Simple, rapid and on spot dye-based sensor for the detection of Vibrio load in shrimp culture farms

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Received: 22 February 2021 / Revised: 9 April 2021 / Accepted: 11 April 2021 / Published online: 3 May 2021
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Abstract
For the detection of Vibrio bacteria, a kit involving two-step method was developed. In the first step, a specific media was added in the water sample which selectively promote the growth of vibrios and inhibit the growth of other bacteria. The second step involved addition of dye-based sensor (already developed in our previous work) in the sample which detect the active Vibrio and changed the colour of the sample to red/pink. The vibrio detection kit was optimised on five different species of Vibrio (V. cholerae, V. parahaemolyticus, V. campbellii, V. harveyi and V. proteolyticus) and two negative control bacteria (Escherichia coli and Bacillus subtilis). The kit was further evaluated on aquaculture pond water and probiotics used in aquaculture farms. It successfully estimated Vibrio concentration of all the five strains in aquaculture ponds. The negative control bacteria and probiotics were not sensed by the kit. Hence, the kit developed here is perfect for the detection of Vibrio, especially in aquaculture farms.

Keywords Kit · Water · Vibrio · Aquaculture · Probiotics · Detection

Introduction
The members of Vibrio genus are asporogenous Gram-negative rods that may have a single, rigid curve or are straight rods. Vibrio are motile and possess single polar flagellum. They are glucose fermenters and do not produce gas. Most of them produce oxidase and catalase (Kaysner et al. 2004). Among them, V. parahaemolyticus, V. cholerae and V. vulnificus are well-recognised human pathogens (Bonnin-Jusserand et al. 2019). V. cholerae causes cholera and it is indirectly transmitted through contaminated water supplies. Apart from drinking water, vibrios are also the major microbiota of the ecosystems such as marine water, estuarine ecosystem and aquaculture farm. Many of them are potent pathogens for the animals reared in aquaculture (Chatterjee et al. 2012 and Haldar et al. 2011). The Vibrio spp. causes vibriosis, which is a most predominant diseases among organisms bred in aquaculture and other fishes. It is also accountable for mortality of cultured aquaculture organisms globally (Lavilla-Pitogo et al. 1998 and Chen et al. 2000). The species of Vibrio which are generally related with shrimp diseases are V. parahaemolyticus, V. anguillarum, V. harveyi, V. alginolyticus, V. splendidus and V. vulnificus (Chatterjee et al. 2012 and Haldar et al. 2010). Therefore, considering the epidemiological significance of the Vibrio spp. both in public health and aquaculture, there is an earnest need to develop a rapid and portable kit to detect them in the water samples in simpler way to prevent any disease outbreak which leads to spread of disease and huge economic loss, respectively.

Our group has developed a multiplex PCR based method for accurate identification of some of the important Vibrio spp., but this method requires initial screening in selective media, subsequent DNA extraction and amplification through PCR (Haldar et al. 2010 and Neogi et al. 2010). However, this method needs sophisticated laboratory facility which is generally not available in the shrimp farms. Similarly, many techniques and methods have been established
for the detection of *Vibrio* spp. for example, typing methods, molecular based detection methods, culture-based identification, and antibody-based assays (Ramamurthy et al. 2020; Bisha et al. 2012; Kaysner et al. 2004). Although, many of these methods are rapid but also very expensive and need skilled persons to operate them. The traditional microbiological methods which are used commonly to detect them are very tedious and time-consuming. In addition, onsite testing without microbiological laboratory and skilled manpower is not possible for any of these methods.

Recently, our group has developed a dye-based sensor which senses the total bacteria in the water just by dipping 1 cm² coated membrane in 1 mL of water sample (Binod et al. 2018). However, when the kit was introduced in the aquaculture settings; on the basis of feedback from the end user, it was strongly felt that along with the total bacterial count, specific kit was also needed which can specifically detect the selected *Vibrio* load of the pond water. Hence, with the help of this dye-based sensor, we have made an attempt to develop a kit for easy, rapid and affordable detection of *Vibrio* spp. onsite. This kit involves two steps in which the first step selects *Vibrio* and inhibits other bacterial species in the given sample by adding the designed medium. The second step will then detect *Vibrio* in the sample by adding dye-based kit by changing the colour of the sample into pink/red.

Therefore, the main objectives of this study were (1) development of vibrio detection kit based on the dye-based sensor, (2) optimization of the prepared kit on different standard *Vibrio* species taking *E. coli* and *B. subtilis* as negative control and (3) evaluation of the kit in aquaculture pond water.

### Materials and methods

#### Materials

Polyvinylidene fluoride (MW = 573 KDa, Solef, Solvay, France), polyester fabric (Filtration Sciences Corporation, USA), N, N dimethyl formamide (Qualigen, India), d-(+)-Glucose anhydrous (Hi media, India), Graphene oxide (Sigma-Aldrich) and 2, 3, 5-Triphenyltetrazolium chloride (≥ 99.0% SIGMA, Life Science) were used to prepare the asymmetric membranes. Reverse osmosis treated water was used for the membrane preparation.

Nutrient agar, nutrient broth, Luria–Bertani broth and TCBS Agar (Hi media, India) were used for all microbiology studies. *Escherichia coli* (NCIM2065), *Bacillus subtilis* (NCIM2920), *Vibrio cholerae* (N16961), *Vibrio parahaemolyticus* (IDH02640), *Vibrio campbellii*, *Vibrio harveyi*, and *Vibrio proteolyticus* (Isolated from seawater and identified in our laboratory) were used for testing and evaluating the vibrio kit. In addition, commercial probiotic containing *Paracoccus pantotrophus* (PondDtox, Novozymes) was used to test the vibrio kit.

Casein enzymic hydrolysate, yeast extract, proteose peptone, sucrose, sodium thiosulphate, sodium citrate, sodium deoxycholate, sodium chloride, oxgall, sodium lauryl sulphate, bile salts and potassium tellurite (HiMedia, India) were used to prepare *Vibrio* selective medium.

#### Preparation of dye-based sensor for the detection of bacteria

The dye-based sensor was prepared as described in our previous paper (Binod et al. 2018). In brief, PVDF membrane of 7% W/W concentration was prepared using dimethyl formamide as solvent. 5% Glucose (pH 8.6) was dissolved in the non-solvent medium (water). The membrane was then cut into 1 x 1 cm² pieces and autoclaved. The membrane pieces were then dip-coated in the prepared solution of filter sterilised tetrazolium dye (5 mg/mL), sterile graphene oxide (25 µg/mL) and sterile nutrient broth inside laminar air flow for 24 h. Membrane pieces were then taken out from the solution and allowed to dry inside the laminar.

#### Preparation of selective growth media for *Vibrio* species

Selective media of different compositions were prepared which promoted the growth of different *Vibrio* species and inhibited the growth of other bacterial species. Total six types of such media compositions were prepared (Table S1–S5), out of which only one medium was selected for further experiments (Table 1) as the other five media were showing inconsistent results (Table S6–S14).

| Ingredients                     | g/50 mL |
|---------------------------------|---------|
| Proteose peptone                | 1.7     |
| Yeast extract                   | 0.9     |
| Sodium thiosulphate             | 1.7     |
| Sodium citrate                  | 1.7     |
| Oxgall                          | 1.4     |
| Sucrose                         | 3.4     |
| Sodium chloride                 | 1.7     |
| pH at 25 °C                     | 8.6 ± 0.2|

Table 1 Composition of selective growth medium for *Vibrio* spp.
Application of selective growth medium for the detection of different Vibrio species

For the evaluation of selective growth medium, initially two Vibrio species were selected. V. cholerae and V. parahaemolyticus were used as positive control and E. coli and B. subtilis were used as negative control. Subsequently, three more Vibrio strains commonly reported from shrimp culture water (V. campbellii, V. harveyi and V. proteolyticus) were used to validate the efficiency of the prepared medium. Various concentration of the prepared medium (100, 200, 300, 400, 500 and 1000 µL) was standardised on different cell densities (10^2–10^8 CFU/mL) at 5, 15 and 30 min for all the selected bacteria. Sterile water was used as the diluent for the preparation of bacterial solution. All the selected positive and negative control bacteria were grown in nutrient broth for 12 h and washed subsequently with physiological saline (0.9 wt% NaCl) 3–4 times (3500 rpm for 15 min) to remove any traces of the nutrient from the bacterial cells. The cells were then resuspended in the diluent. The washed cells were then diluted for different concentrations and spread on nutrient agar plates simultaneously to find out the bacterial concentration.

The dye-based sensor of size 1 cm² was then dipped into the bacterial solution containing the medium and kept at 37 °C. The colour change in the water was then monitored periodically.

Assessment of vibrio detection kit in the water sample spiked with probiotics

To check whether the kit is detecting probiotics in the water sample, a range of dilutions of Vibrio culture was mixed with a specific cell concentration of probiotics and tested with the vibrio detection kit. In detail, the probiotic was grown in the sterile pond water and was diluted subsequently so that the bacterial concentration reaches to 10^4 CFU/mL approximately. The selected Vibrio strain was grown in the LB media and its different dilutions were prepared in sterile pond water. The diluted probiotic (100 µL) of 10^4 CFU/mL cell concentration was then added to all the different dilutions of Vibrio. All the mixtures were spread on nutrient agar plates and TCBS plates to determine the total bacterial load and Vibrio concentration, respectively. As comparison standards, another set was prepared containing different dilutions of probiotic and Vibrio separately. The dilutions of probiotics and Vibrio were spread on nutrient agar plates and TCBS plates, respectively, to determine the total bacterial load.

Each of the prepared mixtures and separate dilutions was then inoculated in the vibrio detection kit containing 500 µL of the selective growth medium, which were incubated for 15 min and the dye-based sensor of size 1 cm² was then dipped into the kits and kept at 37 °C. The colour change in the water was then monitored periodically. All the experiments were done in duplicates.

Evaluation of vibrio detection kit in two different aquaculture farms

Applicability of the vibrio detection kit was tested on two different aquaculture farms which have unrelated environmental and water parameters and located distantly from each other. Water samples from aquaculture farms were collected from Bhavnagar, Gujarat (India) and Bhimavaram, Andhra Pradesh (India). Both the ponds are practising intensive type of culture (100/m²) with regular use of different probiotics and chemicals to improve the productivity.

For sample collection, polypropylene bottles (for physicochemical parameters) and sterile screw cap vials (for microbiology) were obtained from the local market. Physicochemical parameters (salinity, total dissolved solids and pH) were measured for all the collected aquaculture pond water samples with manual refractometer (ATAGO), digital TDS metre (Eutech) and digital pH metre (Eutech), respectively.

For the total Vibrio count, the water samples were spread on TCBS agar plate.

One millilitre of all the water samples was collected in sterile transparent container (e.g. 1.5/2 mL vials) containing 500 µL of the selective growth medium. It was incubated for 15 min and the dye-based sensor of size 1 cm² was then dipped into the water samples and kept at 37 °C (the samples from Bhimavaram were kept at room temperature, 25–28 °C approximately). The colour change in the water was then monitored periodically. All the experiments were done in triplicates.

Results and discussion

Dye-based bacterial detection kit

In our previous study, the principle and properties of dye-based bacterial detection kit have already been explained (Binod et al. 2018). In brief, the membrane used as the kit was prepared by wet phase separation technique. The replacement of solvent by non-solvent in the formation process of asymmetric membrane results in the creation of macro-voids which gets filled by glucose particles present in the water (non-solvent) during the diffusional exchange. Additives such as glucose, tetrazolium and graphene oxide were impregnated onto the membrane surface so that they could leach in surrounding medium and subsequently get utilised by bacteria present in the water.
Detection of different Vibrio strains with the aid of dye-based sensor and prepared media

The principle behind the vibrio detection kit is that the specific medium added in the water sample allows the growth of only Vibrio spp. and inhibits the growth of other unspecific bacteria. Subsequently, the dye-based sensor detects the active Vibrio and change the colour of the sample to red/pink.

The prepared medium contains proteose peptone and yeast extract which provides nitrogenous compounds, vitamin B complex and other essential growth nutrients. Bile salt derivative, i.e. oxgall and sodium citrate inhibit gram-positive bacteria and coliforms (Howard 1994). A good source of Sulphur is also present in the form of sodium thiosulphate. For the metabolism of vibrios, sucrose is added as a fermentable carbohydrate.

Initially, different concentrations of the selected medium were used for a range of cell densities of positive and negative control bacteria for the optimization of the medium concentration used in the vibrio detection kit. The selected medium quantity from 100 to 400 µL showed the colour change of the negative control bacteria for all the different bacterial concentration (Table 2). Therefore, the medium concentration ranging from 100 to 400 µL was not sensitive for positive control bacteria and hence, cannot be used for vibrio detection kit. Again, the 1000 µL quantity was giving inconsistent results for varying bacterial concentration. For higher bacterial concentration, the colour change was slow and for less bacterial load, the colour change was faster when compared with the results of all the other medium concentration (Table 2). Finally, the 500 µL concentration of medium was selected which was showing delayed or no colour change in the negative control bacteria and had consistent results with respect to the different bacterial concentrations.

For the optimization of medium concentration (explained in the above paragraph), the incubation time of 15 min was selected because short duration of incubation could not inhibit the growth of nonspecific bacteria in the medium effectively and too long incubation might give inconsistent result as the vibrio can grow in the medium exponentially affecting the time of colour change. Therefore, to confirm the optimum incubation time for

| Bacteria                          | Media conc. (µL) | Time taken (in h) for colour development by corresponding bacterial load (CFU/mL) |
|----------------------------------|-----------------|---------------------------------------------------------------------------------|
|                                  | 102             | 103                                | 104                                | 105                                | 106                                | 107                                |
| Vibrio cholera                    | 100             | 14:30                              | 10:30                              | 6:45                               | 3:30                               | 2:00                               | 00:35                             |
|                                  | 200             | 14:30                              | 11:00                              | 6:45                               | 3:30                               | 2:00                               | 00:35                             |
|                                  | 300             | 15:00                              | 11:40                              | 9:00                               | 4:00                               | 2:15                               | 00:40                             |
|                                  | 400             | 15:00                              | 12:00                              | 9:00                               | 4:15                               | 2:15                               | 1:00                              |
|                                  | 500             | 15:30                              | 12:30                              | 10:00                              | 5:00                               | 2:30                               | 00:50                             |
|                                  | 1000            | 11:10                              | 11:00                              | 10:00                              | 8:00                               | 6:30                               | 6:10                              |
| Vibrio parahaemolyticus           | 100             | 14:00                              | 10:00                              | 6:00                               | 3:00                               | 1:30                               | 00:10                             |
|                                  | 200             | 14:00                              | 10:00                              | 6:15                               | 3:00                               | 1:30                               | 00:15                             |
|                                  | 300             | 16:00                              | 10:45                              | 9:30                               | 3:30                               | 1:30                               | 00:15                             |
|                                  | 400             | 16:00                              | 10:45                              | 10:15                              | 3:30                               | 2:00                               | 00:30                             |
|                                  | 500             | 16:30                              | 11:00                              | 10:15                              | 3:30                               | 2:00                               | 00:45                             |
|                                  | 1000            | 8:00                               | 6:10                               | 6:00                               | 4:00                               | 3:00                               | 1:45                              |
| Escherichia coli                  | 100             | 15:30                              | 12:30                              | 8:00                               | 5:00                               | 2:00                               | 00:35                             |
|                                  | 200             | 15:30                              | 12:30                              | 8:15                               | 6:45                               | 2:30                               | 00:35                             |
|                                  | 300             | 16:35                              | 14:00                              | 10:00                              | 7:15                               | 5:00                               | 3:30                              |
|                                  | 400             | 17:00                              | 14:00                              | 10:00                              | 10:00                              | 6:15                               | 5:30                              |
|                                  | 500             | –                                  | –                                  | –                                  | –                                  | 24:00                              | 24:00                              | 16:00                             |
|                                  | 1000            | –                                  | –                                  | –                                  | –                                  | –                                  | –                                 |
| Bacillus subtilis                 | 100             | 18:00                              | 14:00                              | 9:00                               | 8:30                               | 8:00                               | 6:00                              |
|                                  | 200             | 18:00                              | 14:00                              | 9:00                               | 8:30                               | 8:15                               | 6:00                              |
|                                  | 300             | 18:40                              | 15:15                              | 11:00                              | 11:00                              | 10:00                              | 8:00                              |
|                                  | 400             | 20:00                              | 16:00                              | 12:00                              | 12:00                              | 10:00                              | 10:00                             |
|                                  | 500             | –                                  | –                                  | –                                  | –                                  | –                                  | –                                 |
|                                  | 1000            | –                                  | –                                  | –                                  | –                                  | –                                  | –                                 |

(–): No colour development
the selected medium, three different incubation time was selected, i.e. 5 min, 15 min and 30 min.

In case of *Vibrio cholerae* and *Vibrio parahaemolyticus*, faster rates of colour development were observed when the incubation time was increased from 5 to 15 min. However, when the incubation time was increased further to 30 min, it did not significantly increase or alter the rate of colour development in corresponding dilutions of bacterial load (Table 3).

In case of *Escherichia coli*, lesser span of incubation resulted in faster colour development in higher bacterial counts and obtained an irregular pattern for lower bacterial load. This was expected due to reduced time for effective selectivity of the medium. Thus, higher, incubation time of 15 min observed significantly slower rates of colour development. Further, 30 min incubation span observed similar durations of colour development for all the bacterial counts. In case of *Bacillus subtilis*, no colour development was observed at any bacterial load irrespective of incubation span. This may be by virtue of effective selectivity of the medium (Table 3).

The incubation span, may, therefore, be restricted to 15 min as it optimally enhanced colour development rates in case of *Vibrio* spp., and prolonged the same in case *E. coli* and *Bacillus* sp. so as to facilitate a threshold limit.

In conclusion, the vibrio detection kit was prepared with 500 µL medium concentration with incubation time of 15 min. To validate the efficiency of the kit further experiments were done with other positive control bacteria, e.g. *V. proteolyticus*, *V. harveyi* and *V. campbellii*.

Table 4 clearly shows that the vibrio detection kit is very sensitive for *Vibrio* spp. and it is showing delayed/no colour change for negative control bacteria. Hence, the kit can be used effectively for the detection of *Vibrio* in any water source. Figure 1 illustrates the colour change by the kit, in which positive control bacteria changed the colour of water and the negative control bacteria did not show any colour change. All the experiments were performed for three times

### Table 3

| Bacteria                  | Incubation time | Time taken (in hours) for colour development by corresponding bacterial load (CFU/mL) |
|---------------------------|-----------------|----------------------------------------------------------------------------------|
|                           |                 | 10²                  | 10³                  | 10⁴                  | 10⁵                  | 10⁶                  | 10⁷                  |
| *Vibrio cholerae*         | 5 min           | 8:58                | 7:44                | 6:32                | 5:08                | 3:04                | 0:26                |
|                           | 15 min          | 7:23                | 6:39                | 5:51                | 4:50                | 2:55                | 0:17                |
|                           | 30 min          | 7:50                | 6:17                | 5:23                | 4:27                | 2:38                | 0:31                |
| *Vibrio parahaemolyticus* | 5 min           | 6:51                | 6:11                | 5:46                | 4:21                | 3:06                | 1:30                |
|                           | 15 min          | 6:36                | 5:46                | 4:50                | 3:46                | 2:56                | 0:45                |
|                           | 30 min          | 6:50                | 5:40                | 5:05                | 3:35                | 2:40                | 0:50                |
| *Escherichia coli*        | 5 min           | NA                  | –                   | –                   | –                   | –                   | –                   |
|                           | 15 min          | NA                  | 24:20               | 21:40               | 20:40               | 19:40               | 17:10               |
|                           | 30 min          | NA                  | 23:55               | –                   | 23:55               | 23:25               | 19:55               |
| *Bacillus subtilis*       | 5 min           | –                   | –                   | –                   | –                   | –                   | –                   |
|                           | 15 min          | –                   | –                   | –                   | –                   | –                   | –                   |
|                           | 30 min          | –                   | –                   | –                   | –                   | –                   | –                   |

NA: data not available, (–): no colour development

### Table 4

| Bacteria                  | Time taken (in h) for colour development by corresponding bacterial load (CFU/mL) |
|---------------------------|----------------------------------------------------------------------------------|
|                           | 10²                  | 10³                  | 10⁴                  | 10⁵                  | 10⁶                  | 10⁷                  |
| *V. cholerae*             | 8:30                | 8:00                | 6:00                | 5:00                | 3:15                | 00:45               |
| *V. parahaemolyticus*     | 6:00                | 5:15                | 4:30                | 3:15                | 2:00                | 00:45               |
| *V. proteolyticus*        | 6:45                | 5:30                | 4:45                | 3:45                | 2:30                | 1:30                |
| *V. harveyi*              | 6:00                | 5:15                | 4:00                | 3:15                | 1:45                | 00:45               |
| *V. campbellii*           | 5:00                | 4:30                | 3:00                | 2:30                | 1:30                | 00:45               |
| *E. coli*                 | –                   | 24:00               | 22:00               | 21:30               | 20:45               | 16:30               |
| *B. subtilis*             | –                   | –                   | –                   | –                   | –                   | –                   |

(–): No colour development
and time taken to change the colour was same. Therefore, mean of time taken to change the colour was not mentioned.

Finally, after all the standardisation the average time intervals were obtained which correlated to the approximate cell densities of *Vibrio* which is shown in Table 5. Hence, a rough estimate of *Vibrio* in the water samples can be determined using these time periods.

### Testing vibrio detection kit in mixed culture of *Vibrio* and probiotics

Probiotics are commonly used in the aquaculture farms for various purposes such as enhancing the innate immunity of shrimps, achieve better growth and specially to mitigate the proportion of harmful *Vibrio* spp. (Martínez Cruz et al. 2012). Therefore, it is necessary to check whether the presence of probiotic bacteria of non-vibrio origin in the pond is affecting the sensitivity of kit. Hence, one *Vibrio* strain was selected and mixed with the commercially available probiotic to assess the vibrio detection kit.

The total bacterial count and *Vibrio* count of mixed culture (*Vibrio* and probiotics) shows that the probiotics and *Vibrio* both are coexisting in the mixture. Since in the higher dilutions of total bacterial count there is an increase in *Vibrio* cell concentration whereas low bacterial load was observed in the higher dilutions of *Vibrio* count (Table S15). Now to test whether the probiotic present in the mixture was detected by the kit; the timings of colour change of mixed culture dilutions was compared with the timings of the colour change of only *Vibrio* culture dilutions. Table 5 shows that the *Vibrio* cell density (for all dilutions) in both the mixture as well as in the *Vibrio* culture alone was same and the time period of colour change corresponding to these cell concentrations were also comparable. Hence, there was no effect of probiotic on the timing of colour change by the kit. The complete experiment was repeated three times and similar trend was observed.

To further confirm the results, probiotic of different concentrations was prepared and checked with the vibrio detection kit. No colour change was observed in any of the dilutions of probiotics (Table S16). Therefore, the kit did not detect commercial probiotics present in the pond water. Thus, the kit is reliable when used for *Vibrio* contamination detection in aquaculture pond water.

### Application of vibrio detection kit in two different aquaculture farms

It is really required to evaluate any kit in the field to confirm that it is properly working in diverse and fluctuating environmental conditions. The dye-based bacterial detection kit was already tested in wide variety of environmental samples and it perfectly worked in all types of water having different physicochemical properties (Binod et al. 2018). The vibrio testing kit could detect *Vibrio*
in the ponds of Bhavnagar aquaculture farm of having quite high salinity. When compared to the standard chart prepared for Vibrio detection concentration (Tables 4, 5), the kit perfectly estimated the Vibrio load in the different ponds, i.e. for $10^2$–$10^3$ CFU/mL Vibrio concentration and the time of colour change was 5:30–7:50 h approximately (Table S17). Again, the different ponds were having unlike characteristics. For example, the water of Pond 1 had too much hardness and dead fishes were also present in the water for which flocculants (HydroFloc™) and zeolite was added into the pond to improve its water quality. In Pond 2, also HydroFloc and disinfectant were added. Probiotics was supplemented in the Pond 3 and Pond 4. In spite of these many additions to the pond, the kit could correctly estimate the Vibrio concentration in the various ponds.

Further, at Bhimavaram, a wide range of pond water was tested for the presence of Vibrio with the aid of kit, e.g. mixed pond water used for algae culture (Pond 1), effluent treatment pond water (Pond 2), culture pond water (Pond 3), grow out pond water (Pond 4 and 5), brine water reservoir (Pond 6) and fresh water reservoir (Pond 7). Each water had different physicochemical properties. Compared to the Bhavnagar aquaculture farm, the water of Bhimavaram pond water was having lower salinity and TDS. But here also the Vibrio load was $10^2$–$10^3$ CFU/mL for which the kit took 10:00–12:00 h for colour change (Table S18). The delay in colour change was may be due to the incubation temperature (see “Evaluation of vibrio detection kit in two different aquaculture farms”) and/or less salinity of the water. The control sample and the water from freshwater reservoir did not have any Vibrio count on TCBS plates and the kit also did not change its colour which contained those samples (Table S18). This surely indicates that the kit did not give false positive results. Nonetheless, it was confirmed from this result that the kit can even work at room temperature and it can be directly used onsite. Further, the kit can be standardised according to the salinity and temperature of particular field where the kit is to be used and can be distributed directly to the farmers working at the farm. Therefore, when the colour of the kit changes its colour in very less time compared to the normal time of colour change; the farmers can immediately raise alarm about the increase in Vibrio load in pond water.

Hence, there is no influence of different additives and probiotics on the working mechanism of the vibrio detection kit. Moreover, it can work effectively on the field and can evade the problem of transporting samples to the laboratory which are many times very far from the farm and affect the final result.

### Conclusion

For the detection of Vibrio, a unique kit was developed having very simple operating mechanism. It is affordable, simple and does not need any skilled personnel for its operation. The kit requires only 500 μL of the selective medium to inhibit unspecific bacteria within 15 min. Even low concentration ($10^2$ CFU/mL) of the Vibrio can be detected within 6–7 h. Other specific bacterial detection kit can also be prepared on the basis of this kit. This will be very useful for the initial detection of harmful bacteria especially Vibrio spp. from water and can act as an alarm to warn about the presence of particular pathogen in water source. It can be operated onsite and visual analysis is enough for result interpretation. Hence, it is perfect for inexpert people like aquaculture farmers.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02333-3.

### Acknowledgements

The authors sincerely acknowledge analytical division of CSIR-CSMCRI for providing all the instrument facilities. SBK acknowledges Department of Biotechnology, Govt. of India and AHS acknowledges MoES, Govt. of India, for their financial support and BMJ acknowledge CSIR-JRF fellowship, respectively. The manuscript has been assigned CSIR-CSMCRI-2/2020 registration number.

### Author contributions

SBK performed the experiments and interpreted the data. AHS and BMJ performed the experiment and help in writing manuscript. DI performed the part of experiment. SH has conceptualised the work and prepared the manuscript.

### Funding

The work was performed with internal fund of the institute. Sources of fellowships received by SBK, AHM and BMJ has been mentioned in the acknowledgement part.

### Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

### Declarations

#### Conflict of interest

The authors declare that they have no conflict of interest.

#### Ethical approval

The manuscript has not been submitted to any other journal and the work is original and not have been published elsewhere in any form or language (partially or in full). Results have been presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation. Plagiarism has been checked throughout the manuscript.

#### Consent to participate

All the coauthors have given their consent to participate as coauthors in the manuscript.

#### Consent for publication

No third party data has been included without proper citation. Therefore, this is not applicable.
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