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Isolation and characterization of *Pseudomonas syringae* pv. *lachrymans* from angular leaf spot disease of cucumber (*Cucumis sativus* L.) and evaluation of its antibiotic sensitivity

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Abstract
The present investigation was conducted to isolate and characterize the causal organism from angular leaf spot disease of cucumber and evaluation of its sensitivity test against some antibiotics and plant extracts. Different biochemical tests were performed to characterize the isolated bacteria from angular leaf spot disease of cucumber. The growth of the isolated bacteria was obtained in 16 hours of incubation at 37 ºC and colonies were creamy white in color. The isolated bacteria showed gram negative at gram staining and potassium hydroxide (KOH) test. Isolated bacteria showed positive response in catalase test, indole test, Simmons citrate test and MacConkey agar test while negative results revealed in urease test, motility test and methyl red test. Antibiotics and antimicrobial activity of the isolated bacteria were screened by disc diffusion method. The highest antibiotic activity against the isolated bacteria was found to be 40.0±0.5mm diameter of zone of inhibition by standard erythromycin. The highest antimicrobial activity was found to be 8.3±0.5mm diameter of zone of inhibition by *Allium sativum* against the isolated bacteria. This study will be helpful for future research to detect and control of this important bacterial disease of cucumber.

Keywords: Cucumber, angular leaf spot, *Pseudomonas syringae*, biochemical test, biological control measures

Introduction
The cucumber (*Cucumis sativus* L.) belongs to the family of Cucurbitaceae, which is one of the most important vegetable crops throughout the world. Cucumber is found primarily in tropical and subtropical regions of the world (Wang *et al*., 2007) [35]. It is a relatively low-calorie food at just about 15 calories per cup, and contains 95% water. Cucumber contains high levels of vitamin B, vitamin C, vitamin K, cucurbitacins, lignans, antioxidants such as beta carotene and other trace elements and minerals (Mukherjee 2013) [24]. Cucumber slices offer many benefits to the eyes and surrounding tissues through their hydrating properties and their high levels of vitamin K that helps reduce dark circles (Lopes *et al*., 2007) [20]. Moreover, cucumbers have been used to treat wrinkles and sunburns of skins (Hooda 2015). [16].
Production of cucumber in Bangladesh is very low due to different types of disease. Different types of disease of cucumber are caused by bacteria, fungus and viruses. Bacterial diseases of cucumber are most common in Bangladesh. Angular leaf spot disease is one of them which cause 37-40% yield reduction in Bangladesh (Bradbury 1986) [8]. *Pseudomonas syringae* pv. *lachrymans* is the causal agent of cucumber angular leaf spot (Lelliott and Stead 1987) [19]. *Pseudomonas syringae* pv. *lachrymans* is one of 50 pathovars belonging to the heterogeneous species *Pseudomonas syringae* (Young *et al*., 1996) [37]. The symptoms of the disease include vein-limited, water-soaked lesions on the cucumber leaves, with or without a chlorotic halo, and water-soaked lesions on fruits, which may be misshapen (Bradbury 1986) [8]. The spots first appear as water soaked lesions on leaves. The lesions usually expand until they are delimited by larger secondary veins, which give the lesions an angular appearance.
To overcome this problem, there is a constant need for new and effective infection fighting strategies. Therefore, there is a need to develop alternative therapeutic agents for the treatment of infectious diseases (Tumpa *et al*., 2015) [33]. There are few works have done in Bangladesh regarding the cucumber diseases. But most of the works focused on isolation and characterization of causal organism of angular leaf spot of cucumber.

Shila *et al*. [29] reported to identify and characterize harmful pathogen *Pseudomonas syringae* from seeds of eight different cucurbits. Alzoreky and Nakahara [4] reported antibacterial activity of extracts from some edible plants commonly consumed in Asia. There is no clear report to detect and control of the angular leaf spot disease of cucumber in Bangladesh.

Therefore, the present investigation was design to isolate bacteria from angular leaf spot disease of cucumber and to characterize the isolated bacteria through different types of biochemical test. Some standard antibiotic and antimicrobial activities were also tested against the isolated bacteria.

Materials and Methods

Plant materials

In the present study, disease infected cucumber plant leaves were collected from the Khorkhori bypass region of Rajshahi, Bangladesh and were identified by Bangladesh Council of Science and Industrial Research (BCSIR), Binodpur, Rajshahi. Angular leaf spot disease infected leaves of cucumber were used as plant material.

Isolation of causal organism

Disease infected leaves were disinfested using a dilute sodium hypochlorite solution (10%) and rinsed thoroughly. We cut the infected area and placed on Lauri and Bourtani (LB) liquid media and incubated for 12 to 16 hours at 37 ºC for allow to growing bacteria. After the bacteria have grown into LB liquid medium, used a sterile loop to streak the bacteria onto a solid nutrient agar media plates and incubated for 12 hours at 37 ºC. One of creamy white colony was picked by wire loop and streaked on another media plate for pure culture.

Biochemical test of the isolated bacteria

Isolated bacteria were characterized by some morphological and biochemical test. Bacteriological analysis was performed using selective media and method (Prescott *et al*., 2002; Sherman and Cappuccino 2005) [27] [28]. Colony morphology, size, shape, color, and growth pattern were recorded after 12 to 16 hours of growth on LB Agar plate at 37°C. Cell size was observed by light microscopy. The Gram reaction was performed as described by Vincent and Humphrey [34]. A series of biochemical tests were conducted to characterize the isolated bacteria using the criteria of Bergey's Manual of Systematic Bacteriology (Bergey *et al*., 1994) [6].

Potassium hydroxide test (KOH): For the KOH solubility test, bacteria were aseptically removed from petridishes with an inoculating wire loop, mixed with 3% KOH solution on a clean slide for 1 minute and observed for formation of a thread-like mass. It was done according to Halebian *et al*. [14]

SIM Test: Using a needle, strains were introduced into test tubes containing SIM medium and were incubated at room temperature until the growth was observed. Turbidity away from the line of inoculation was a positive indicator of motility. Indole production from tryptophan was tested using the method of Clarke and Cowan [10]. We used SIM medium manufactured by High Media Lab. Pvt. Ltd. from India. 36.23gm medium were suspended in 1L distilled water as per the directions.

Catalase Test: The catalase production was determined by adding the H_20_2 (3% vol/vol) to a bacterial culture and the presence of catalase indicated by bubbles of free oxygen gas (Cappuccino and Sherman 2001) [9].

Simmons citrate test: The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source (MacFaddin 2000) [22].To determine whether the isolated bacteria better suited to aerobic or anaerobic environments, the citrate test was done according to Simmons [30] method, using Simmons citrate agar medium (Oxoid ltd., Basingstoke, Hampshire, England). For working solution 23gm powder medium was dissolved in 1L distill water.

Kovac Oxidase test: For Kovac oxidase test, a loopful inoculum from pure culture was picked up by sterilized platinum loop. The inoculum was smeared over the area of filter paper containing oxidize reagent to develop deep blue or purple color within ten seconds indicating the oxidation of the reagent (Kovac 1956) [18].

Methyl Red (MR) test: Bacteria were inoculated into the MR broth medium in test tubes for methyl red test. Test tubes were incubated at 37^0C for 16-18 hours. After incubation, 2-3 drops of Methyl red reagent was added.

MacConkey agar test: MacConkey agar test was performed for isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non fermenting gram negative bacteria (MacConkey 1905**)** [21]. MacConkey agar was inoculated with bacteria using streak plate technique and incubated at 37^0C for 16-18 hours.

Kligler Iron Agar test (KIA): KIA medium was prepared by using usable amount in 1 liter distilled water and sterilized at 121°C for 20 minutes (Taylor and Silliker 1958) [32]. The tube cooled in a slanted position to obtain a butt of 1.5-2.0 cm. The 24 hours of old culture of each isolate were stabbing the butt and streaking the surface of the tube. Tubes were incubated aerobically at 37ºC for 12-16 hours.

Triple Sugar Iron (TSI) and Urease test: Bacteria were inoculated in TSI medium and incubated at 37°C for seven days. For the observation of urease activity of isolated bacteria, urease test was performed as described by Cowan [11].

Antibiotic sensitivity test

Susceptibility of isolates to different antibacterial agents was determined *in-vitro* by employing a modified disc diffusion method (Hasan and Sikdar 2016) [15]. The isolated bacterial strain was incubated overnight in nutrient broths that were placed in the shaker at 37°C and 150 rpm for the antibiotic sensitivity test. A serial dilution technique was made for the test respective. LB agar medium was prepared for making culture plates and the sterile liquid medium was distributed in sterile conical flasks when the temperature cooled down to 40-50°C. Approximately, 15-20 ml of the medium was poured in each petridish and left the airflow cabinet for solidification. Using a loop we streaked the colony on LB agar culture. Commercially available and frequently prescribed standard antibiotics namely, amoxycillin, erythromycin, gentamycin, penicillin, chloramphenicol, clarithromycin, ciprofloxacin tetracycline, carbenicillin, neomycin, streptomycin, azithromycin, kanamycin, doxycycline, cefotaxime were used to test antibiotic sensitivity of the two isolated bacteria. Antibiotic disc were placed centrally on the respective plates

and incubate overnight at 37°C. After overnight incubation the diameter of zone of inhibition was observed and measured with the help of millimeter scale (mm).

Screening of antimicrobial activity

The screeningof antimicrobial activities were investigated by moderate disc diffusion method (Hasan and Sikdar 2016) [15]. Four medicinal plants namely, *Allium sativum, Allium cepa, Gingiber officinale,* and *Momordica charantia* methanol extracts were used for antimicrobial screening against the isolated bacteria. Different parts of the selected plants were collected from the local market. Plant materials were washed and air-dried for 15 days. The dried plant materials were grind to a fine powder in a grinding machine. A measured quantity of 20g of dried powder was soaked in 200ml methanol in round bottom flask at room temperature for seven days with occasional shaking. The extract was filtered by cotton white cloth followed by Whatman No.1 filter paper (Whatman, USA). The filtrate was evaporated at 45^{0}C to dryness and the dried substance was kept in sterile bottle under refrigerated condition until use. An inoculum suspension was swabbed uniformly to solidified 20mL LB agar media for bacteria and the inoculum was allowed to dry for 5 minutesand 6mm diameter paper discs were used. Aliquot of 10, 20, 40µL from each plant crude extract (500 mg mL^{-1}) was added into each disc on the seeded medium and allowed to stand on the bench for 1 hour for proper diffusion and thereafter incubated at 37 ºC for 24 hours. The resulting inhibition zones were measured in millimeters (mm).

Statistical analysis

All the above assays were repeated threes for consistency of results and statistical purpose. The data were expressed as mean and standard error (Mean±SE).Data were analysis using Microsoft Excel software 2010.

Results

Isolation of bacteria

Liquid culture of bacteria was obtained after 16 hours of incubation at 37ºC. From liquid culture, subculture was done by streaking onto the Lauria and Bertani (LB) agar medium in 90mm petridishes.Visual observation was identified the colony morphology of the bacteria.

Biochemical Characterization

The colony color of the isolate was creamy white (Fig: a).The size and shape of colonies were found to be small to medium, smooth, convex and mucoid. KOH solubility test (Fig: b) and gram staining reaction showed isolated bacteria was gram negative. In the SIM medium test no H_2S was produced no motility was (Fig: c) found and indole ring was formed for the isolated bacteria (Fig: d). After inoculating the bacteria with Hydrogen peroxide, the bubbles resulting from production of oxygen gas clearly indicated a catalase positive result for isolated bacteria (Fig: e). In Simmons citrate test, the inoculated bacterial medium showed dark blue color and bacteria showed positive result against citrate medium(Fig: f). In kovac oxidase test, isolated bacteria did not produce any purple colour (Fig: g), so it was negative to Kovac oxidase test. After inoculated the bacteria into Methyl Red medium it produced less acid and give yellow colour (Fig: h). Bacteria showed negative result against methyl Red. Bacteria produced no colour round the colony in MacConkey agar, so it was lactose no fermenting. After inoculating bacteria in the medium it produced dark red colour after overnight and showed negative result against KIA medium. Isolated bacteria showed negative result to TSI and urease test. The responses of isolated bacteria in different biochemical test are given in the Table 1.

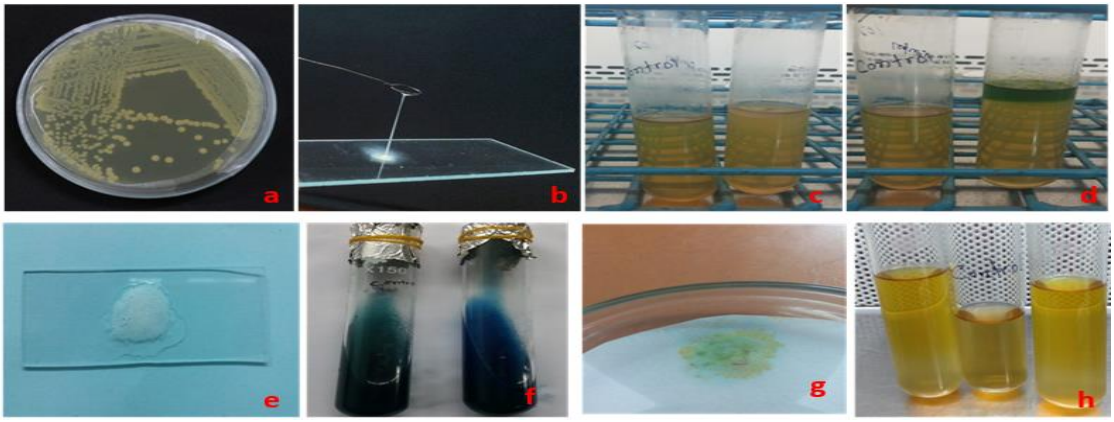

Fig 1: (a) Colony morphology of *Pseudomonas syringae pv. Lachrymans* (b) Thread like viscous appearance indicated KOH positive for *P. syringae* (c) In SIM medium no H_2S was produced and no motility was found (d) No indole ring was formed after adding kovac reagent (e) Bubble formed for catalase test (f) Formation of blue color in simmon citrate test indicated bacteria used citrate as energy (g) No purple color was formed for oxidase test (h) No red ring was formed in methyl red test.

Table 1: Effect of different biochemical tests on the isolated bacteria

Name of the Test	Results	Optimization	Remarks
Gram staining	-ve	Small rod shaped, pink color colony	Isolated bacteria was gram negative
Potassium hydroxide	+ve	Bacterial smear becomes viscous, stringy, sticky mess.	Isolated bacteria was gram negative
H_2S production	-ve	Absence of blackening along the line of inoculation	Bacteria was negative to H_2S production
Indole formation	+ve	A pink to red color band is formed at the top of the medium.	A positive indole test for isolated bacteria
Motility	-ve	Growth of the inoculum is confined to stabling	No motility was recorded
Catalase	+ve	Copious bubbles produced	Bacteria was able to produce catalase enzyme
Simmon citrate	+ve	Production of medium color from green to light blue	Isolated bacteria can utilize citrate
Kovac oxidase	-ve	No color form within 5-10 seconds after inoculation,	Isolated bacteria are negative to oxidase reagent.

Methyl red	-ve	Yellow color	No red color was formed in methyl red
MacConkey agar	-ve	No color for colony	Lactose non fermenting
Kligler Iron Agar	-ve	Red butt and slant	Isolated bacteria can ferment glucose
Triple Sugar Iron	-ve	Red butt and slant	Isolated bacteria could not ferment sugars
Urease	-ve	Yellow color	No magenta color was formed

Antibiotic susceptibility assay

In the present investigation, different types of standard antibiotic discs were used against the isolated bacteria. The highest antibiotic activity with 40.0±0.5mm diameter of zone of inhibition was showed by erythromycin at 10µg/disc concentration following by chloramphenicol with 32.0±0.5mm diameter of zone of inhibition at 30µg/disc against isolated bacteria. On the other hand, tetracycline and kanamycin showed the lowest 10.0±0.5mm diameter of zone of inhibition at 30µg/disc concentration against the isolated bacteria. The standard azithromycin, ciprofloxacin and clarithromycin showed moderate inhibition zone against the isolated bacteria (Table 2)

Table 2: Effect of some standard antibiotics against the isolated bacteria

Name of antibiotic	Symbol	Disc potency (µg/disc)	Diameter of zone of inhibition(in mm, M±SE)	Sensitivity pattern
Amoxycillin	AML	10	12.0±0.5	Intermediate
Erythromycin	E	10	40.0±0.5	Susceptible
Gentamycin	GEN	10	20.0±0.5	Susceptible
Penicillin	P	10	15.0±0.5	Intermediate
Chloramphenicol	C	30	32.0±0.5	Susceptible
Clarithromycin	CLR	15	25.0±0.5	Susceptible
Ciprofloxacin	CP	5	26.0±0.5	Susceptible
Tetracycline	TE	30	10.0±0.5	Resistant
Carbenicillin	CB	100	24.0±0.5	Susceptible
Neomycin	N	30	20.0±0.5	Susceptible
Streptomycin	S	10	20.0±0.5	Susceptible
Azithromycin	AZM	15	30.0±0.5	Susceptible
Kanamycin	K	30	10.0±0.5	Resistant
Doxycycline	DO	30	17.0±0.5	Susceptible
Cefotaxime	CTX	30	26.0±0.5	Susceptible

Note: Resistant =<10 mm; Intermediate = <10-15 mm; Susceptible = >15 mm

Screening of antimicrobial activity

For microbial activity screening, four different medicinal plant extracts were used against the isolated bacteria. The highest antimicrobial activity with 8.3±.5mm diameter of zone of inhibition at 40µl/disc concentration was showed by *Allium sativum* methanol extract followed by *Allium cepa* with 5.0±0.5mm diameter zone of inhibition against the isolated bacteria. On the left hand, methanol extract of *Gingiber officinale* showed the lowest 2.0±.5mm diameter zone of inhibition at the concentration of 10µl/disc against the isolated bacteria (Table 3).

Table 3: Screening of antimicrobial properties of some medicinal plant extract against the isolated bacteria

Name of plants	Diameter of zone of inhibition (in mm)			Sensitivity pattern of isolated bacteria
	10µl/disc	20µl/disc	40µl/disc	
Allium sativum	5.0±0.5	6.0±0.5	8.3±0.5	Resistant
Allium cepa	4.0±0.5	3.0±0.5	5.0±0.5	Resistant
Gingiber officinale	2.0±0.5	4.0±0.5	4.0±0.5	Resistant
Momordica charantia	2.5±0.5	3.5±0.5	4.5±0.5	Resistant

Note: Resistant =<10 mm; Intermediate = <10-15 mm; Susceptible = >15 mm

Discussion

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries (Al-bari *et al.* 2006) [2]. To overcome this problem many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs (Akinpelu and Onakoya 2006) [1]. In the present study, bacteria isolated from the angular leaf spot disease of cucumber showed characteristics of the genus *Pseudomonas* and confirmed all the criteria for inclusion in the group of plant pathogenic *Pseudomonas* (Misaghi and Grogan 1969) [23].The phenotypic description of isolated bacteria was the same as genus *Pseudomonas.* The morphological characters of several isolates of *Pseudomonas syringae pv. lachrymans* isolated from all over Japan was reported that all were gram negative, aerobic, non-sporing, straight rods and motile with one to five polar flagella (Kagiwata 1990) [17]. Narayanasamy [25] reported the characteristics of *Pseudomonas syringae* isolated from apicot trees were creamy color on nutrient agar medium.We also found colonies of the isolated bacteria from angular leaf spot infected cucumber plant were creamy in color on nutrient agar medium.We observed that, bacterial colonies in sucrose medium were round shaped as reported by Aksoy [3].In potassium hydroxide test isolated bacteria showed viscous appearance. Shila *et al.* [29] reported viscous appearance and positive response to KOH solubility test against *Pseudomonas syringae* pv. *lachrymans* in the seeds of eight cucurbits. This result supports our present findings. In SIM test isolated bacteria did not produced H_2S but it formed indole and non-motile in the medium which was similar to

Baron *et al.* [5] report. Smith [31] also reported that *Pseudomonas syringae* pv. *lachrymans* was negative for the production of hydrogen sulphide. In catalase test, the isolated bacteria showed bubbles formation in the medium. This result was supported by Facklam and Elliott [13] in their previous report**.** In Simmons citrate test, the inoculated bacterial medium showed dark blue colour and bacteria showed positive response against citrate medium. Baron *et al.* [5] found similar result for Simmons citrate test in his work**.**In kovac oxidase test purple colour was observed which indicate oxidase negative. Misaghi and Grogan [23] reported that *Pseudomonas syringae*pv. *lachrymans* isolates were negative for oxidase test. Methyl Red medium produce less acid and give yellow colour in methyl red agarmedium. In the present study, the result of methyl red test was confirmed by Crown and Gen [12]. In antibiotic susceptibility assay, highest antibiotic activity was 40.0±0.5mm diameter of zone of inhibition by erythromycin against the isolated bacteria. Hasan and Sikdar [15] found similar zone of inhibition by standard kanamycin against *Pseudomonas sp*. A similar diameter of zone of inhibitions by erythromycin against *Pseudomonas aeruginosa* was reported by Bharathi *et al.* [7]. In screening of antibacterial assay, the highest antimicrobial activity with 8.3±.5mm diameter of zone of inhibition at 40µl/disc concentration was showed by *Allium sativum* methanol extract. Though *Allium sativum* showed highest 8.3±.5mm zone of inhibition against the isolated bacteria but it was resistance. Praba and Kumaresan [26] found minimum zone of inhibition by *Allium sativum* extract against *Pseudomonas aeruginosa* at 50% concentration.This findings also confirmed by Whitemore and Naidu [36] who found inhibitory action of garlic against gram positive and gram negative bacteria.

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Authors' contribution: MFH, SMZH, ZFZ, MFH, MAI and BS designed the experiments and developed the methodology. MFH, SMZH, and MFH prepared the manuscript. MFH, SMZH, ZFZ, MFH and BS collected the data and carried out analysis. MAI and UKA assisted with data analysis and manuscript preparation.

Conflict of interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

1. Akinpelu DA, Onakoyo TM. Antimicrobial activities of medicinal plants used in folklore remedies in southwestern. African Journal of Biotechnology. 2006; 5(11):1078-1081.
2. Al-Bari MAA, Sayeed MA, Rahman MS. Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces bangladeshiensis*, a novel species collected in Bangladesh. Res J Medicine and Med Sci. 2006; 1:77-81.
3. Aksoy HM. Occurrence of *Pseudomonas syringae*pv. *lachrymans* at Bafra province Greenhouses. Plant Pathology Journal. 2006; 5:80-82. doi: 10.3923/ppj.2006.80.82
4. Alzoreky NS, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology. 2003; 80:223-30. doi.org/10.1016/S0168-1605(02)001691
5. Baron E, Finegold S. Bailey and Scott's diagnostic microbiology. 8th edn. The Mosby Company, St. Louis, 1990, MO.
6. Bergey DH, Holt JG, Noel RK. Bergey's Manual of Systematic Bacteriology, 9th Edn. 1994; 1:1935-2045.
7. Bharathi T, Kolanjinathan K, Saranraj P. Antimicrobial activity of solvent extracts of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus amarus* against clinical Pathogens. Global Journal of Pharmacology. 2014; 22:33-45. doi: 10.5829/idosi.gjp. 2014.8.3.83191
8. Bradbury JF. Guide to Plant Pathogenic Bacteria. London, UK: CAB International Mycological Institute, 1986, 154-177.
9. Cappuccino JG, Sherman N. *Microbiology*: A Laboratory Manual. Benjamin Cummings, San Francisco, 2001, 185.
10. Clarke PH, Cowan ST. Biochemical methods for microbiology. J. Gen. Microbiol. 1952; 6:187.
11. Cowan ST. Cowan and Steel's Manual for the Identification of Medical Bacteria. Cambridge University Press, 1974.
12. Crown ST, Gen J. Micro method for the methyl red test Microbiol. 1998; 9:101-109.
13. Facklam, Elliott JA. Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. Clin. Microbiol. Rev. 1995; 8:479-495.
14. Halebian S, Harris B, Finegold SM, Rolfei RD. Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. Journal of Clinical Microbiology. 1981, 444-448.
15. Hasan MF, Sikdar B. Screening of antimicrobial, cytotoxic and pesticidal activities of *Cocciniagrandis* (l.) Voigt. J Microbiol. Biotech. Food Sci. 2016; 5(6):584-588. doi: 10.15414/jmbfs. 2016.5.6.584-588.
16. Hooda R. Antiwrinkle herbal drugs. Journal of Pharmacognosy and Phytochemistry. 2015; 4:277-28.
17. Kagiwata T. Bacteriological characteristics of cucumber angular leaf spot pathogen *Pseudomonas syringae* pv. *lachrymans*. Journal of Agricultural Science. 1990; 35:116-128.
18. Kovacs N. Identification of Psedudomonas pyocyanae by oxidase reaction. Nature. 1956; 178:703. doi:10.1038/178703a0
19. Lelliott RA, Stead DE. Methods for the diagnosis of bacterial diseases of plants. Methods in Plant Pathology. 1987; 2:44-56.
20. Lopes L, Speretta F, Bentley M. Enhancement of skin penetration of vitamin K using monoolein-based liquid crystalline systems. European Journal of Pharmaceutical Sciences. 2007; 32:209-215. doi.org/10.1016/j.ejps.2007.07.006
21. Macconkey A. Lactose fermenting bacteria in faces. J Hygiene. 1905; 5:333-379. doi.org/10.1017/S002217240000259X
22. Macfaddin. Biochemical tests for identification of medical bacteria, 3rded. Lippincot, Williams & Wilkins, Philadelphia PA, 2000.
23. Misaghi I, Grogan RG. Nutritional and biochemical comparison of plant pathogenic and saprophytic fluorescent pseudomonads. Phytopathology. 1969; 59:1436-1450.

24. Mukherjee PK. Phytochemical and therapeutic potential of cucumber, Fitoterapia. 2013; 84:227-236. doi.org/10.1016/j.fitote.2012.10.003
25. Narayanasamy P. Plant Pathogen Detection and Disease Diagnosis. Marcel Dekker, New York, NY, US, 1997.
26. Praba SK, Kumaresan R. Journal of Chemical and Pharmaceutical Research. 1997; 6:677-679.
27. Prescott LM, Harley JP, Klein DA. Microbiology, 5theds. McGraw-Hill, New York, 2002; 1014.
28. Sherman N, Cappuccino JG. Microbiology: a laboratory manual. 6th eds, ISBN. 2005; 81:265-267.
29. Shila SJ, Islam MR, Ahmed NN, Dastogeer KMG, Meah MB. Detection of *Pseudomonas Syringae* pv. *lachrymans* Associated with the seeds of cucurbits. Universal Journal of Agricultural Research. 2013; 1:1-8. https://doi: 10.13189/ujar.2013.010101
30. Simmons JS. A culture medium for differentiating organisms of typhoidcolonaerogenes groups and for isolation of certain fungi. Journal of Infectious Disease. 1926; 39:209. doi:10.1093/infdis/39.3.209
31. Smith MA. Bacterial spot of honeydew melon, Phytopathology. 1946; 36:943-949.
32. Taylor WI, Silliker JH. Isolation of Salmonellae from Food Samples: III. Dulcitol Lactose Iron Agar, a New Differential Tube Medium for Confirmation of Microorganisms of the Genus Salmonella. Applied microbiology. 1958; 6:335-338.
33. Tumpa SI, Hossain MI, Ishika T. Antimicrobial activities of *Psidium guajava, Carica papaya and Mangifera indica* against some gram positive and gram negative bacteria. Journal of Pharmacognosy and Phytochemistry. 2015; 3:125-129.
34. Vincent JM, Humphrey B. Taxonomically significant group antigens in Rhizobium. Journal of General Microbiology. 1970; 63:379-382. doi:10.1099/00221 287-63-3-379.
35. Wang YH, Joobeur T, Dean RA, Staub JE. Cucurbits. Genome Mapping and Molecular Breeding in Plants, Vegetables, 2007, 5.
36. Whitemore BB, Naidu AS. Thiosulfinates. In: Naidu A.S. (Ed.), Natural food antimicrobial systems. Boca Raton, FL: CRC Press, 2000, 265-380.
37. Young JM, Saddler GS, Takikawa Y, DeBoer SH, Vauterin L, Gardan L, *et al.* Names of plant pathogenic bacteria. Review of Plant Pathology. 1996; 7:721-63.