Polyaniline-Pectin nanoparticles immobilized paper based colorimetric sensor for detection of *Escherichia coli* in milk and milk products

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ABSTRACT

In the food quality and safety arena, there is a need to develop novel and sustainable methodologies that can help in the prevention of foodborne diseases. Herein, we report the development of a rapid conducting polymer strip-based sensor using Polyaniline-pectin (PANI-PEC) for the detection of *Escherichia coli* in milk and milk products. Polyaniline-pectin nanoparticles stabilized with biopolymer pectin were synthesized and its characterization studies such as FTIR, UV-Vis spectroscopy, electrical conductivity and particle size analysis were done. The assay parameters were optimized for the selective detection of *E. coli* in milk and milk products. The concentration of PANI-PEC solution immobilized/strip was optimized to be 3 mg/mL as it exhibited good sensitivity and colour intensity. Based on acid production and selectivity for *E.coli*, concentrations of media components like lactose, tryptophan, yeast extract, chondroitin sulphate, sodium lauryl sulphate, potassium chloride, tergitol-7, gentamycin sulphate and ampicillin trihydrate were optimized as 0.9, 0.1, 0.45, 0.015, 0.1, 2, 0.0125, 0.00016 and 0.015 respectively and sample volume was optimized to 500 μL. The developed PANI-PEC colorimetric strip-based sensor detects 0.52 ± 0.17 log CFU/mL *E. coli* within 10: 21 h (h). Further shelf-life study revealed that the developed PANI-PEC colorimetric sensor strips are stable at room temperature up to six months exhibiting the same sensitivity. The results obtained here indicate that this novel and simple paper based colorimetric sensor holds potential for application in food industries as a reliable and rapid method for detection of *E. coli* in milk and milk products at various stages of production and processing.

1. Introduction

Milk and milk products play an important role in human diet across the world. The important task and challenge faced by dairy industries is promising the microbiological safety of milk and milk products as they can easily get contaminated with various pathogenic agents during its journey from farm to fork. Oliver et al. (2005) reported the global public health hazards and related food-borne disease outbreaks associated with the consumption of raw milk and milk products. One of the frequently reported contaminating microorganisms in milk and milk products is *Escherichia coli* (*E. coli*). It is considered as an indicator of fecal contamination and hence indicates deteriorated microbiological quality of milk and milk products. A fair number of studies revealed the presence of *E. coli* in milk and dairy products like cream, butter, ice cream,

chhana (milk coagulated with citric acid/lactic acid at 80–85 °C) based milk products like rasomalai, sandhesh and rasagolla, flavored milk, milk powder, sweetened condensed milk and khoa based sweets (Pal et al., 2012). Rapid detection and identification of *E. coli* is a major challenge faced by food processing industries. Thus development of sensitive method for rapid detection of *E. coli* to accurately identify their presence has become highly desirable (Xu et al., 2016). Traditionally used conventional methods for the detection of *E. coli* are mainly culture-based methods and are regarded as gold standards but are laborious and time consuming (Franco-Duarte et al., 2019). Polymerase chain reaction (PCR) based molecular tools can provide results within 12–48 h but it demands expensive equipments and experienced personnel to carry out the detection protocols (Sue et al., 2014; Rajapaksha et al., 2019). Detection approaches using paper-based

colorimetric sensors has gained extensive popularity because of their simplicity, selectivity, rapidity and cost effectiveness (Yan-Qi and Liang, 2020). Conducting polymers are one of the excellent candidates for developing paper-based colorimetric sensors. A large number of studies have been reported on application of conducting polymers in colorimetric biosensors development. Song et al. (2002) demonstrated the application of a biochromic conjugated polymer (BCP) sensor for detection of pathogens. Fabrication of mixed polydiacetylene/phospholipid vesicles incorporated with glycolipid for the detection of *E. coli* was studied by Su et al. (2005). Majumdar et al. (2005) reported the preparation of gold nanoparticles (AuNPs) and polyaniline (PANI) on cation-exchange resin beads for the detection of glucose in water. Silbert et al. (2006) reported a chromatic polymer polydiacetylene (PDA) based sensor for the detection of both Gram positive and Gram-negative bacteria. A polydiacetylene-based detection system was developed by Scindia et al. (2007) in which PDA facilitates visual detection and colorimetric fingerprinting of bacteria. The sensor could detect bacterial concentration of 10^6 CFU/mL in 7 h (Scindia et al., 2007). Antony and Jayakannan (2011) have developed a self-doped polyaniline-based sensor for the colorimetric detection of cysteine and vitamin-C. The colorimetric sensing was based on electron transfer that takes place in water soluble polyaniline substrate (Antony and Jayakannan, 2011). A novel polyaniline based colorimetric food package label was developed by Kuswandi et al. (2012) for fish spoilage detection. The developed sensor film could monitor the breakdown products of bacterial metabolism in the headspace of fish package (Kuswandi et al., 2012). Li et al. (2014) developed a highly stable paper based colorimetric sensor using polyaniline-poly (sodium 4-styrenesulfonate/-PANI: PSS colloid for the detection of volatile amines.

Porcel-Valenzuela et al. (2015) developed a disposable electrochromic biosensor using polyaniline for the detection of ascorbic acid. When exposed to ascorbic acid solution at pH 3.0 the electrochromic polyaniline film changes its color from blue-violet to green and the color change was monitored using a conventional digital camera (Porcel-Valenzuela et al., 2015).

Thakur et al. (2015) reported a novel and simple polyaniline nanoparticle based colorimetric sensor for real-time monitoring of growth of bacteria. The developed sensor is generic in nature and suggested its applications for detection of all types of pathogenic bacteria by introducing selectivity in the sensing system by addition of specific selective agents in the growth media (Thakur et al., 2015). In another study an intelligent packaging system for the detection of *Salmonella* by incorporating phage PVP-SE1 in PDA vesicles embedded in methylcellulose was developed and in the presence of *Salmonella*, the PVP-SE1-PDA methylcellulose film shows color change from blue to red in 24 h (Taila et al., 2017). Thornton et al. (2018) reported the use of PANI-grafted nylon nano-fibers for the colorimetric detection of HCl. All these studies suggest the potential use of conducting polymers as biosensors and chemo-sensors.

Herein, we report the development of a simple and cost-effective paper based colorimetric sensor using Polyaniline-Pectin nanoparticles (PANI- PEC NPs). The working of this developed sensor is based on the pH and conductivity sensing ability of polyaniline. On doping with acids emeraldine base (EB) form of polyaniline which is in blue color gets converted to green colored emeraldine salt (ES) form which results in increased conductivity. Thus, PANI shows reversible color change on acid-base reaction with reactive compounds. In the present study the metabolic end products of glycolytic pathway of *E. coli* such as lactate, acetate, succinate, malate, etc. induce color change of PANI- PEC film from blue to green which can be monitored visually.

2. Materials and methods

2.1. Synthesis of PANI- PEC nanoparticles

2.1.1. Chemicals and reagents
Aniline-99% (Sigma-Aldrich), Ammonium persulfate (Sigma-Aldrich), Hydrochloric acid (HCl)- 37% (Sigma-Aldrich), Ethanol-HPLC grade (Sigma- Aldrich), NaOH crystals (Himedia), Pectin (Himedia) and Milli-Q water obtained from Milli-Q water purification system (Millipore, USA). PANI- PEC nanoparticles were prepared by the methodology given by Thakur et al. (2015) with some modification using aniline, pectin, HCl, ammonium persulfate (APS) and ethanol. Precipitated PANI- PEC colloidal dispersion was lyophilized at -84 ± 1 °C and 1 ± 0.5 Torr for 4 h and was re-dispersed in Milli-Q water by ultrasonication for 4 h to the required concentrations.

2.2. Characterization of PANI- PEC nanoparticles

2.2.1. Instruments
FTIR spectrum of powdered samples of PANI- PEC nanoparticles was analyzed using Shimadzu, IR Affinity-1S model FTIR spectrometer from the range of 4000–400 cm^{-1} with 4 cm^{-1} resolution at room temperature. UV–Vis spectrum was measured using multimode plate reader of TECAN Infinite M200 PRO model which uses monochromator-based wavelength selection with necessarily no filters, silicon photodiode detector and high energy xenon flash lamp as light source. Then UV-Vis spectra were recorded for the wavelength from range 300–1000 nm with 25 numbers of flashes at room temperature. Electrical conductivity of ES form of PANI- PEC solutions was measured using Eutech PC 2700 multi-parameter bench meter. The average hydrodynamic radius (d.nm) and polydispersity index (PDI) of PANI- PEC particles was measured using Zetasizer nano ZS90 instrument based on principle of DLS. The test was carried out using software Zetasizer version 7.11.

2.3. Construction of colorimetric sensor strips

PANI- PEC solution of optimized concentration was printed on Whatman filter paper grade 4 using Easy Printer (Model LPM-02). It was then dried at 45 ± 1 °C/25 ± 5 min and cut into strips. The strips were exposed to UV light for surface sterilization.

2.4. Optimization of assay protocol for selective detection of E. coli

2.4.1. Optimization of media components
Targeting the β-galactosidase and β-glucuronidase activity of *E.coli*, medium components used for the assay were optimized for the selective enrichment of *E.coli*. Different concentrations (0.1–5%) of lactose, tryptophan, yeast extract, chondroitin sulphate, sodium lauryl sulphate, potassium chloride, tergitol-7, gentamycin sulphate and ampicillin trihydrate were weighed and 100 mL of broth media with pH 7.0 ± 0.1 was prepared. Cell suspensions of different cell levels (~8–~0.5 log CFU/mL) of *E. coli* ATCC 25992 were prepared in normal saline solution (0.85%) by serial dilution and were inoculated into media prepared. Concentrations that gave maximum growth of *E.coli* with minimum time for detection and maximum inhibition of possible contaminants were selected.

2.4.2. Optimization of sample volume
Different volumes of normal saline solution spiked with ~0.5 log CFU/mL *E. coli* were inoculated into optimized media. The sample volume in which the strip gave rapid color change was taken as the optimized sample volume that has to be used in the assay.

2.5. Detection of E. coli using PANI- PEC colorimetric sensor strip

Cell suspensions of *E. coli* with different concentrations from ~8.0

log CFU/mL to ~0.5 log CFU/mL were prepared. The assay was carried out as per the optimized protocol for the detection of *E. coli*. The time taken for change in color of strip from blue to green for different log CFU/mL of *E. coli* was observed at regular intervals of time (30 min–12 h). The same protocol was carried out in sterile milk, raw milk and pasteurized milk to evaluate the sensitivity of the assay in real milk system. The assay was also carried out in 10 samples of butter by preparing a 1:10 dilution of the samples.

2.5.1. Selectivity study of PANI- PEC colorimetric strip-based sensor

Selectivity of the developed sensor was determined by following the same protocol using various Gram positive (*Listeria monocytogenes* ATCC 13932, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579

and *Enterococcus faecalis* ATCC 29212 and Gram-negative (*Salmonella arizonae* ATCC 15611, *Enterobacter aerogenes* ATCC 13048, *Shigella flexnerii* NCTC 9199, *Citrobacter freundii* ATCC 43864, *Yersinia enterocolitica* ATCC 23715, *Proteus vulgaris* ATCC 6380, *Klebsiella pneumoniae* ATCC 27736 and *Serratia marcescens* ATCC 13880) contaminants in normal saline system.

2.6. Shelf-life study of PANI- PEC colorimetric strip

The developed strips were kept in air tightened containers and stored at room temperature, 4 °C and −20 °C and checked the sensitivity and color intensity up to 6 months in 15 days interval.
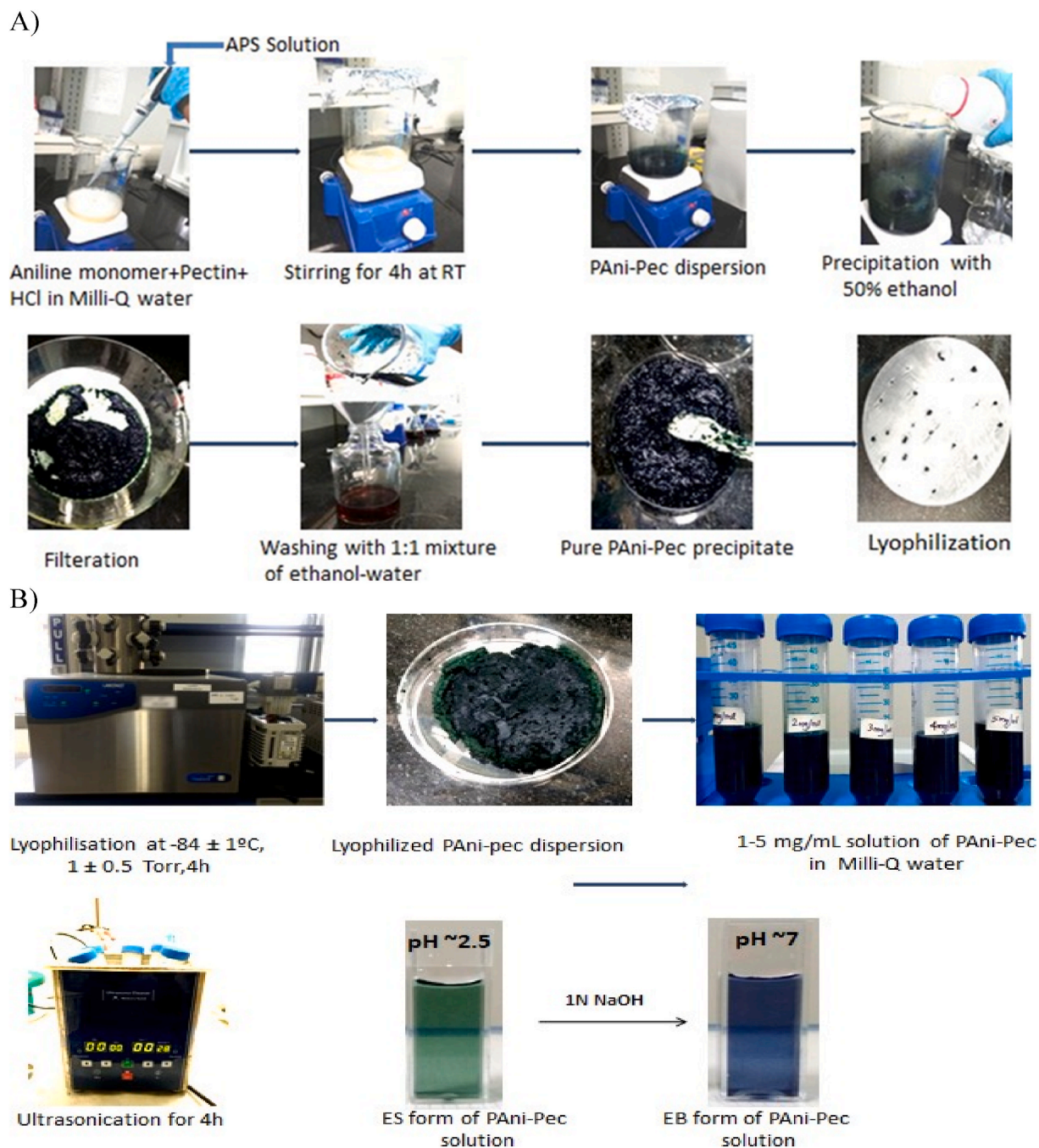
Fig. 1. Synthesis of PANI-PEC nanoparticles. (**A**) Preparation of PANI-PEC precipitate; (**B**) Preparation of PANI-PEC solution in Milli-Q water (APS- Ammonium persulphate; RT- Room temperature).

3. Results

3.1. Synthesis and characterization of PANI- PEC nanoparticles

PANI- PEC nanoparticles were synthesized and the homogenous dispersion prepared was in emeraldine salt form of polyaniline with green color. Concentrated HCl acted as the doping agent and ammonium persulphate (APS) initiated the polymerization reaction of the monomer aniline to polyaniline. Pectin helped in the synthesis of uniformly sized particles with improved solubility and stability. PANI- PEC solutions of different concentrations were prepared in Milli-Q water. The solution obtained was in emeraldine salt (ES) form of polyaniline with pH around 2.5 ± 0.2 and it was converted to emeraldine base (EB) form by addition of few drops of NaOH (1 N) to pH 7 (Fig. 1(A) and (B)).

FTIR spectroscopy of PANI- PEC nanoparticles (Fig. 2 A) revealed same characteristics peaks as that of polyaniline. The additional peaks obtained indicate the presence of pectin. The first extra peaks at 2920 cm^{-1} are due to the asymmetric stretching of aliphatic C–H in pectin. The O–H stretching vibration band of pectin was observed at 3420 cm^{-1}. The band due to the complex formation between carboxyl pectin and protonated amine of polyaniline was appeared at 1724 cm^{-1}. The extra band at 1690 cm^{-1} represents C=O stretching of carboxyl group of pectin and C=C stretching of quinoid rings of PANI merged together with pectin.

The UV-Vis absorption spectra (Fig. 2 B) of emeraldine salt and emeraldine base forms of PANI- PEC solutions showed gradual transition in color from green to deep blue with increase in pH. This indicates structural change of polyaniline from ES to EB form. The first peak observed at 320–330 nm in both ES and EB forms of PANI- PEC is assigned to π-π^* transition of the benzenoid rings, indicating the transition of electron from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The almost similar absorbance at this peak for both ES and EB forms shows the presence of equimolar ratios of amine and imine in EB form of polyaniline.

The electrical conductivity of ES-PANI- PEC dispersed in Milli-Q water of concentrations 1–5 mg/mL was measured and the result of the same has given in Table 1. The particle size measurement of PANI- PEC showed an average hydrodynamic radius of 370 ± 10 nm with polydispersity index (PDI) of 0.225 ± 0.002 (Fig. 2 C).

### 3.2. Construction of colorimetric sensor strips

PANI- PEC colorimetric sensor strip was developed with Whatman filter paper grade 4 immobilized with PANI- PEC solution. A concentration of 3 ± 0.5 mg/mL solution of PANI- PEC solution was selected for its immobilization on paper strips as it gave accurate color intensity and visible color change in less time (Fig. 3 (A and B)). For concentrations $\leq$ 3 mg/mL colour change from blue to green was observed within 2–25 min in 3–5 pH range where as in case of $\geq$ 4mg/mL the time taken for colour change was longer ranging from 15 to 210 min (Table 2). Therefore, 3mg/mL concentration of PANI- PEC solution was selected for its immobilization on paper strips.

Table 1
Electrical conductivity (S cm^{-1}) of PANI-PEC solution.

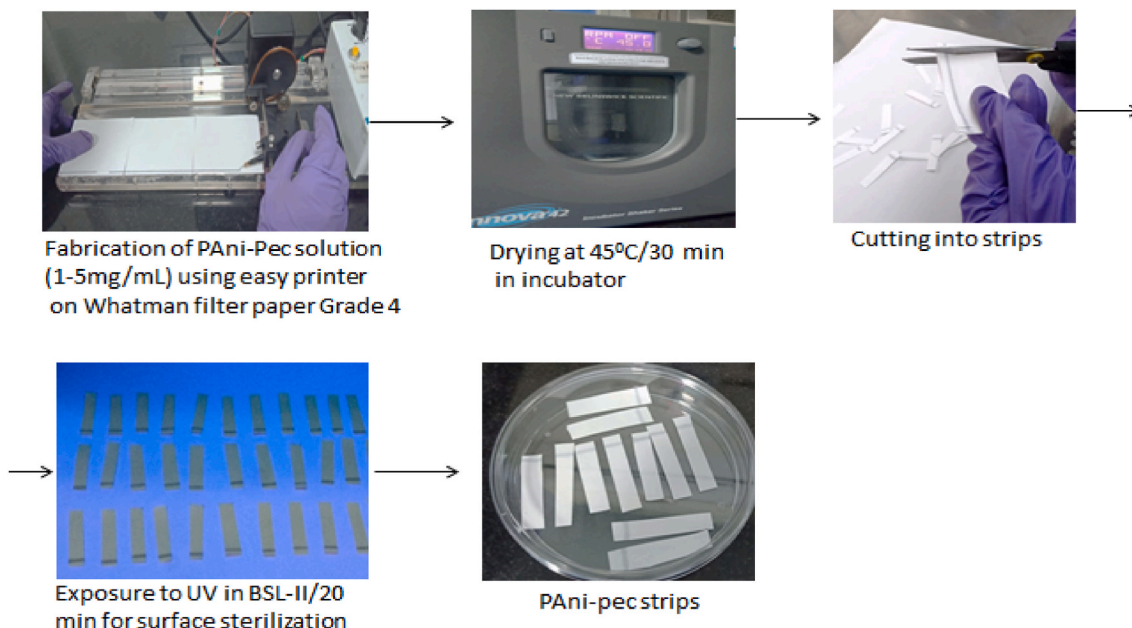| Concentration of ES-PANI-PEC (mg/mL)	Conductivity (S cm^{-1})
1	$2.74 \pm 0.01 \times 10^{-4}$
2	$4.84 \pm 0.01 \times 10^{-4}$
3	$6.53 \pm 0.01 \times 10^{-4}$
4	$7.84 \pm 0.01 \times 10^{-4}$
5	$8.66 \pm 0.01 \times 10^{-4}$

Values are mean $\pm$ standard deviation of triplicate trials (n = 3) for each sample.

A)

B)

C)
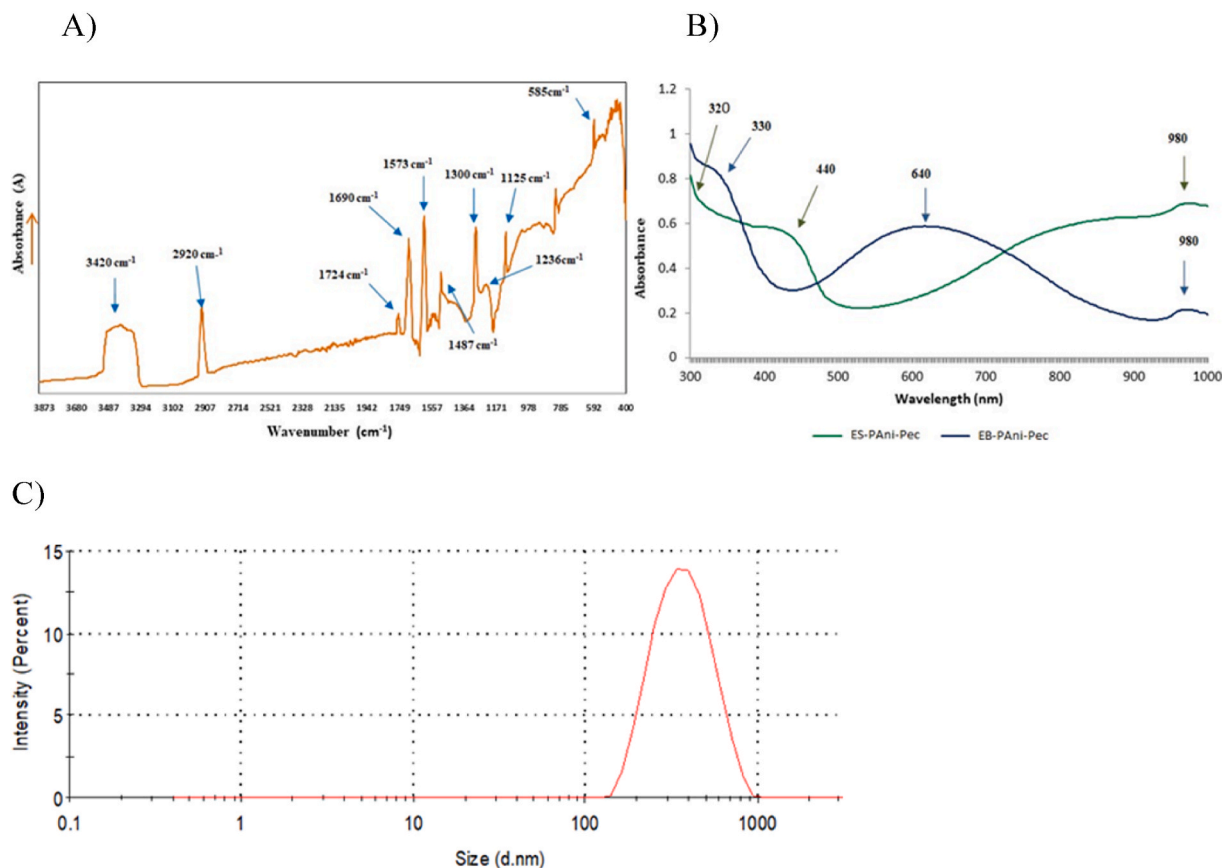
Fig. 2. Characterization of PANI-PEC nanoparticles. (A) FTIR spectroscopy; (B) UV-Vis absorption spectra; (C) Size distribution profile.

A)

B)

Fig. 3. Construction of colorimetric sensor strips. (A) Procedure of PANI-PEC paper based colorimetric sensor strips development; (B) Optimization of PANI-PEC nanoparticles concentration.

Table 2
Time taken for color change of strips with different concentrations of PANI-PEC solution (X- No color change).

PANI-PEC solution (mg/mL)	Time taken for color change of PANI-PEC strips (Min)									
	pH1	pH2	pH3	pH4	pH5	pH6	pH7	pH8	pH9	pH10
1	0	0	2.5 ± 0.4	5.03 ± 0.04	10.1 ± 0.08	X	X	X	X	X
2	0	0	4.2 ± 0.08	8.5 ± 0.4	15.2 ± 0.12	X	X	X	X	X
3	0	0	6.1 ± 0.06	15 ± 0.08	25.03 ± 0.04	X	X	X	X	X
4	0	0	15.02 ± 0.04	40 ± 0.09	150.1 ± 0.08	X	X	X	X	X
5	0	0	30.1 ± 0.08	120.2 ± 0.2	210.6 ± 0.4	X	X	X	X	X

Values are mean $\pm$ standard deviation of triplicate trials (n = 3) for each sample.

3.3. Optimization of assay protocol for selective detection of E. coli

Concentration of different media components (%) were optimized on the basis of acid production and selectivity for *E.coli*. The optimized medium composition was lactose (0.9%), tryptophan (0.1%), yeast extract (0.45%), chondroitin sulphate (0.015%), sodium lauryl sulphate (0.1%), potassium chloride (2%), tergitol-7(0.0125%), gentamycin sulphate (0.00016%) and ampicillin trihydrate (0.015%). The sample volume for the assay was optimized to 500 μL. The detection mechanism and protocol of the developed assay for the detection of *E. coli* is schematically represented in Fig. 4 (A and B).
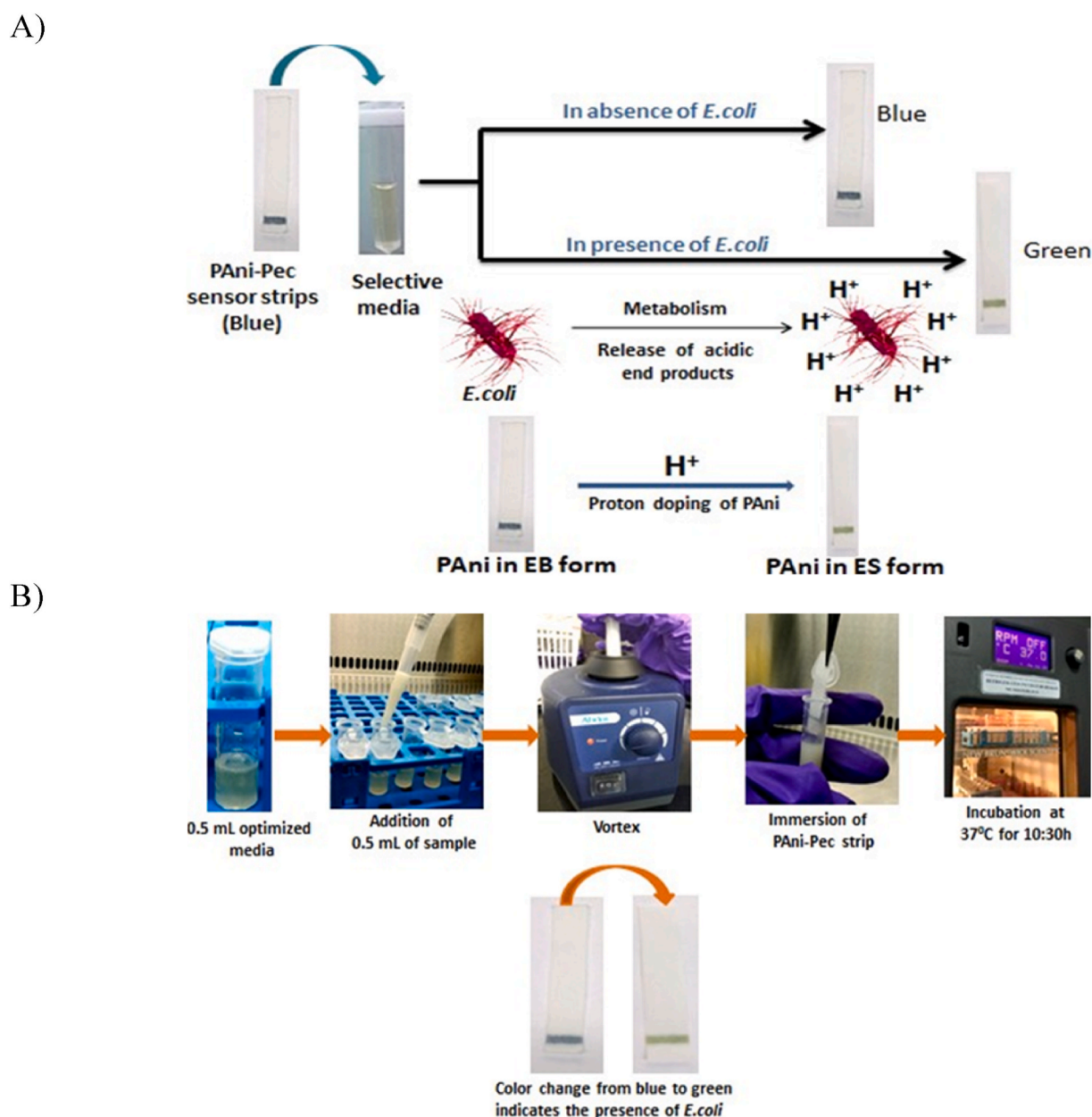
3.4. Detection of E. coli using optimized PANI- PEC colorimetric sensor strip assay

The sensitivity study shows that the developed assay could detect 8.56 ± 0.17 log CFU/mL *E. coli* in 1: 22 ± 0: 07 h and 0.52 ± 0.17 log

CFU/mL *E. coli* in 10: 21 ± 0: 10 h (Table 3, Fig. 5A–C) on incubation at

Table 3
Summary of evaluation of PANI- PEC paper strip sensor assay with IS: 5887 (Part-1):1976 method and two stage enzyme assay\.

No. of Samples		Analytical methods			
		PANI-PEC strip based sensor assay	IS 5887 Part-1 method	Two stage enzyme assay	Outcome
Raw milk	24	+ve	+ve	+ve	Positive for *E. coli*
	16	-ve	-ve	-ve	Negative for *E. coli*
Pasteurized milk	20	-ve	-ve	-ve	Negative for *E. coli*
Butter	10	-ve	+ve	+ve	Positive for *E. coli*

Fig. 4. Detection of *E. coli* using optimized PANI-PEC colorimetric sensor strip assay. (A). Mechanism of detection; (B) Protocol for detection of *E. coli* using paper strip sensor.
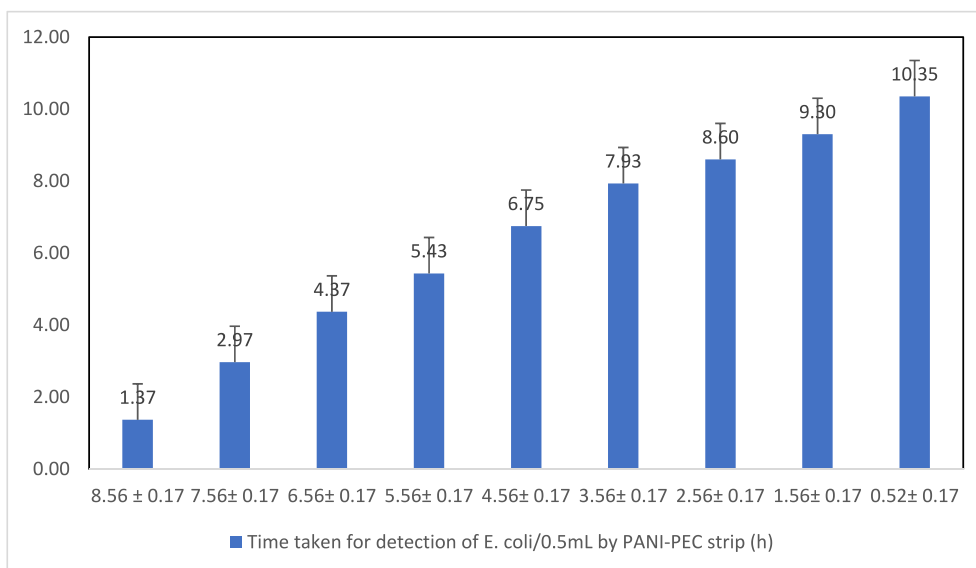
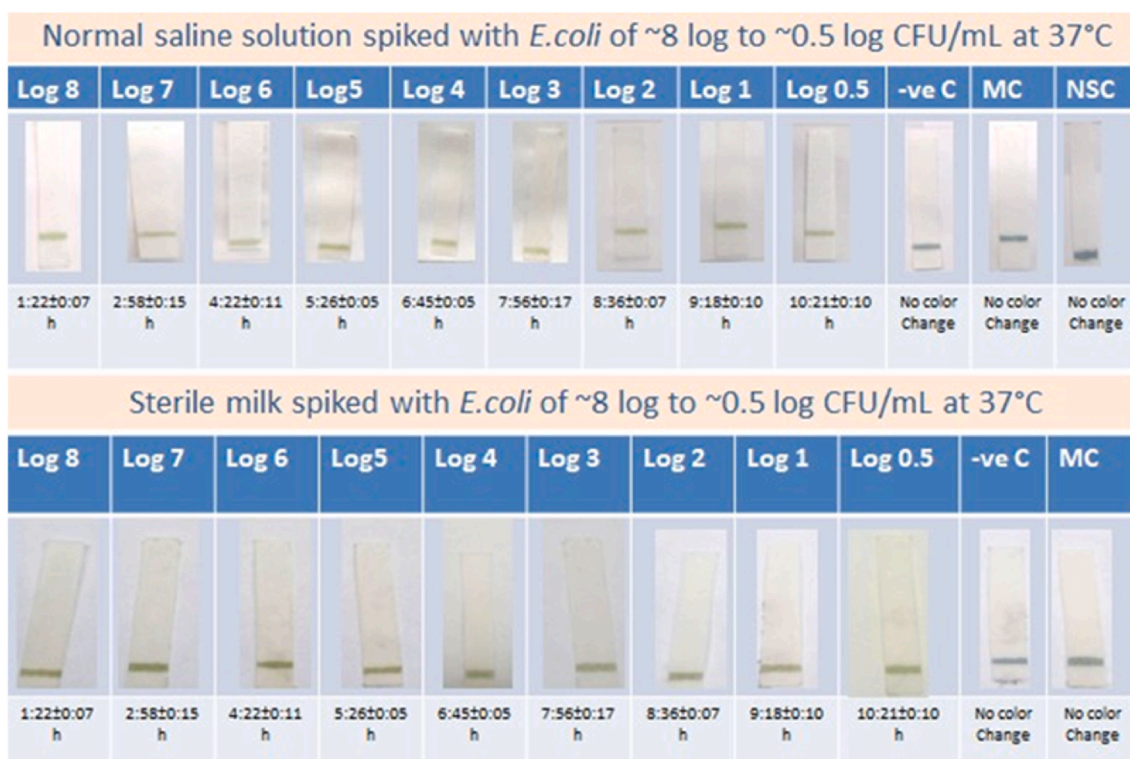**Fig. 5A.** Plot showing sensitivity of PANI-PEC strip based sensor assay for detection of *E. coli*.

Fig. 5B. Sensitivity of PANI-PEC paper strip for the detection of *E. coli*. (-ve C - Negative control; MC- Media control; NSC: Normal saline control).

37 °C both in normal saline system as well as milk system (Fig. 6).

The developed assay was evaluated with 40 raw milk samples, 20 pasteurized milk samples and 10 butter samples and the samples were simultaneously analyzed with approved IS: 5887-1 (1976) method for the detection of *E. coli*. Out of 40 raw milk samples analyzed, 24 samples showed positive for *E. coli*. All the pasteurized milk samples showed negative for *E. coli* whereas all the 10 butter samples showed positive for *E. coli* (Table 4). Similar results were obtained when the samples were analyzed with IS: 5887 (Part I)-1976 method (Fig. 7 A), and two stage enzyme assays for detection of *E. coli* (Fig. 7 B).

3.5. Selectivity study of PANI- PEC colorimetric strip-based sensor

The result of the selectivity study is given in Table 4. The developed assay was found to be highly selective for *E.coli* detection as the optimized medium could inhibit the growth of other Gram-negative bacterial contaminants. Among the organisms studied, *K. pneumonia* showed most sensitivity compared to others (4.5 log). For Gram positive bacterial contaminants like *L. monocytogenes, S. aureus, B. cereus* and *E. faecalis* inhibition was observed up to ~8 log CFU/mL at 37 °C whereas *E. faecalis* showed sensitivity to ~8 log CFU/mL *E.coli* on 12 h 15 min incubation at 37 °C.
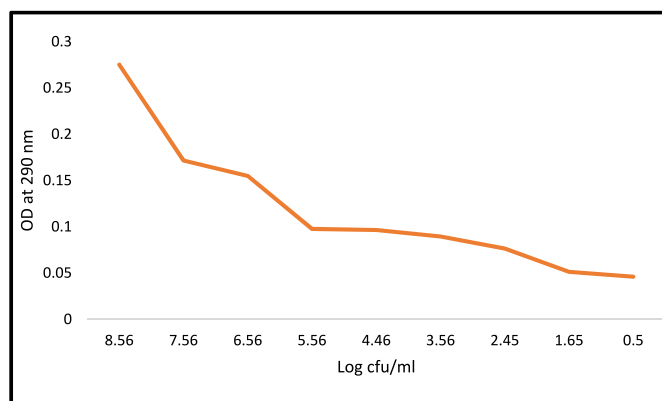
Fig. 5C. Optical density (290 nm) of different log CFU/ml of *E.coli* cells to quantify the switch from blue to green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.6. Shelf-life study of PANI- PEC colorimetric strip

Shelf-life study revealed that the developed PANI-PEC colorimetric sensor strips are stable at room temperature up to six months exhibiting same sensitivity and color intensity as that of freshly prepared strips as well as strips kept at 4 °C and −20 °C.

4. Discussion

Many studies have reported the remarkable electrochromic properties of PANI- PEC particles which are extremely sensitive to surrounding pH resulting in reversible colour change from blue to green and vice-versa (Hu et al., 2018). In our present study the homogenous dispersion of PANI- PEC prepared was in emeraldine salt form of polyaniline with green colour. Amarnath et al. (2014) also reported the synthesis of water dispersible polyaniline-pectin using HCl, ammonium persulphate and pectin as doping agent, polymerization initiator and stabilizer respectively. Characterization studies such as FTIR, UV-Vis spectroscopy, electrical conductivity and particle size analysis of synthesized polyaniline nanoparticles stabilized with biopolymer pectin were done. The characteristics peaks of PANI- PEC nanoparticles studied by FTIR spectroscopy were similar to previously reported studies showing the presence of pectin in PANI- PEC nanoparticle preparation (Amarnath et al., 2014; Kavitha et al., 2016; Thakur et al., 2014). The UV-Vis absorption spectra study revealed that the peak due to π bipolaron electronic transitions was appeared at 960–980 nm (Li et al., 2014; Dai and Jia, 2011; Michira et al., 2007). The peak at 420–440 nm corresponds to polaron band to π^* transition which reflects the protonation (doping) on the nitrogen of imine in ES form (He et al., 2015). The peak at 790–820 nm revealed the presence of localized polarons in a coil-like conformation of the polyaniline chain (Danesh, 2014; Tao, 2010). The absorption peak observed at 620–640 nm for EB form is related to the n-π^* transition or quinoid form structure. This reveals that at high pH range, the benzenoid segments into quinoid segments (Shreepathi, 2006). The decrease in absorbance is due to the decrease in protonation of the polymer backbone that results in decrease in the number of polarons. The absorption maxima of the doped and de-doped polymer remain unchanged with the change in concentration, supporting the stability of electronic structures of the self-doped and de-doped structures in water irrespective of their concentration (Antony and Jayakannan, 2011; Amarnath et al., 2014; Rao et al., 2017; Koh, 2014; Li et al., 2011).

The electrical conductivity measurements of ES-PANI- PEC solution of concentrations 1–5 mg/mL displays that with increases in concentration the conductivity also increases. The enhanced conductivity of PANI- PEC solutions than the conventional PANI solutions is due to the presence of more entrapped PANI nano-particles in pectin chain

(Kavitha et al., 2016; Mostafaei and Zolriasatein, 2012).

The particle size of the PANI- PEC particles were evaluated using dynamic light scattering (DLS) particle size analyzer. The hygroscopic nature of pectin that swells up on water absorption is responsible for the bigger size of PANI- PEC particles compared to PANI particles (Thakur et al., 2014, 2015; Amarnath et al., 2014).

PANI- PEC colorimetric paper based sensor strip was developed with PANI- PEC solution of optimized concentration 3 mg/mL. Immobilization of PANI- PEC solution with easy printer helped in getting sharp and clear colour development. The immobilized PANI- PEC solution in emeraldine base form with blue colour exhibited strong binding with the filter paper surfaces and there was no leaching of PANI- PEC particles from the paper when immersed in buffer solution of pH 7. Gomes et al. (2012) described the printing process of polyaniline on photographic papers and bond papers using a desktop inkjet thermal printer for the application of PANI/paper based devices as humidity sensors. Inkjet printing was done by making a solution containing polyaniline, ethylene glycol, n-methyl-2-pyrrolidone and alcohol in water and doping of printed PANI was done by HCl vapour exposure of paper (Gomes et al., 2012). Based on the colour intensity and time taken for colour change, concentration of PANI- PEC solution for immobilization on paper strips was optimized and it was found that as the concentration and volume of PANI- PEC solution increases the time taken for colour change from blue to green also increases. Similar to the method followed in the study, dos-Santos et al. (2011) constructed cellulose strip incorporated with TDER/PCDA vesicles by spreading 500 μL of TDER/PCDA vesicular suspension on to cellulose strips of size 2 × 10 cm followed by drying for 1 h using air flow at 35 °C for the development of colorimetric biosensor for foodborne pathogen detection (dos-Santos et al., 2011). In medium components optimization study, incorporation of tryptophan, chondroitin sulphate and antibiotics (gentamycin sulphate and ampicillin trihydrate) improved the selectivity of the medium for *E.coli*. Pederson and Skinner (1955) reported the inclusion of tryptophan in the enrichment medium for *E. coli*. Similar results were obtained in a study conducted by Pellock and Redinbo (2017) in which *E.coli* β-glucuronidase was found capable of hydrolyzing chondroitin sulphate and release glucuronic acid. The selective growth of *E.coli* in presence of gentamycin sulphate and ampicillin trihydrate was reported by Aly et al. (2012) and Tadesse et al. (2012).

The sensitivity of the optimized PANI- PEC colorimetric strip based sensor assay for detection of *E. coli* was evaluated by using spiked normal saline and spiked sterile milk system. The sensitivity of the developed sensor was lower compared to the colorimetric sensor developed by Thakur et al. (2015) in which ~10^8CFU/mL and 10^6 CFU/mL of *E. coli* could be sensed within 5 min and 120 min respectively. However they conducted the experiment using lysogeny broth, which is a general purpose medium used for cultivation and growth of bacteria in general and not specific for *E.coli* and also the sensors were not analyzed for its selectivity with other bacterial contaminants or in other food matrixes. They constructed the sensor using PANI-pectin-agarose dispersion films glued on PET strips, which has difficulty in preparation, handling and storage (Thakur et al., 2015). Further the results obtained in the present study had no interference with factors like physico-chemical properties of milk or different constituents of milk. This shows the potential of application of the developed assay as a rapid technique for monitoring the presence of *E. coli* in milk and milk products by dairy industries. The developed sensor exhibited excellent shelf life and the high shelf life of the strips can be attributed to the absence of any bio-recognition elements such as antibody, DNA or any enzymes in the strips. This dramatically improves its shelf life and makes the strips capable of withstanding rough handling (Thakur et al., 2015).

5. Conclusion

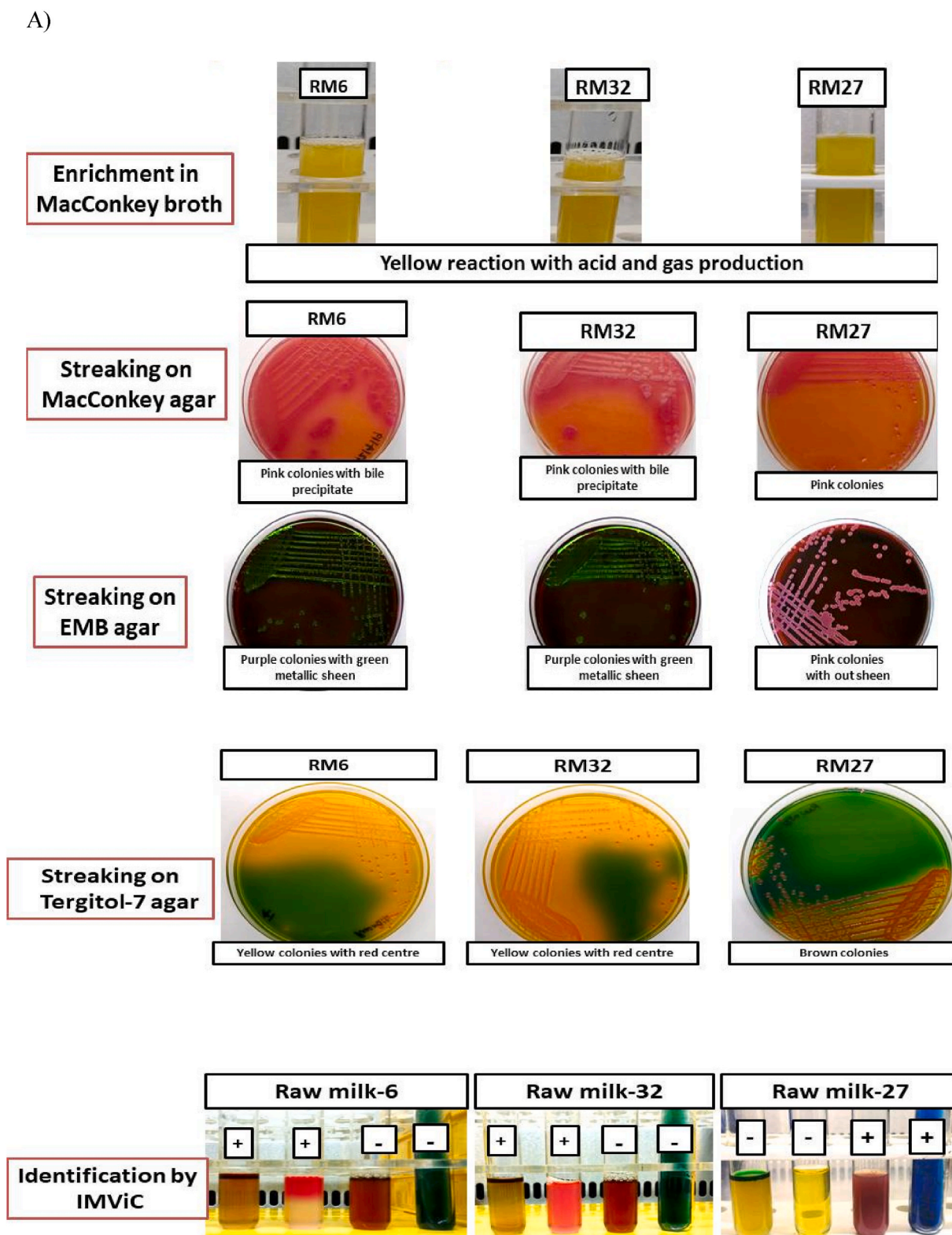The developed PANI- PEC colorimetric strip-based sensor assay

A)

Fig. 6. Evaluation of PANI-PEC paper strip sensor assay. (A) With IS: 5887 (Part-1):1976 method; (B) With two stage enzyme assay developed at ICAR-NDRI.

works on the basis of selective enrichment of *E. coli* and the interaction of their acidic metabolites with Polyaniline-pectin colorimetric paper strip. Paper strips immobilized with 3 mg/mL PANI-PEC solution were prepared and assay parameters like sample volume (500 μL) and various media components (lactose - 0.9%, tryptophan - 0.1%, yeast extract-

0.45%, chondroitin sulphate - 0.015%, sodium lauryl sulphate - 0.1%, potassium chloride - 2%, tergitol-7 - 0.0125%, gentamycin sulphate - 0.00016% and ampicillin trihydrate - 0.015%) were optimized in the range of 0.9, 0.1, 0.45, 0.015, 0.1, 2, 0.0125, 0.00016 and 0.015. The assay exhibited good sensitivity and selectivity for detection of *E. coli*
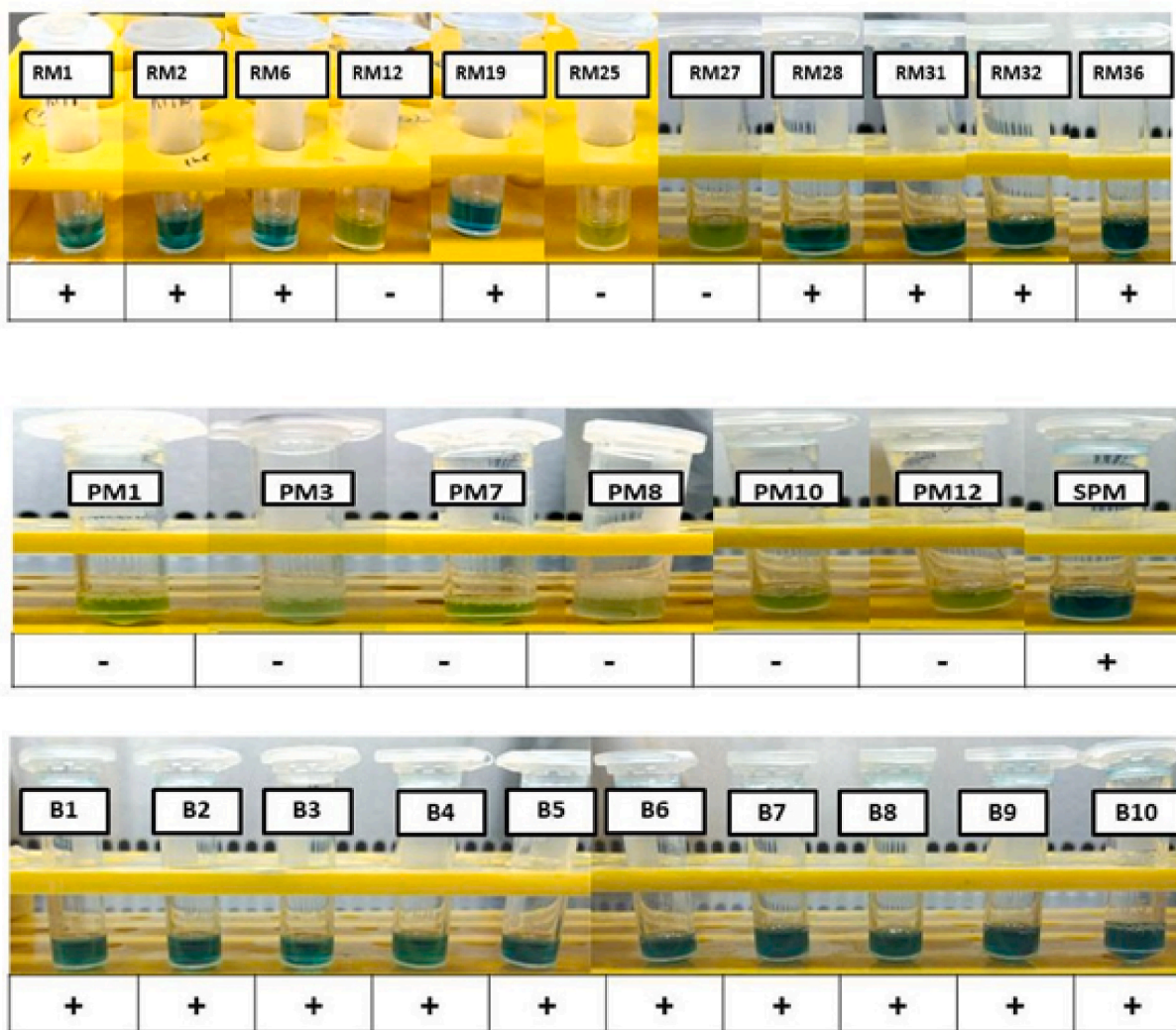
B)

Fig. 6. (*continued*).

Table 4
Inhibition level of Gram negative contaminants in the developed assay.

Gram negative contaminants	Log CFU/mL
Salmonella arizonae	5.4 ± 0.12
Enterobacter aerogenes	5.2 ± 0.12
Shigella flexneri	7.4 ± 0.12
Citrobacter freundii	6.3 ± 0.76
Yersinia enterocolitica	6.5 ± 0.15
Proteus vulgaris	7.6 ± 0.11
Klebsiella pneumonia	4.5 ± 0.15
Serratia marcescens	5.7 ± 0.07

Values are mean $\pm$ standard deviation of triplicate trials (n = 3) for each bacteria.

without interfering with factors like physico-chemical properties of milk or different constituents of milk, promising its applicability in milk system. Absence of any bio-recognition elements such as antibody, DNA or any enzymes in the strips along with the excellent thermal and environmental stability of polyaniline dramatically improves its shelf life and makes the strips capable of withstanding rough handling. Based on the above findings, it can be concluded that the developed PANI- PEC colorimetric strip-based sensor assay is a sensitive, selective and simple to use method for the detection of *E. coli*. The developed assay was found to inhibit the growth of major gram positive pathogens and most of the potential gram negative bacteria used in the study that are commonly found in milk. The developed assay has lot of industrial importance enabling routine monitoring of *E. coli* in milk and milk products at various stages of production and processing.

CRediT authorship contribution statement

M.K. Anjali: conducted experimental design, experimental work, Formal analysis, and was the primary author of this manuscript. **G. Bharath:** assisted with experimental work. **H.M. Rashmi:** Formal analysis. **Jaswal Avinash:** assisted with experimental work. **Kumar Naresh:** assisted with writing, editing, Formal analysis. **P.N. Raju:** Data analysis and shelf life studies. **H.V. Raghu:** conducted experimental design, experimental work, Formal analysis, and was the corresponding author of this manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial

Fig. 7. Sensitivity of PANI-PEC colorimetric strips stored at different temperatures.

interests or personal relationships that could have appeared to influence the work reported in this paper.

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