_3$, 3 g; $FeSO_4.5H_2O$, 0.01 g; vitamin B_{10}, 10 µg and distilled water up to 1000 mL. these results are in agreement with the results obtained by Naki *et al*. (2000), Toshio *et al*. (2000), Yutaka *et al*. (2001), Gupte and Kalkarni (2001), Ammar *et al*. (2003), Ghaly *et al*. (2005), Abou El-Hawa *et al*. (2006) and El-Sherbiny *et al*. (2007).

Table 3: Effect of different incubation periods on the production of antialgal substance by *S. neyagawaensis* No. 60

	Inhibition zone (mm) broth extract		
Incubation period (day)	*Anabaena* sp.	*Anabaena flos-aquae*	*Nostoc* sp.
2	0	0	0
3	13	0	11
4	18	13	14
5	22	17	19
6	22	27	22
7	35	30	32
8	30	24	26
9	24	18	20
10	19	12	15
11	12	0	0
12	0	0	0

Table 4: Effect of different pH values on the production of antialgal substance by *S. neyagawaensis* No. 60

	Inhibition zone (mm) broth extract		
pH values	*Anabaena* sp.	*Anabaena flos-aquae*	*Nostoc* sp.
6.0	21	14	17
6.5	36	30	34
7.0	30	22	23
7.5	28	21	22
8.0	20	12	15

Table 5: Effect of different incubation temperature on the production of antialgal substance by *S. neyagawaensis* No. 60

	Inhibition zone (mm) broth extract		
Incubation temperature	*Anabaena* sp.	*Anabaena flos-aquae*	*Nostoc* sp.
15	20±0.10	14±0.06	18±0.15
20	30±0.15	24±0.12	27±0.08
25	32±0.00	26±0.10	29±0.11
28	37±0.07	32±0.00	35±0.00
30	36±0.11	32±0.00	34±0.13
35	33±0.00	28±0.15	30±0.14
40	27±0.30	21±0.17	25±0.00
45	No growth	0.00	0.00

Table 6: Effect of different carbon sources on the production of antialgal substance by *S. neyagawaensis* No. 60

Carbon sources	Inhibition zone (mm) broth extract		
	Anabaena sp.	*Anabaena flos-aquae*	*Nostoc* sp.
Starch	37±0.31	32±0.16	35±0.19
D-glucose	29±0.05	20±0.09	21±0.00
D-fructose	32±0.00	25±0.15	28±.31
D-galactose	22±0.14	18±0.13	19±0.02
Sucrose	22±0.21	13±0.00	15±0.14
Maltose	39±0.00	32±0.14	36±0.03
α-lactose	20±0.03	12±0.21	14±0.07

Table 7: Effect of different nitrogen sources on the production of antialgal substance by *S. neyagawaensis* No. 60

Nitrogen sources	Inhibition zone (mm) broth extract		
	Anabaena sp.	*Anabaena flos-aquae*	*Nostoc* sp.
KNO_3	39±0.05	32±0.10	36±0.11
$NaNO_3$	33±0.03	26±0.12	28±0.06
NH_4Cl	41±0.11	33±0.09	36±0.00
NH_4NO_3	30±0.03	22±0.11	25±0.12
$(NH_4)_2SO_4$	35±0.13	28±0.08	30±0.13
Peptone	36±0.11	30±0.03	34±0.11
Yeast extract	38±0.60	31±0.14	34±0.07
Beef extract	35±0.14	29±0.90	32±0.21
Asparagines	40±0.31	32±0.11	36±0.02

Table 8: Effect of different phosphorus sources on the production of antialgal substance by *S. neyagawaensis* No. 60

Phosphorus sources	Inhibition zone (mm) broth extract		
	Anabaena sp.	*Anabaena flos-aquae*	*Nostoc* sp.
K_2HPO_4	41	33	36
KH_2PO_4	36	30	32
Na_2HPO_4	38	31	33
NaH_2PO_4	33	28	3
$(NH_4)_2HPO_4$	40	32	35

Table 9: Effect of different microelements on the production of antialgal substance by *S. neyagawaensis* No. 60

Microelements	Inhibition zone (mm) broth extract		
	Anabaena sp.	*Anabaena flos-aquae*	*Nostoc* sp.
Control	41	33	36
$FeSO_4$	42	36	37
$CuSO_4$	38	33	34
$MnCl_2$	38	34	36
$NiCl_2$	34	29	30
$ZnSO_4$	40	35	36
Na_3BO_3	35	29	30
$Na_2WO_4.2H_2O$	30	25	26
$COCl_2$	37	32	33
$(NH_4)_6MO_7O_{24}$ $4H_2O$	32	27	29

Table 10: Effect of different vitamins on the production of antialgal substance by *S. neyagawaensis* No. 60

Vitamins	Inhibition zone (mm) broth extract		
	Anabaena sp.	*Anabaena flos-aquae*	*Nostoc* sp.
Vitamin C	42	36	37
Vitamin A	42	35	37
Vitamin B_2	37	31	33
Vitamin B_6	40	34	36
Vitamin B_{10}	38	31	34
Vitamin B_{12}	44	37	40
Vitamin B_{12}	43	36	38
Vitamin B-complex	44	36	39

Effect of different concentrations of antialgal substance produced by *S. neyagawaensis* No. 60 on chlorophyll "a" and nucleic acids content of Anacystis nidulans: Results in Table 11 revealed that, the content of chlorophyll "a" decreased with increasing the concentration of the antialgal substance of *S. neyagawaensis* No. 60. The maximum sharp decline in chlorophyll "a" content was obtained with a concentration of 40 µg mL^{-1}. The percentage of inhibition in chlorophyll "a" content was 74.3%, when compared with the corresponding control.

Results in Table 12 showed that, the content of nucleic acids decreased with increasing the concentration of lytic substance up to 40 µg mL^{-1}. The maximum percentage of inhibition in nucleic acid of *Anacystis nidulans* was 64.1% in DNA content and 45.1% in RNA content as compared with the concentration of the corresponding contents. These results are in agreement with the results of Rodrigues *et al*. (1974).

Extraction and purification of the antialgal substance: The experimental organism was cultured in the optimized liquid-shaken medium in 250 mL Erlenmeyer flask, each contain 50 mL. After autoclaving, each flask was inoculated with 1 mL of dense spore suspension of *S. neyagawaensis* under a septic and conditions. All flasks were incubated at 28°C for 7 days. After the expiry of the incubation period, the fermented media were collected and the biomass separated from the broth by filtration to obtain cell-free filtrate. Aliquot of broth was concentrated to about 500 mL by dialysis process (cellulose bag), the active substance was extracted by xylene at pH 7.0 (Ahmed *et al*., 2002; El-Shirbiny *et al*., 2007) and the organic layers were collected and concentrated under vacuum by using rotary evaporator till dryness. The obtained residual fraction was purified by using Thin Layer Chromatography (TLC), which

Table 11: Effect of *S. neyagawaensis* metabolites on chlorophyll "a" of Anacystis nidulans

Concentration of purified substance (µg mL^{-1})	Chlorophyll "a" (µg g^{-1} d.wt.)
0	41.65±3.10
10	38.08±1.50
20	21.42±1.15
30	16.66±1.00
40	10.71±2.10

Table 12: Effect of *S. neyagawaensis* metabolites on nucleic acid of Anacystis nidulans

Concentration of purified substance (µg mL^{-1})	DNA content (µg g^{-1} d.wt.)	RNA content (µg g^{-1} d.wt.)
0	0.935±0.03	0.510±0.04
10	0.868±0.02	0.434±0.02
20	0.632±0.00	0.338±0.05
30	0.452±0.10	0.326±0.04
40	0.336±0.05	0.280±0.00

manifested through ultraviolet lamp one spot at $R_f = 0.65$. The spots were collected by its elution and the physico-chemical characteristics, IR, Mass spectrum, NMR spectrum and elementary analysis were determined (Stefani and Agodi, 2000). These microanalyses are carried in microanalysis center in Cairo University.

Biological and physico-chemical Characteristics of the antialgal substance produced by *S. neyagawaensis* No. 60

Solubility: The lytic substance was readily soluble in acetone, diethyl ether, chloroform, benzene, petroleum ether, acetic acid and xylene; slightly soluble in water and ethyl acetate and insoluble in ethanol, methanol, n-butanol, n-hexane and isopropanol.

Melting point: The antialgal substance is liquid.

Elementary analysis: The antialgal substance extracted from *S. neyagawaensis* No. 60 was found to contain, carbon (C = 61.7%), hydrogen (H = 8.46%), nitrogen (N = 3.79%) and oxygen (O = 25.98%).

Infra-red spectrum (IR): Based the elementary analysis, the identification of the compounds were also confirmed by spectroscopic measurements. The infra-red spectrum of the present of the antialgal substance showed a free OH band in 3593 cm^{-1}; NH group in 3448 cm^{-1}; CH-aliphatic in 2923, 2866 cm^{-1}; CH-aromatic in 3025 cm^{-1}; keton group in 1703 cm^{-1}; aromatic ring in 1608, 1563 and 1494 cm^{-1} (Fig. 2).

Molecular weight: The FD-MS spectrum showed the molecular peak at m/z 369.45 (Fig. 3).

The molecular formula: The molecular formula was determined on the basis of the results obtained from the mass spectrometric and elementary analysis of the extracted compound as $C_{19}H_{21}NO_6$.

Nmr spectrum of the antibiotic: The ^{1}H-NMR of the antialgal substance produced by *S. neyagawaensis* No. 60 was investigated by Nuclear Magnetic Resonance (NMR). It could be deduced from (Fig. 4) that the antibiotic molecular is characterized by the following:

- Presence of four protons of the sugar molecules (multiplete at 0.87-1.31)
- Presence of four CH_3 protons (triplet at 2.23)
- Presence of aliphatic chain (sharp signlet at 3.36)
- Presence of aromatic ring (multiplete at 6.78-7.23)
- Presence of NH proton (signlet at 7.84)
- Presence of free OH proton (signlet at 14.85)

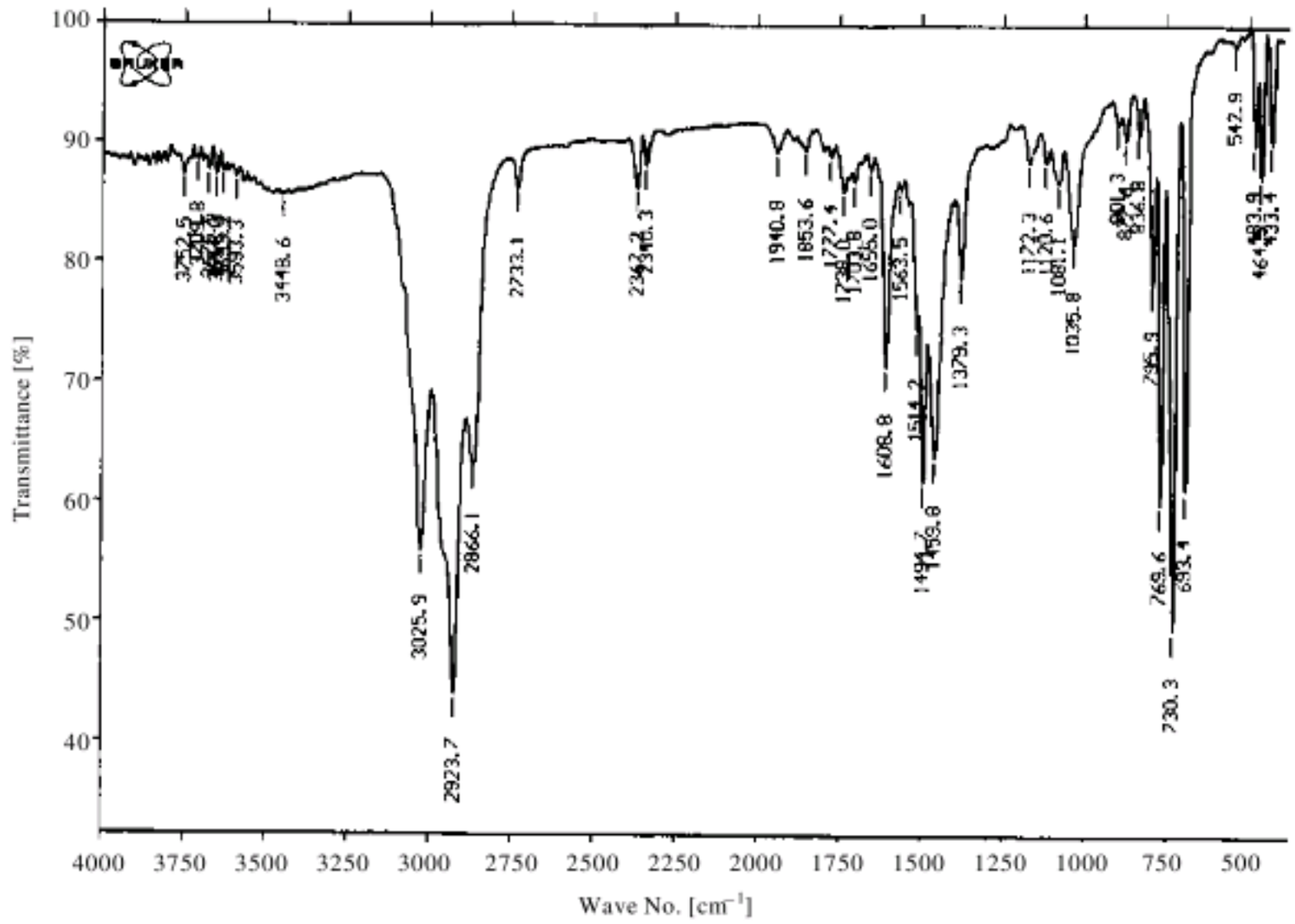


Fig. 2: Mass spectrum of *Streptomyces neyagawaensis* N60 metabolite

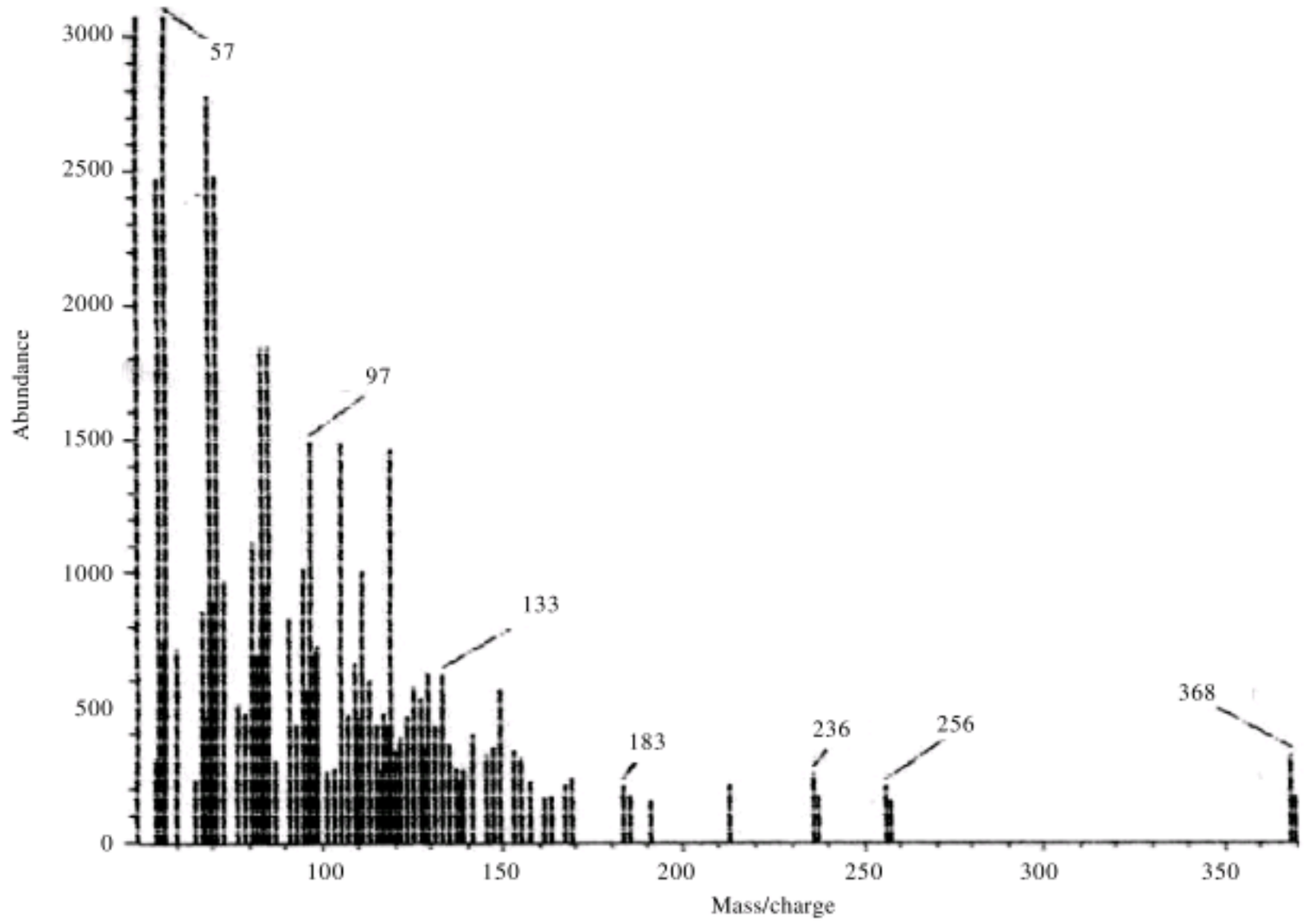


Fig. 3: Mass spectrum of *Streptomyces neyagawaensis* N60 metabolite

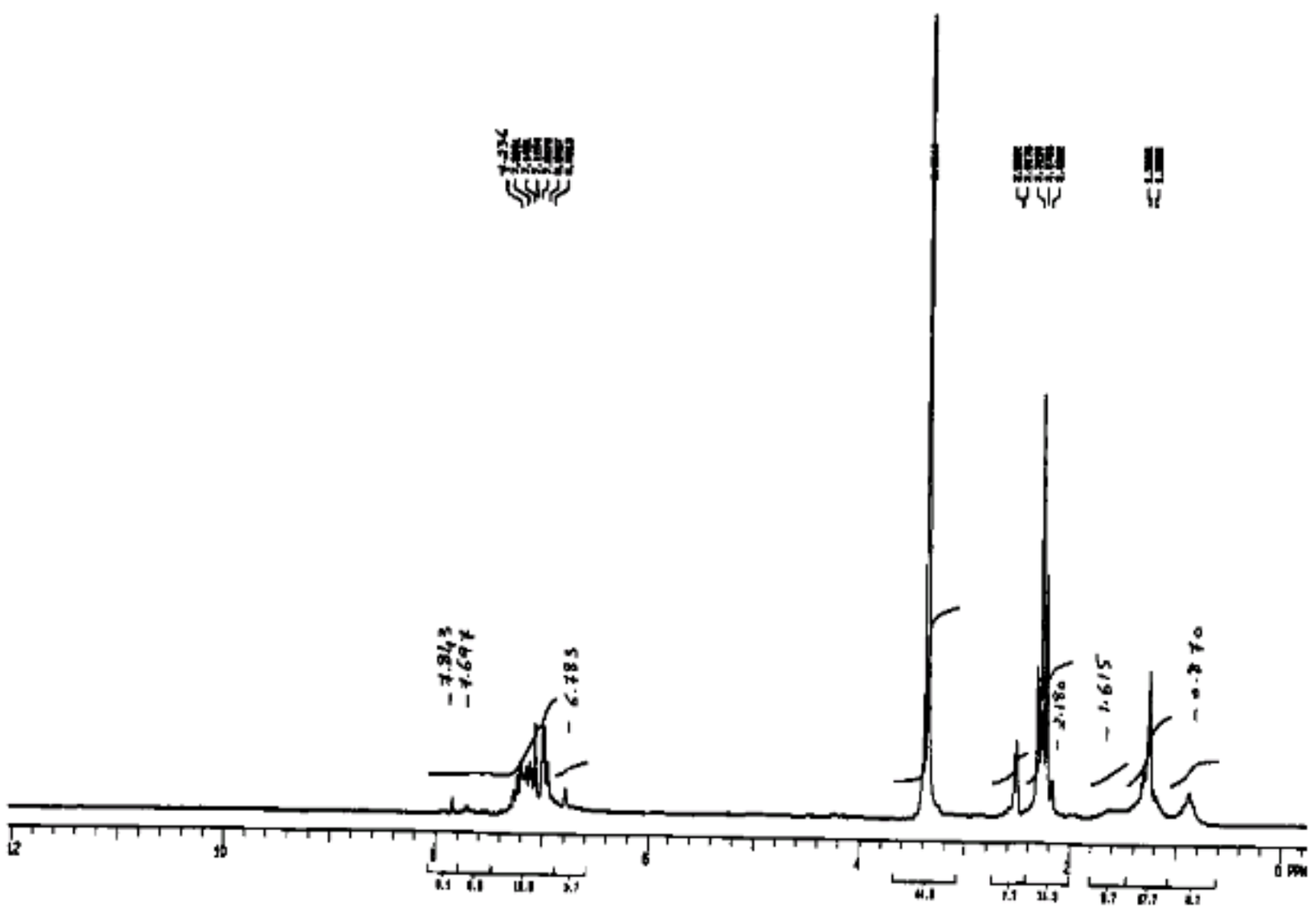


Fig. 4: NMR spectrum of *Streptomyces neyagawaensis* N60 metabolite

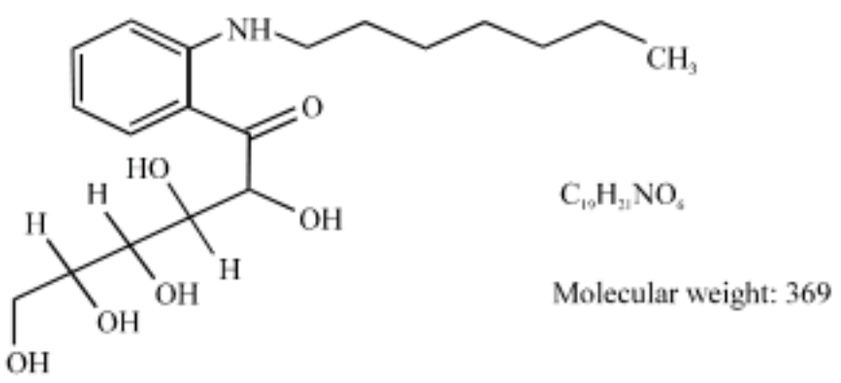
Table 13: A comparative study of the characteristic properties of antialgal substance extracted from *S. neyagawaensis* No. 60 in relation to reference antibiotic (Anthracidin A)

Chemical analysis	Antracidin A	Extracted substance
C	66.09	61.71
H	8.04	8.46
N	3.85	3.79
O	22.01	25.98
Molecular weight	363.44	369.00
Formula	$C_{20}H_{29}NO_5$	$C_{19}H_{31}NO_6$

Table 14: Effect of different concentration of Anthracidin A of *S. neyagawaensis* No. 60 on the growth of *Anacystis nidulans* (unicellular alga)

Concentration of anthracidin A ($\mu g\ mL^{-1}$)	Absorbing reading (mm)								
	Zero time	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day
Control	0.05	0.06	0.11	0.12	0.16	0.14	0.23	0.46	0.80
10	0.13	0.60	1.10	0.46	0.39	0.30	0.26	0.40	0.75
20	0.08	0.60	0.90	0.42	0.32	0.22	0.13	0.31	0.42
30	0.06	0.56	0.66	0.32	0.20	0.15	0.12	0.27	0.37
40	0.04	0.24	0.29	0.22	0.18	0.12	0.10	0.23	0.36

Taking into consideration, the elementary analysis, IR spectra, Mass and NMR spectra, the structure of the present antibiotic could be suggested as follows:



All these results were applied according to Williams and Fleming (1987), Hayakawa *et al*. (1994) and Stefani and Agodi (2000).

Identification of the antialgal antibiotic produced by *S. neyagawaensis* No. 60: Taking into consideration the elementary analysis, IR, mass and NMR spectra and also on the basis of the recommended keys for the identification of antibiotics and in view of the comparative study of the recorded properties of the antibiotics, it could be stated that the antialgal substance extracted from *S. neyagawaensis* is suggestive of being Anthracidin A (Table 13). The identification is carried out according to Berdy (1980) and Umezawa (1977).

Effect of different concentrations of purified antibiotic against *Anacystis nidulans*: Different concentrations of the purified antialgal substance produced from *S. neyagawaensis* No. 60 were tested against *Anacystis nidulans* by serial dilution technique as follows; 10, 20, 30, 40 $\mu g\ mL^{-1}$ in 50 mL liquid algal media. Successive growth was indicated every 48 hours using spectrophotometer at λ 665 nm. The data in Table 14 revealed that there was gradual inhibition in the growth of alga as treated with the previous concentrations. The percentage of inhibition of the antialgal substance which extracted from *S. neyagawaensis* No. 60 after 16 days were 75, 81.3, 85 and 88.8% at different concentrations, respectively.

DISCUSSION

Blooms of cyanobacteria represent a trouble shouting problems among these are secretion of toxic substance such as hepatotoxins, neurotoxins, cytotoxins, endotoxins and other potential inhibitors (Sasner *et al*., 1994; Zurawell *et al*., 2005; Ross *et al*., 2006; Dai *et al*., 2008; Kim *et al*., 2009). Most of them may be responsible for dermatitis when people are exposed to this kind of water. In addition, they produce unpleasant odor, color and taste of water (Choi *et al*., 2005; Liu *et al*., 2008). Great efforts have been done for producing new substances with antimicrobial activities from microorganisms instead of chemicals (Liu *et al*., 1996). It was found that the biological control is the best mean that replace the chemical control (Kim *et al*., 2009; Boudjella *et al*., 2006; Volka and Furkert, 2006). Biological controls for solving these problems involve isolation of streptomycete isolates from different localities of Egypt and screened against some cyanobacteria (El-Shirbiny *et al*., 2007).

Out of 107 strains, only 33 isolates showed antialgal activity against different algal species under investigation. *Streptomyces neyagawaensis* No. 60 was superior to induce a lytic efficiency against the tested cyanobacterial strains (*Nostoc* sp.; *Anabaena* sp.; *Anabaena* flos-aquae; *Anacystis nidulans* and cyncoccus).

Before application of an antialgal agent to freshwater systems, there should be information on, (1) the antialgal activity against the target alga, (2) the effects on the other organisms in the freshwater ecosystem and (3) a forecast of the algal dynamics after the removal of the target alga (Choi *et al*., 2005). Here, *Streptomyces neyagawaensis* No. 60 release an algicidal substance and its concentration was time dependent. The maximum inhibitory concentration was obtained after 16 days that equal 89% compared with its corresponding control. Present results are in agreement with the results of Yamamoto and Suzuki (1990) and Mitsutani *et al*. (1988), who stated that the proteins such as lysozymes and protease extracted from streptomyces which cause cell lysis of *Microcystis aeruginosa*. At the same time,

El-Sherbiny *et al.* (2007) showed that niromycin A produced by *Streptomyces endus* No. 40 causing extensive cell lysis of *Nostoc* sp., *Anabaena* sp., *A. flos-aquae* and *Anacystis nidulans*. However, Sigee *et al.* (1999) found that the formation of the lytic agent by *Streptomyces exfoliates* occurs independently with the presence of cyanobacteria, the ability to destroy these organisms is probably due to the antagonist of streptomycetes. Also, Liang *et al.* (2003) clear that the crud extract of hormaomycin antibiotic showed very strong activity against three microalgae (*Chlorella sorokiniana*, *Chlorella vulgaris*, *Senedesmus subspicatus*).

Choi *et al.* (2005) studied the effects of the antialgal bacterium *S. neyagawaensis* on several dominant algae in the Daechung Reservoir and Naktong River and found that *S. neyagawaensis* had an effect on *A. flos-aquae* and *A. cylindrical* but not *A. macrospore* and *A. aynis* it also affected strains within a species differently. The antialgal activity was 38.8% on *M. aeruginosa* NIES.44 and 70.2% on *M. aeruginosa* NIES-298. Also, Choi *et al.* (2005) reported that, there are two possible explanations: (1) the culture conditions utilized in this experiment were not suitable for bacterial growth and (2) *M. aeruginosa* exudates may suppress bacterial growth. The first explanation is based on the divergence between the optimum culture conditions for the antialgal bacterium and the cyanobacterium. The bacterium did not grow well at pH 9 and 25°C. The second explanation is that the toxicity of microcystin from *M. aeruginosa* is known to inhibit growth of organisms such as *Chadocerans*, *Copepdos* and mosquito larvae (Sathiyamoorthy and Shnmugasundaram, 1996; Singh *et al*., 2003; Volk and Furkert, 2006).

In the present study, the results showed that 40 μg mL^{-1} of the antialgal substance extracted from *Streptomyces neyagawaensis* No. 60 inhibits both chlorophyll "a" (74.3%) and DNA (64.1%) and RNA contents (45.1%) in *Anacystis nidulans* alga.

In this investigation, the purified antialgal substance was identified using the elementary analysis, I.R., Mass and NMR spectra and also on the basis of the recommended keys for the identification of known antibiotics. Also on the basis of this data and their comparison with the published data of the known antibiotics (Umezawa, 1977; Berdy, 1980). The present antibiotic can be named as Anthracidin A.

In conclusion, the maximum inhibition rate of growth of *Anacystis nidulans* was recorded after 16 days of incubation by the 40 μg mL^{-1} with total percentage of inhibition about 88.8%.

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