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EXPERIMENTAL ARTICLES

Isolation, Characterization, and Identification of Two Methomyl-Degrading Bacteria from a Pesticide-Treated Crop Field in West Bengal, India[1]

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Abstract—Two methomyl-degrading bacteria (initially named Disha A and Disha B) were isolated from a pesticide-treated crop field in Baruipur, 24 Parganas (South), West Bengal, India. Both strains could not grow in mineral salt (MS) medium but showed efficient growth in the presence of methomyl. The highest growth was observed in the MS medium containing 0.16% methomyl. When methomyl was supplemented with glucose, no further enhancement of growth was observed, whereas supplementation with yeast extract had a positive effect on growth of both strains, indicating that methomyl could be utilized as the sole source of carbon but not that of nitrogen. In Nutrient Broth and Luria Bertani medium, these strains could tolerate 0.4% methomyl. Optimum pH and temperature for growth of both bacteria in the methomyl-containing MS medium were 7.0 and 30°C, respectively. Protein concentration in the cell-free extracts of bacterial cultures was directly proportional to methomyl concentration in the medium. Disha A was more resistant to the antibiotics amoxicillin and penicillin, as indicated by minimum inhibitory concentration (600 and 500 μg/mL, respectively), which were higher than those obtained for Disha B (350 and 300 μg/mL, respectively). Both Disha A and Disha B were plasmid-bearing, gram-positive, endospore-producing, rod-shaped bacteria. Biochemical studies, 16S rDNA sequencing, and phylogenetic analysis indicated maximum similarity of Disha A to *Bacillus cereus* ATCC 14579, whereas Disha B showed maximum similarity to *Bacillus safensis* F0-36b ATCC BAA-1126. The HPLC analysis clearly indicated that *B. cereus* and *B. safensis* showed 88.25 and 77.5% of methomyl (Sigma) degradation, respectively within 96 h of growth. This is the first report of *Bacillus* species that can degrade the carbamate pesticide methomyl and thrive in presence of its high concentrations.

Keywords: Bacillus sp., carbamate, methomyl, pesticide, 16S rRNA

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Agricultural revolution due to increased market demand for agricultural products has led to widespread use of pesticides for pest management and vector control. Consequently, pesticides have become an integral part of global agriculture. According to Tomlin (2003), an ideal pesticide should rapidly kill the target pest at a very low concentration and degrade into non-toxic, or at least less toxic, substances as quickly as possible. Even though large-scale application of pesticides is an essential part of augmenting agricultural production, the excessive use of pesticides leads to the accumulation of their massive residual amounts in the environment. The unwarranted presence of pesticides in the environment causes microbial imbalance, environmental pollution, and health problems (Alexander, 1999). Moreover, when pesticides accumulate in the drinking water and food chain in extremely toxic concentrations, they not only pose a considerable health hazard to the present generation, but also threaten the existence of future generations (Aislabie and Lloyd-Jones, 1995).

Methomyl (*S*-methyl-*N*-[(methyl-carbamoyl) oxy] thio-acetimidate), is a systemic carbamate insecticide introduced in 1966 and widely used in agriculture worldwide to protect a wide range of crops, including vegetables, grain, soya beans, cotton, fruit, vines, hops, and ornamentals, against a large variety of insects (Cox et al., 1993; Farre et al., 2002; Chang et al., 2008; Xu et al., 2009). Methomyl acts as an ovicide against budworms and cotton bollworms (Shanthalata et al., 2012; Van Scoy et al., 2013). It inhibits acetylcholinesterase, an enzyme of the nervous system, and is considered as one of the extremely toxic pesticides to mammals, including humans (Xu et al., 2009; Van Scoy et al., 2013). The United States Environmental Protection Agency classified methomyl as a

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Class 1 or "Restricted Use" pesticide (Farre et al., 2002). Methomyl is also considered a ground water contaminant due to its high water solubility and low soil/organic carbon partition coefficient (Farre et al., 2002). In view of the extreme toxicity of methomyl, its full degradation is essential for environmental safety (Chang et al., 2008).

In recent years, there have been many attempts to use soil microorganisms for degradation of various xenobiotic compounds to harmless or less harmful intermediate(s). *Azotobacter, Arthobacter, Burkholderia, Rhodococcus, Pseudomonas*, as well as representatives of other bacterial genera, can tolerate and degrade a large number of pesticides (Chennappa et al., 2014). Many rhizospheric bacteria show plant growth promoting or biocontrol activity, which may be diminished or completely suppressed due to application of pesticides at high concentrations into the soil. Although several methomyl-degrading bacteria have been reported, the search for new bacterial species able to tolerate and efficiently degrade large amounts of pesticides within a shorter time and producing environment-friendly by-products remains a topic of considerable scientific and practical interest. In this study, we sought to isolate efficient methomyl tolerating/degrading bacteria from the rhizospheric soil of a pesticide-treated crop field. We report identification of two *Bacillus* species that can tolerate high concentrations (0.4%) of methomyl in laboratory conditions.

MATERIALS AND METHODS

Media and chemicals. The culture media, including Nutrient Broth (NB), Nutrient Agar (NA), and Luria Bertani (LB), were purchased from Hi Media, India. Methomyl (Lannate®) used most in the experiments was purchased from E.I. Dupont India Pvt. Ltd., Haryana unless otherwise mentioned. HPLC grade Methomyl was purchased from Sigma. All other reagents used were of analytical grade. Mineral salt (MS) medium contained (g/L distilled water) NaCl 1.0, $(NH_4)_2SO_4$ 1.0, $MgSO_4 \cdot 7H_2O$ 0.05, K_2HPO_4 0.5, pH 7.0 (Tang and You, 2012).

Isolation of soil microorganisms from a pesticide-treated crop field by enrichment culture. Rhizospheric soil was collected into a sterile plastic bag from an agricultural field located in a village of Baruipur of the 24 Parganas (S) district of West Bengal, India. The soil was dried in an incubator at 37°C. One gram of the soil was mixed with 9 mL of sterile distilled water. The suspension was vortexed and centrifuged at 5000 rpm for 5 min. The supernatant was collected and stored as stock solution. The mineral salt (MS) medium with or without 2% agar were used for further enrichment experiments (Tang and You, 2012). The supernatant was inoculated into the MS medium containing 0.01% methomyl and incubated at 37°C for 24 h. Pesticide-tolerant colonies were selected in methomyl containing MS-agar medium grown again in the methomyl-containing MS medium for several generations, and finally kept in the methomyl-supplemented MS-agar medium for further characterization.

Effect of methomyl on the growth of soil bacteria. Initially, the bacterial filter-sterilized stock solution of 40% methomyl (250 mg/mL) was prepared either in sterile distilled water or in liquid sterilized media (NA/LB/MS). Each nephelometric flask contained 25 mL of the culture medium (NA/LB/MS) and the required amount of methomyl. The flask without the pesticide was treated as control, whereas the flask without bacteria was treated as blank. To prepare the inoculum, 1 mL of bacterial culture grown for 96 h in the MS medium (optical density at 600 nm, $OD_{600} \approx 0.4$) was centrifuged at 10000 rpm for 15 min, washed in sterile water, and finally vortexed in 1 mL of sterile water or medium (NB/LB/MS). To inoculate 25 mL of the medium, 0.1 mL of the inoculum was used. All flasks were incubated at 37°C in stationary condition, unless otherwise mentioned, and OD_{600} measurements were made at different time intervals.

Growth of Disha A and Disha B on methomyl as sole carbon/nitrogen source. Disha A and Disha B were cultured separately in presence of different concentrations of methomyl in the MS medium, unless otherwise mentioned, with or without glucose (carbon source) or yeast extract (nitrogen source) Growth curves of Disha A and Disha B were obtained in the NB, LB, or MS medium in presence or absence of methomyl at different concentrations.

Effect of temperature and pH on methomyl tolerance of Disha A and Disha B. Seven test tubes containing 10 mL of the NB/MS medium were prepared and sterilized in an autoclave. Then, 0.1 mL of the liquid fresh culture of experimental bacteria was inoculated in presence or absence of methomyl. The test tubes were incubated at different temperatures in shaking or stationary conditions. OD_{600} measurements were made in 24 h. For the determination of optimum pH, the bacteria were grown separately in the NB/MS medium at different pH levels (5.0–9.0) at 30°C, unless otherwise mentioned.

Protein concentration. Protein concentration was determined according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

Characterization of Disha A and Disha B bacteria. Gram staining, acid-fast staining, endospore staining, flagella staining, and capsule staining were done using the standard methods. Biochemical tests for the presence of starch hydrolysis, catalase, lysine decarboxylase, urease, phenylalanine production, citric acid uti-

lization, indole production, nitrate reduction, acid and gas production, as well as the methyl red-Voges Proskauer test, were done according to Dubey and Maheswari (2006).

Plasmid isolation. Plasmid isolation was performed using a PureSol™ Plasmid isolation kit according to the manufacturer's instructions.

Antibiotic sensitivity and minimum inhibitory concentration test. Antibiotic sensitivity and the minimum inhibitory concentration (MIC) test were carried out as described by Dubey and Maheswari (2006).

Isolation of genomic DNA. Genomic DNA was isolated using the sodium dodecyl sulfate (SDS) lysis method. In brief, approximately 9 mL of the bacterial isolate A/B (Disha A/Disha B) cultured overnight in the LB medium was pelleted by centrifugation of tubes at 5000 rpm for 5 min. The pellet was lysed in 3.6 mL of the lysis buffer (10 mM Tris, 1 mM EDTA, pH 8.0, 0.6% SDS, and 120 μg/mL proteinase K) and, after brief vortexing, the tubes were incubated at 37°C for 2 h. Following the phenol-chloroform extraction step, DNA was precipitated from the aqueous phase using a 3-M sodium acetate solution and ice-cold ethanol. DNA was eluted in the TE buffer (Tris 10 mM, EDTA 1 mM, pH 8.0), aliquoted, and stored at 4°C. The concentration of genomic DNA was determined by using UV-visible spectrophotometry.

Designing PCR primers and PCR reaction. In order to amplify and sequence the 16S rRNA genomic region of the isolated bacteria, several primers were obtained (Bioserve, India) (Table 1). The primer sequences, optimized to amplify diverse genera of bacteria and archea, were obtained from previous studies (Edrn et al., 1991; Baker et al., 2003). The strategy to amplify various regions of the 16S rDNA sequence was presented. PCR reactions were carried out using Phusion polymerase (Finzymes, USA) as described previously (Sasi et al., 2014). PCR products were electrophoresed and gel-purified using a HiPura Silica gel kit for DNA isolation (HiMedia, India). DNA was eluted in sterile water and assessed for the correct size by agarose gel electrophoresis. PCR products were sequenced using either forward or reverse primers listed in Table 1 by automated Sanger dideoxy chain termination method.

Identification of bacterial strains and phylogenetic analysis. Partial 16S rRNA gene sequences of the bacterial culture isolates A and B (Disha-A and Disha-B) were compared with the 16S ribosomal RNA sequences from bacterial and archeal sequence NCBI database by using NCBI online server (http://www.ncbi. nlm.nih.gov/blast). The sequences that were more than 99% identical to those of the isolates were used for the phylogenetic analysis. The phylogenetic tree was generated using either Blast tree view (http://www.ncbi.nlm.nih.gov/blast) or Phylogeny.fr (http://www.phylogeny.fr/) (Dereeper et al., 2008).

HPLC (high performance liquid chromatography). To confirm the probable degradation of methomyl, HPLC was done with different standard solution of methomyl (Sigma HPLC grade) like 1, 2, 3, 4, 5, and 6 ppm concentration. Disha A and Disha B was inoculated in methomyl (6 ppm; Sigma HPLC grade) containing MS media, and incubated for 96 h at 30°C. Then the cell free culture filtrate was membrane filtered (0.22 μm Millipore) and ready for HPLC (Shimadzu LC solution Analyser).

Statistical analysis. Data are presented as the mean of individual experiments performed in triplicate. Comparisons with control groups were done using GraphPad Prism software (v. 5.0).

RESULTS

Isolation of soil microorganisms from a pesticide-treated crop field. Several distinct colonies have been observed to grow in the methomyl-containing MS-agar medium in 72 h after plating. In the enrichment culture, two of such colonies showed vigorous growth. These two strains, named Disha A and Disha B, were finally selected for further characterization and evaluation of their ability to tolerate and degrade methomyl.

Growth of Disha A and Disha B in the presence of methomyl. To observe the tolerance level to methomyl, growth of both bacteria was tested in the presence of different concentrations of the pesticide in the MS medium up to 96 h. It was found that both bacteria exhibited optimum growth on fourth day in the MS medium containing 0.16% methomyl (Fig. 1). Disha B growth rates were higher than those of Disha A at all tested methomyl concentrations with the exception of 0.20%. The growth time courses of the isolated bacteria in absence or presence of different concentrations of methomyl in the MS medium are illustrated in Fig. 2. No growth was found in the MS medium without methomyl even after 96 h, whereas in the presence of 0.04–0.16% methomyl, growth of both bacteria was increased. Characteristic growth curves of Disha A and Disha B were observed in the presence of different methomyl concentrations (Fig. 2). The optimum growth of Disha B was observed after 78 h in the MS medium containing 0.16% methomyl whereas the optimum growth of Disha A was found after 84 h in the same methomyl conc. (Figs. 2 and 3).

To observe the effect of glucose and yeast extract on growth and methomyl tolerance, the isolated bacteria were grown in the MS medium containing 0.16% methomyl and different concentrations of glucose and yeast extract (Fig. 4). In presence of methomyl, glucose showed inhibitory effect on growth of Disha B

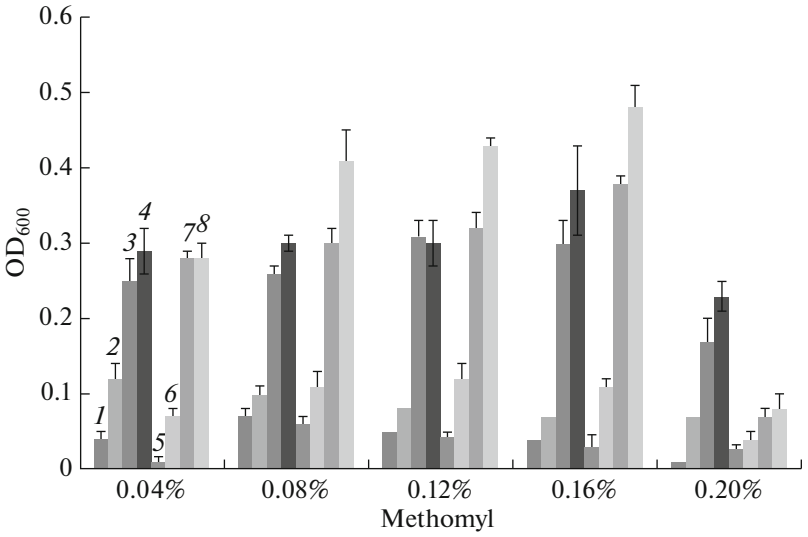

Fig. 1. Graphical representation of growth of Disha A and Disha B in different conc. of methomyl containing MS medium at 24 , 48, 72 and 96 h, respectively. (*1*) 24 h Disha A, (*2*) 48 h Disha A, (*3*) 72 h Disha A, (*4*) 96 h Disha A, (*5*) 24 h Disha B, (*6*) 48 h Disha B, (*7*) 72 h Disha B, (*8*) 96 h Disha B.

whereas slight increase in growth of Disha A was observed in 0.5% glucose. Yeast extract had a stimulating effect on growth of both Disha A and Disha B (Fig. 4).

Protein concentration in the extracellular medium positively correlated with the concentration of methomyl (Fig. 5). Protein content of the methomyl-containing extracellular culture filtrate was the highest (6 mg/mL) in the presence of 1% yeast extract, whereas in the presence of 1% glucose, protein content was 3 mg/mL. The optimum growth of Disha A and Disha B in the presence of 0.16% methomyl occurred at pH 7.0 (data not shown).

To examine the tolerance level to methomyl in a nutrient-rich medium, bacterial growth was observed in the NB medium containing different concentrations of methomyl. The optimum growth was observed in 48 h. Characteristic growth curves of Disha A and Disha B are presented in Fig. 6. Both bacterial strains tolerated 0.40% methomyl in the NB medium (Fig. 7). Disha A growth was similar in the absence or presence of various concentrations of methomyl during 24 and 48 h of the experiment. In contrast, Disha B growth positively correlated with methomyl concentrations after 24 and 48 h. Whereas, Disha A growth was higher than that of Disha B after 24 and 48 h, this situation was opposite after 72 h of culturing the bacteria in the methomyl-containing NB medium because at that time point, Disha-B growth was more intensive than that of Disha-A (Fig. 7).

The growth curve of both isolated strains in the LB medium (Fig. 8) demonstrated that the optimum growth of Disha A was observed in 42 h, whereas that of Disha B occurred in 48 h. Both bacteria tolerated 0.40% methomyl in the LB medium (Fig. 9). As in the case of experiments in the NB medium, Disha A growth in the methomyl-containing LB medium in 24 and 48 h was higher than that of Disha B, whereas in 72 h, Disha B grew better than did Disha A.

Characterization of the bacterial strains. Both strains were plasmid-bearing, gram-positive, endospore-producing, rod-shaped bacteria. The average sizes of Disha A and Disha B were 5.76 × 1.59 and 6.25 × 1.48 μm^2, respectively. The optimum temperature for growth of both bacteria was determined to be 30°C. Both bacteria were positive for catalase, lactase, lysine decarboxylase, and urease, but negative for phenylalanine deaminase. In addition, Disha A hydrolyzed gelatin, whereas Disha B did not.

Antibiotic sensitivity test. Both bacteria were tested for the sensitivity to a number of antibiotics. Disha A and Disha B were resistant to amoxicillin and penicillin, but sensitive to cephalexin, erythromycin, levofloxacin, azithromycin, and doxycycline. The MIC of amoxicillin was 600 μg/mL and that of penicillin was 500 μg/mL in Disha A, whereas in Disha B, these values were 350 and 300 μg/mL, respectively.

Identification of the bacterial isolates using the 16S rDNA sequence. Three PCR products of different sizes were obtained (Fig. 10) using three primer pair combi-

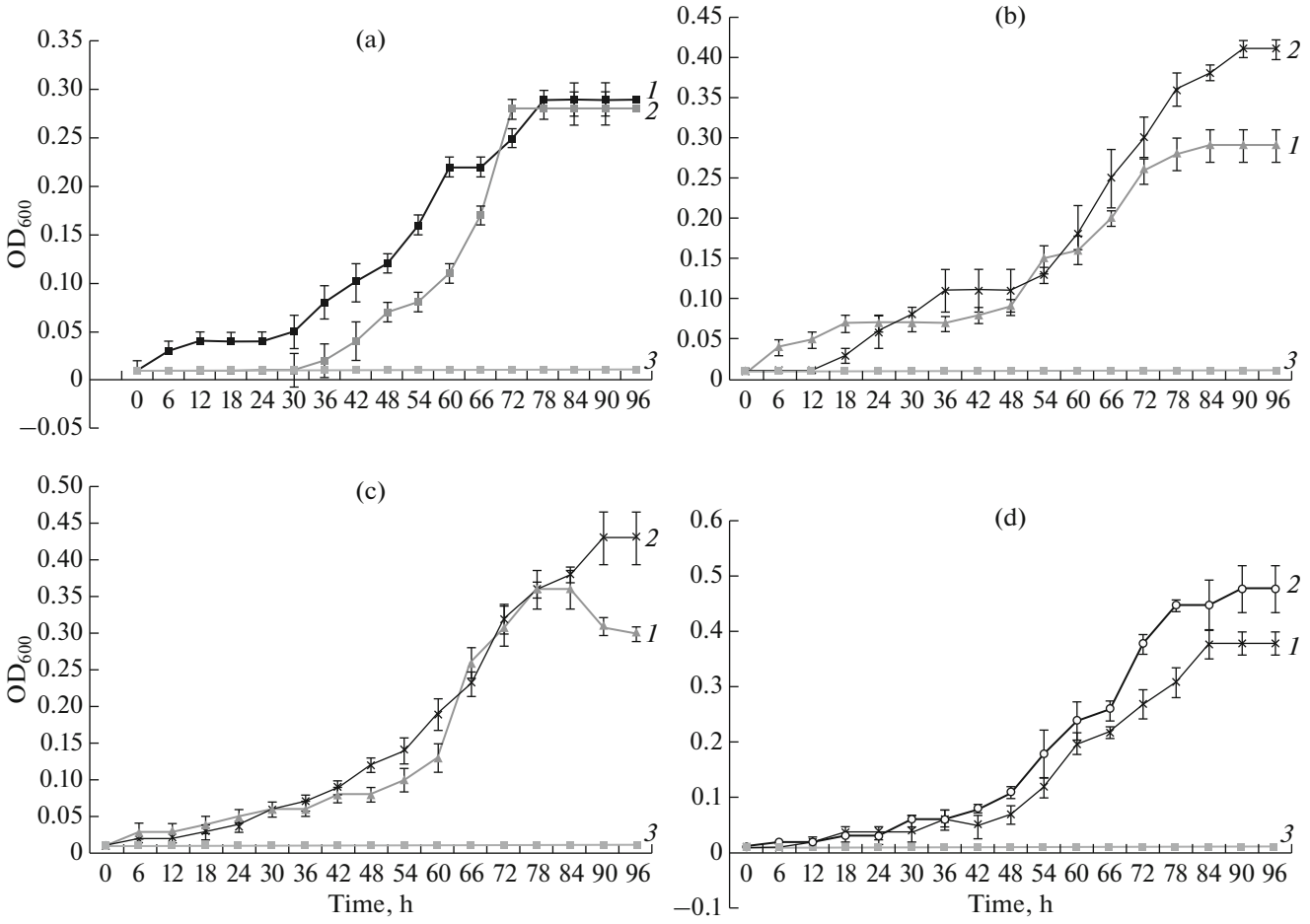

Fig. 2. Growth curve of Disha-A and Disha-B in the MS medium containing different conc. (a) 0.004%, (b) 0.008%, (c) 0.12%, (d) 0.16% of methomyl. *1*—Disha A, *2*—Disha B, *3*—control Disha A/Disha B.

nations (Table 1). The PCR products were gel-extracted, purified, and sequenced. BLAST analysis showed that the sequences of the isolates A and B had maximum similarity with database sequences of the genus *Bacillus*. Phylogenetic analysis showed that Disha A had maximum similarity to *Bacillus cereus* ATCC 14579 (nucleotide accession no. NR_074540.1; Fig. 11a), whereas Disha B exhibited maximum similarity to *Bacillus safensis* F0-36b (nucleotide accession no. NR_041794.1; Fig. 11b).

High performance liquid chromatography. The chromatogram of standard Methomyl in different concentration showed the variable peak size at 7.099 min in 233 nm. The control methomyl showed larger peak than other two the area of control peak is 121760 whereas after 96 h growth *B. cereus* and *B. safensis* showed peak areas of 14302 and 27332, respectively (Figs. 12a–12c).

DISCUSSION

In 1978, the US Environmental Protection Agency classified methomyl as a "Restricted Use" insecticide (US EPA 1989). However, it is still heavily used throughout the world, especially in developing countries, such as India. It is highly soluble in water and has a half-life of 30–45 days in the soil and over 50 weeks in the ground water (Toxonet, 1993; Farre et al., 2002). Like other pesticides, methomyl may also affect beneficial soil microorganisms. Therefore, degradation of methomyl is very much essential for the protection of the environment.

Several microbes have been reported to be able to degrade methomyl. Nyakundi et al. (2011) isolated a white rot fungus (WR 2), which could degrade methomyl and its metabolite in a slow process (~42 days). The degradation could be accelerated in the presence of WR9, another white rot fungal isolate. Farre et al. (2002) reported degradation of methomyl by *Vibrio fischeri,* which was isolated from activated sludge in

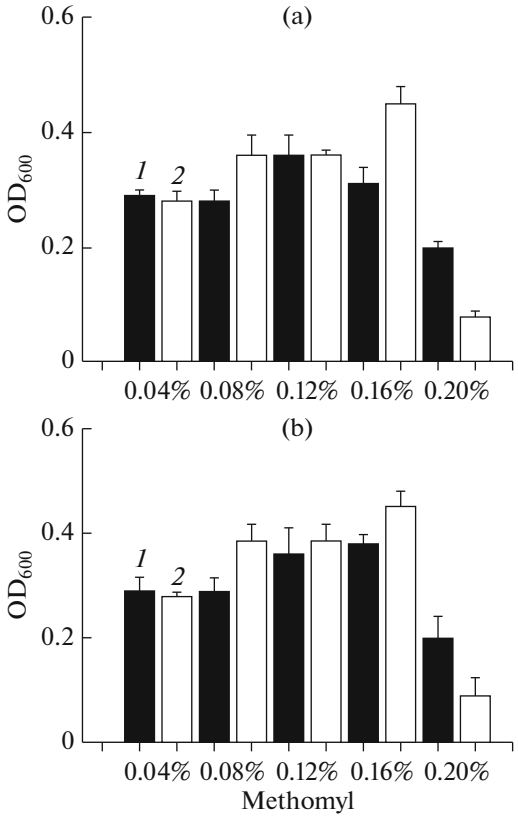

Fig. 3. Graphical representation of growth of Disha A and Disha B in the MS medium containing different conc. of methomyl in 78 h (a) and 84 h (b). *1*—Disha A, *2*—Disha B.

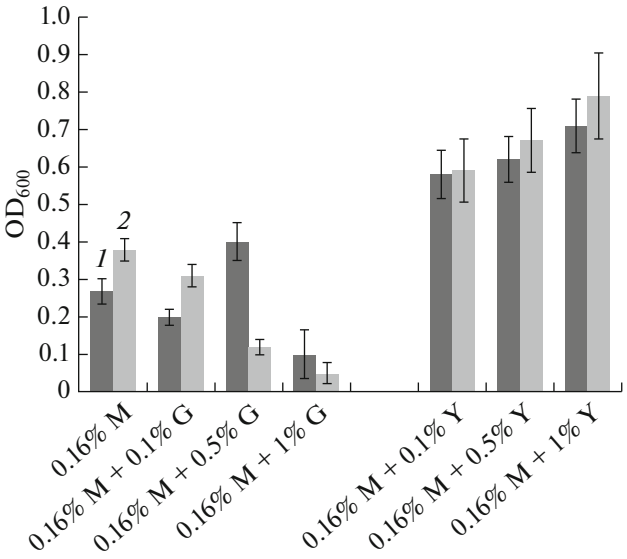

Fig. 4. Graphical representation of growth of Disha A and Disha B in the MS medium containing 0.16% methomyl and different conc. of glucose/yeast extract in 84 h (M = methomyl, G = glucose, Y = yeast extract). *1*—Disha A, *2*—Disha B.

aerobic conditions. Mohamed (2009) reported degradation of methomyl by the gram negative bacterium *Stenotrophomonas maltophilia* M1, which contained a gene encoding a methomyl-degrading protein in its plasmid. Xu et al. (2009) isolated *Paracoccus* sp. Mdw-1, a gram-negative bacterium, which could degrade methomyl into *S*-methyl-*N*-hydroxythioacetamidate within 10 h of incubation. El-Fakharany et al. (2011) reported isolation of *Pseudomonas* spp. (EB20), which could degrade 77% of methomyl within two weeks. Because microbial degradation of methomyl appears to be the most effective bioremedi-

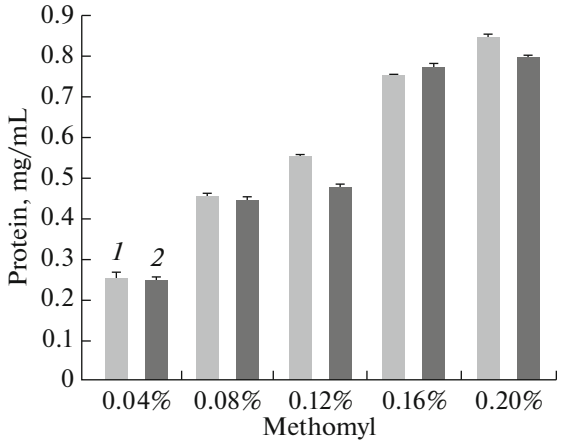

Fig. 5. Estimation of protein of Disha A and Disha B in the MS medium containing different conc. of methomyl. *1*—Disha A, *2*—Disha B.

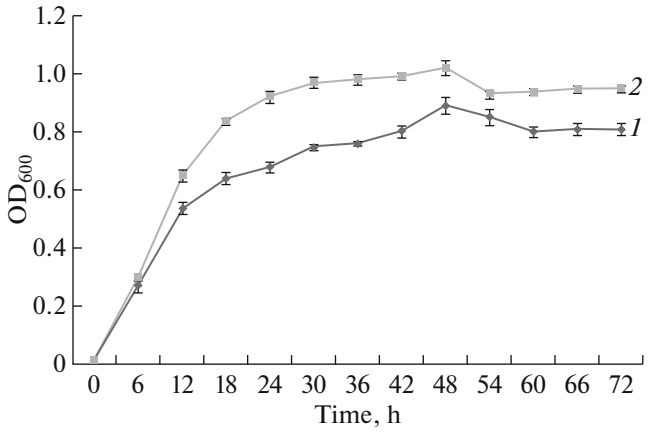

Fig. 6. Growth curve of bacteria in the NB medium. *1*—Disha A, *2*—Disha B.

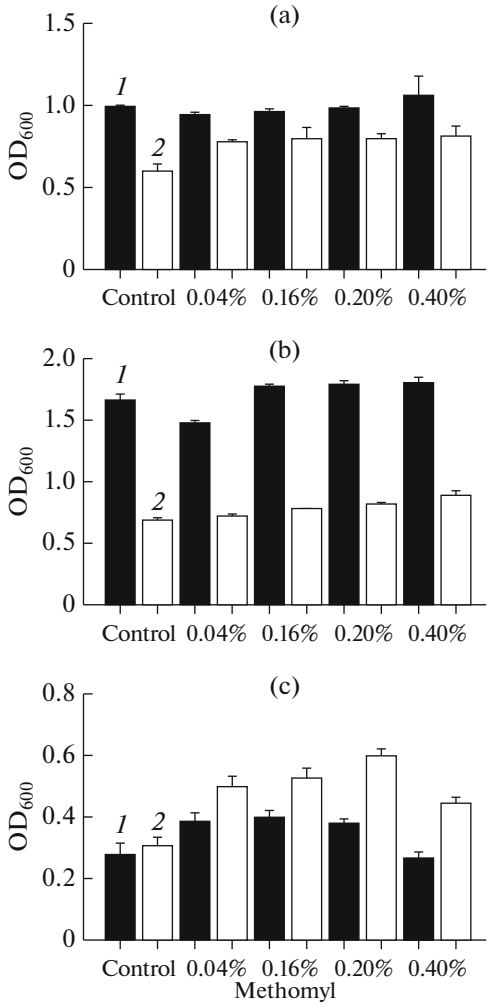

Fig. 7. Comparison between the growth of Disha A and Disha B in the NB medium in presence of different conc. of methomyl at different time (a) 24 h, (b) 48 h, (c) 72 h. *1*—Disha A, *2*—Disha B.

ation method (Van Scoy et al., 2013), environmental microbiologists constantly look for microbial species that can tolerate and efficiently degrade methomyl.

The organisms that can degrade methomyl must also tolerate high concentrations of this pesticide. In the present study, we have isolated two methomyl-tolerant bacteria, Disha A and Disha B, through enrichment culture from a pesticide-treated rhizospheric soil of brinjal cultivation field from Baruipur (West Bengal, India). According to Sadashiv and Kaliwal (2016), in the presence of pesticides and antibiotics, new genes evolve in bacteria and thus, resistance occurs.

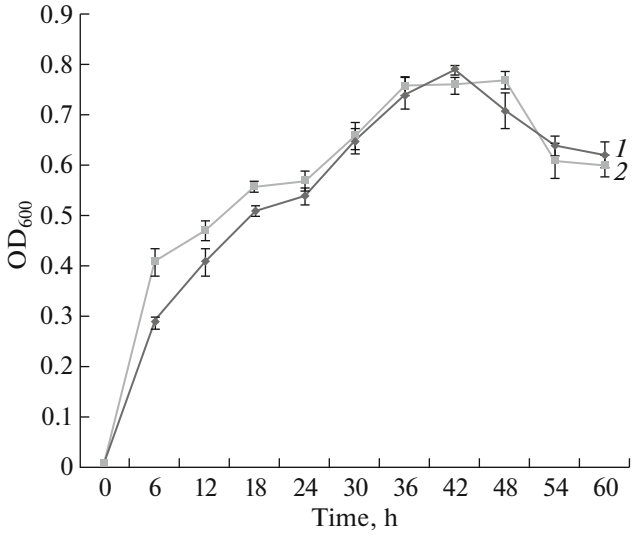

Fig. 8. Growth curve of bacteria in the LB medium. *1*—Disha A, *2*—Disha B.

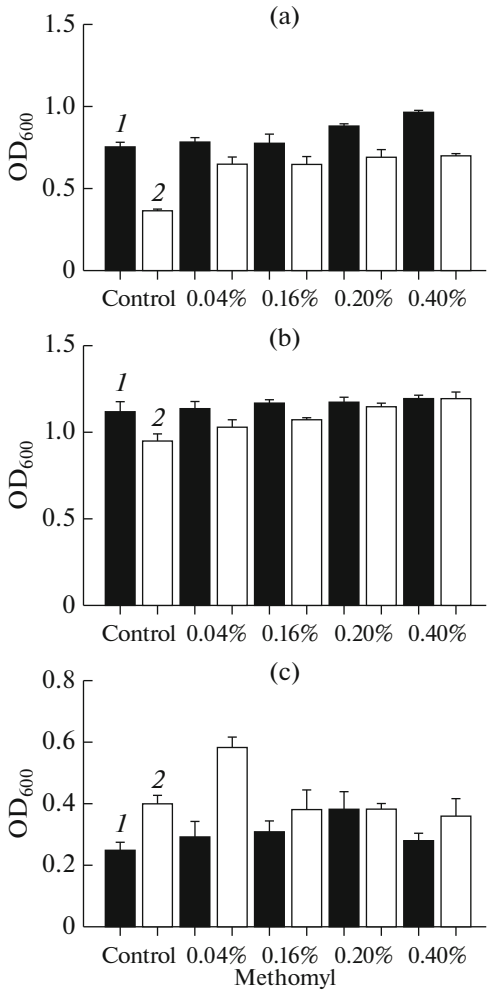

Fig. 9. Comparison between the growth of Disha A and Disha B in the LB medium in presence of different conc. of methomyl at different time ((a) 24 h, (b) 48 h, (c) 72 h). *1*—Disha A, *2*—Disha B.

We found that neither of the two isolated strains could grow in the MS medium devoid of carbon or nitrogen sources, whereas efficient growth was observed in the methomyl-containing MS medium. The growth rate increased proportionally with the increase in methomyl concentrations up to 0.16%, and then, it became lower at 0.2% methomyl. Disha B exhibited higher tolerance to 0.2% methomyl than Disha A. When glucose was added in addition to methomyl, there was no significant increase in growth, whereas the supplementation with the yeast extract enhanced growth of both bacteria. Therefore, although methomyl contains both carbon and nitrogen, it is possible that these two bacteria can utilize methomyl only as the source of carbon but not that of nitrogen.

Both isolated soil bacteria efficiently grew in the NB and LB media, where the levels of their tolerance to methomyl were higher than those in the MS medium. This finding is probably explained by the fact that NB and LB media are highly rich in the nutrient content, so bacterial growth was also intensive, as evidenced by high OD values. In contrast, in the MS medium, there is no source of carbon or nitrogen. Therefore, because growth of both bacteria was detected in the methomyl-containing MS medium, this suggests possible degradation of methomyl by these bacteria, which utilized it as a source of carbon. Similar findings were observed by Xu et al. (2009).

The growth and methomyl tolerance of both strains were optimal at pH 7.0. Xu et al. (2009) reported growth of *Paracoccus* sp. in the presence of methomyl and efficient degradation of the latter at pH 7–9. The optimum temperature for growth in the MS medium containing methomyl was 30°C. *Paracoccus* sp. also showed the same temperature preference for optimal growth and maximal methomyl degradation (Xu et al., 2009).

The amount of protein in the cell-free extracts was directly proportional to the methomyl concentration in the medium, although bacterial growth was inhibited at 0.2% methomyl. This indicates that there may be a correlation between concentration of methomyl and protein synthesis. Kulkarni and Kaliwal (2012) reported higher production of stress-related proteins in *Escherichia coli* when methomyl concentration and duration of treatment were increased. Asghar et al. (2006) reported the induction of stress protein expression in *E. coli* in the presence of the pesticides bifenthrin, carbofuran, cypermethrin, and zeta-cypermethrin.

Biochemical characterization and rDNA analysis were carried out to identify the bacterial isolates. It was found that Disha A showed maximum similarity to *Bacillus cereus* ATCC 14579, whereas Disha B demonstrated maximum similarity to *Bacillus safensis* F0-36b (ATCC BAA-1126). The strains isolated in this study have been deposited in the National Centre for Industrial Microorganisms (NCIM), Pune, India with accession numbers NCIM 5557 and NCIM 5558, respectively.

Although *Bacillus* strains have been reported to degrade some organophosphorus pesticides, such as triazophos (Tang and You, 2012), our study is the first report of *Bacillus* species that can tolerate high concentrations of the carbamate pesticide methomyl. The tolerance to methomyl and resistance to high antibiotic concentrations may be explained by the presence of a plasmid in these bacteria. Don and Pemberton (1981) reported that plasmids present in the bacteria of

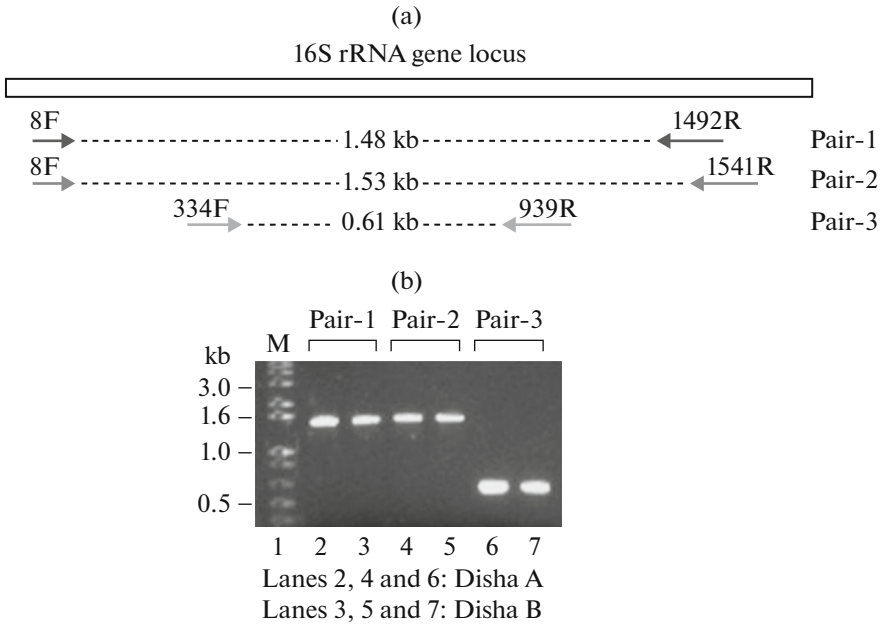

Fig. 10. (a) 16S rRNA gene locus, (b) PCR products of Disha A and Disha B.

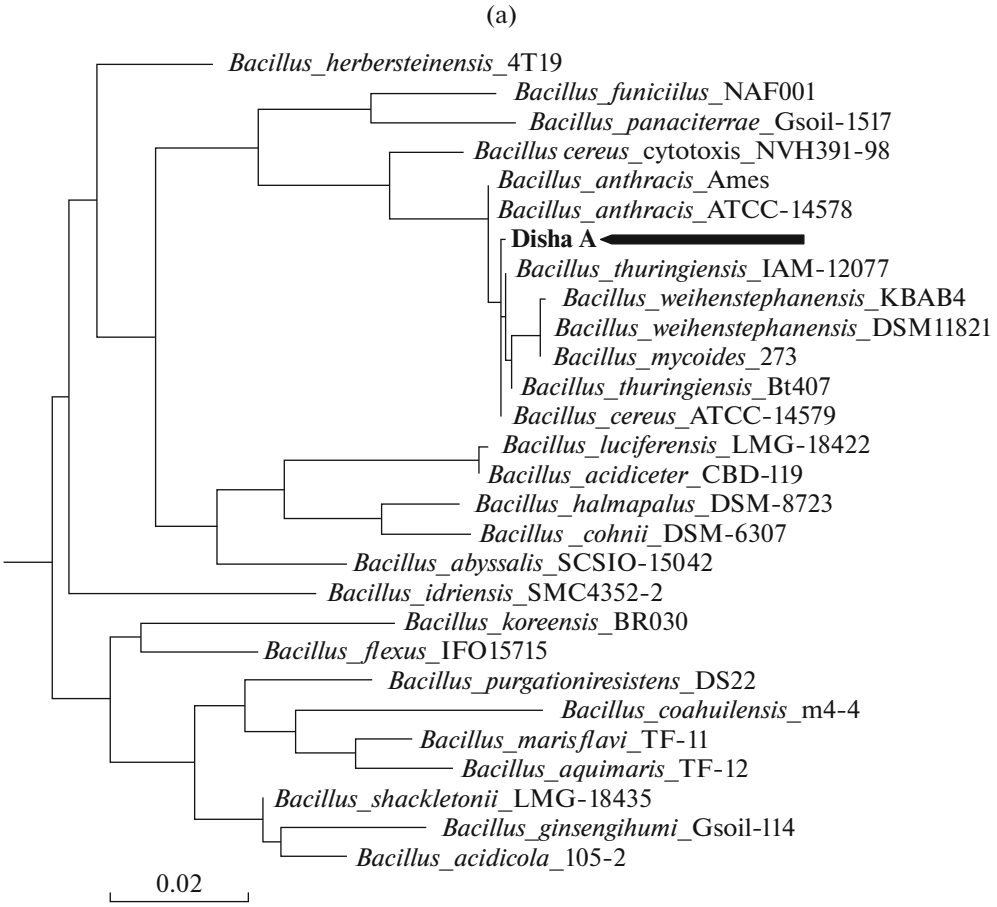

Fig. 11. Phylogenetic tree generated using Phylogeny. Fr (a) Disha A; (b) Disha B.

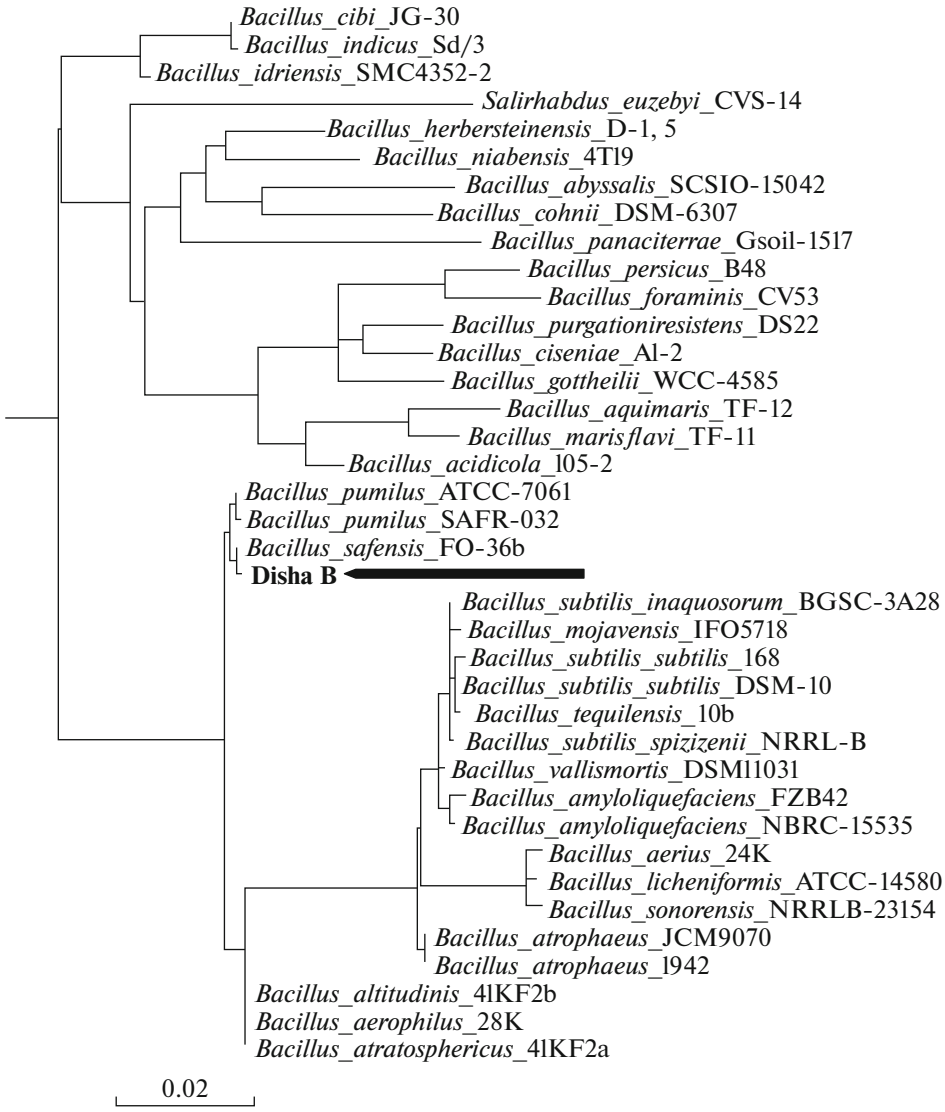

Fig. 11. (Contd.)

the genera *Bacillus, Pseudomonas*, and *Escherichia* may be responsible for the degradation of a number of aromatic and aliphatic compounds.

HPLC analysis clearly indicated the degradation of methomyl by both the bacteria *B. cereus* and *B. safensis*. *B. cereus* showed comparatively better degradation (88.25%) of methomyl (Sigma) than *B. safensis* (77.5%). Xu et al. (2009) showed that *Paracoccus* sp. degraded methomyl into *S*-methyl- *N*-hydroxythioacetamidate. Kulkarni and Kaliwal also showed that *Pseudomonas aeruginosa* can degrade methomyl as the source of carbon (Kulkarni and Kaliwal, 2014). In the present study, although we did not examine whether methomyl was degraded into *S*-methyl-*N*-hydroxythioacetamidate, it can be assumed that the pesticide was degraded by both these bacterial strains very efficiently within very short time period (96 h) and used as the carbon source for the growth of the bacteria. Thus the soil bacteria *B. cereus* and *B. safensis* can efficiently degrade carbamate pesticide methomyl and may be commercially utilized in methomyl bioremediation.

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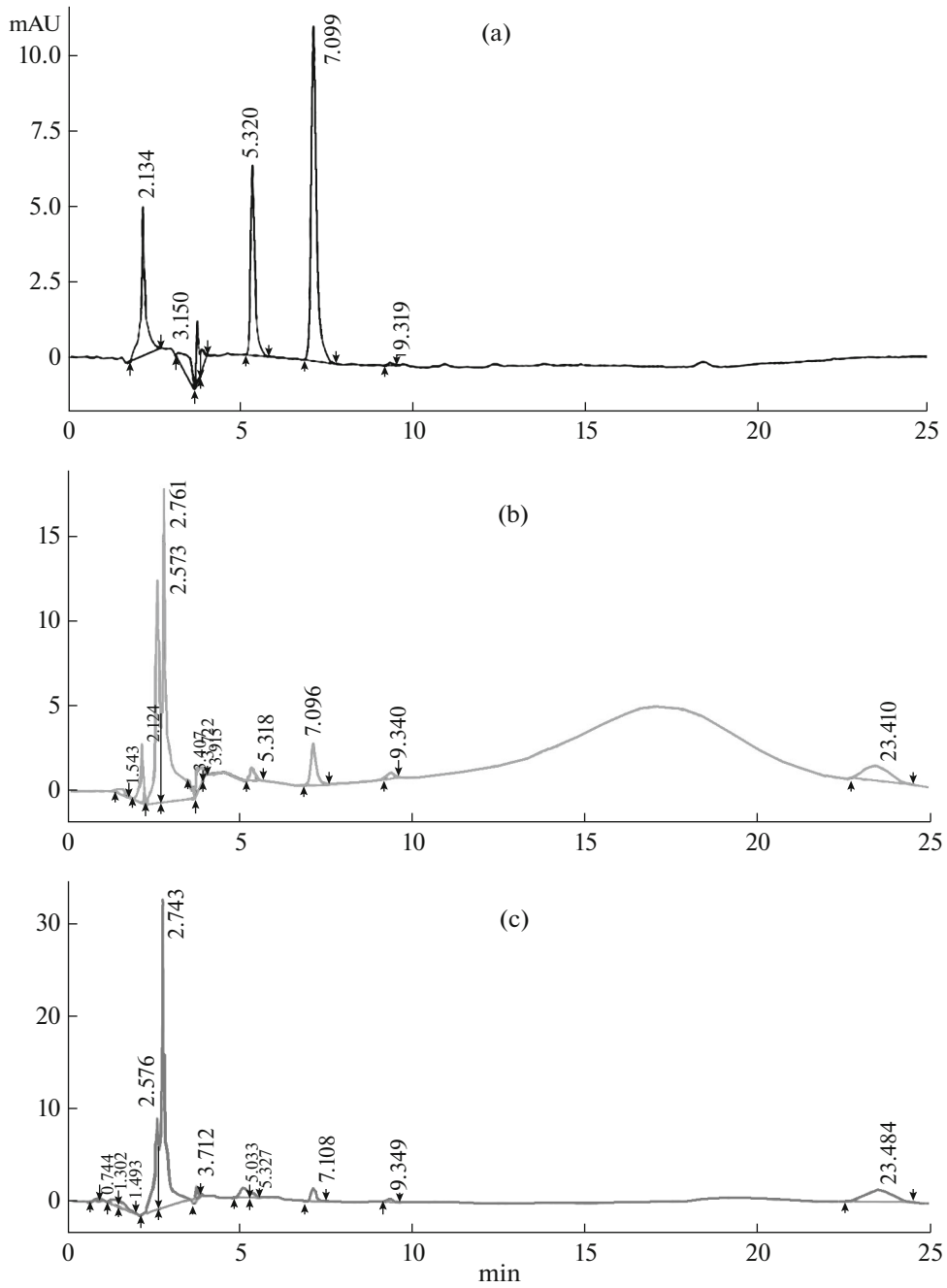

Fig. 12. Chromatogram of standard methomyl solution (a), culture filtrate of Disha B (b), culture filtrate of Disha A (c). (Column size—250 × 4.6 mm, eluent—20% acetonitrile and 80% water, flow rate—1 mL/min, type—isocretic, detector—UV-233 nm, injection volume—20 μL).

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