Composition of microflora associated with the total psychrotrophic plate in Indian Mackerel (*Rastrelliger kanagurta*) stored at chilled condition is a major reflection of the peptone iron agar count

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Fish ensures the world population's nutritional security. In the food sector, the quality of the fish is quite crucial. *Rastrelliger kanagurta*, or Indian Mackerel, is one of India's most significant pelagic fish resources in terms of national food security. During 2016-2017, the resource collected 2.49 lakh tonnes, accounting for 6.9% of total marine fish output in India. Indian mackerel is a medium-fat fish that is in great demand in both domestic and international markets. It is generally known that fatty and semi-fatty species such as mackerel are more prone to lipid oxidation because significant levels of haemoglobin and lipids coexist in their muscles (Richards and Hultin 2002; Viji et al., 2016). As a result, mackerel is susceptible to fast decomposition during refrigerated or chilled storage, the most common preservation technique used in the domestic market.

The quality of fish food degrades during storage due to chemical and microbial changes. In general, the microbiological quality of fish held at chilled temperatures is evaluated using traditional reference plating techniques such as Total plate count (mesophilic), Total plate count (psychrotrophic), and additional counts such as peptone iron agar (PIA), Pseudomonas agar base supplemented with cephaloridine, fucidin, and cetrimide (CFC), and streptomycin-thallous acetate-actidione agar (STAA) at regular storage intervals. Total mesophilic and total psychrophilic counts that exceed the permissible limit (6 logCFU/g) are typically considered spoiled. In many circumstances, the total mesophilic aerobic bacterial count is not a clear reflection or sign of spoiling and stays lower than the acceptance limit of 6 log cfu/g, at which point the total Psychrotrophic count surpasses the acceptance limit.

The spoiling of fish food is often seen as a reflection of the specific spoilage organisms found first in the gut and moving towards the muscle of the fish throughout the decomposition process, causing the loss of fish quality and rendering fish unfit for further consumption. *Shewanella* sp., *Pseudomonas* sp., *Aeromonas* sp., *Vibrio* sp., *Achromobacter* sp., *Psychrobacter sp*, *Moraxella sp*, *Acinetobacter* sp., *Pseudoalteromonas sp*, *Flavobacterium sp*, *Photobacterium sp*, *Brochothrix* sp., *Lactobacillus* sp., *Micrococcus sp*, *Corynebacterium sp*, *Vagococcus sp*, *Bacillus sp*, and *Clostridium* sp have been recognized as spoilage organisms in fish spoilage (Hubbs, 1991; Boziaris & Parlapani, 2017). Despite the fact that many studies have been conducted in the microbiological quality assessment in terms of spoilage with many methods of reference plating such as TPC, PIA, CFC, STAA, MRS. Therefore, determination of spoilage association bacteria in total Psychrotrophic plate count to other media is highly essential to understand and predict the shelf-life of the products (Dalgaard, 2000). And there is a scarcity of information on how much percentage of colonies observed in total Psychrotrophic count plate are reflection of counts of PIA, CFC, STAA, SAA, TCBS which are reflection of individual flora of spoilage potential.

A pilot study was conducted in this context to establish a link between the total Psychrotrophic count and other counts such as Peptone Iron Agar count (H_2S formers), Pseudomonas agar base supplemented with cephaloridine, fucidin, and cetrimide, streptomycin-thallous acetate-actidione agar (STAA); starch ampicillin agar (SAA); thiosulfate-citrate-bile-sucrose (TCBS). To comprehend the percentage of microflora in the entire Psychrotrophic count with the other media.

To understand this, the total plate count (Psychrotrophic) obtained from Indian Mackerel (*Rastrelliger kanagurta*) was taken on spoilage date on 15th day of chilled storage at the time of sensory and microbiological rejection (Mol et al., 2007; Kunjulakshmi et al., 2020). The study was a storage study conducted for another experiment. The total plate count (psychrotrophic count) was performed in accordance with ISO 17410:2019 standard methodology, with minor modifications. In brief, fish samples (25 g) were aseptically weighed and homogenised for 60 seconds at room temperature in

stomacher 400 with 225 ml sterile physiological saline (Seward Medical, London, UK). The homogenates were serially diluted in saline for microbiological analysis, spread plated in plate count agar and incubated at 7°C for 10 days. For checking the ability of the colonies from TPC (psychrotrophic plate count) to grow in the other media was performed in PIA, STAA, TCBS, SAA, CFC and incubated at 25°C for 48h and observed for 5 more days (Gardner 1966; Gram et al. 1987; Gennari and Campanini 1991; Lalitha and Surendran, 2006).

Fifty-three morphologically diverse colonies were selected from Total Psychrotrophic Count plates and tested for purity, Gram reaction, spore forming ability, motility, oxidation/fermentation test, and capacity to generate oxidase and catalase enzymes. A TSI test was also performed to assess the capacity to produce H2S. The colonies were tested for spoiling potential using the TMAO reduction assay and H2S generation (Gram et al., 1987). These assays were carried out in order to categorise these isolates as fermentative or oxidative bacteria with grammes positive and negative features (Sneath et al. 1986; Gram et al. 1987).

On the day of sensory rejection, 53 colonies were collected and evaluated for their spoilage potential and how much percentage it is represented in the other medium widely used for spoilage evaluation and based on cultivable bacteria reported in the rotting process of finfish. On the 15th of storage in iced condition, the total aerobic counts on Indian Mackerel at 20 °C were 6 log10 cfu/g. 96% (51 colonies) of the bacteria recovered from the Total Psychrotrophic count plate, they were gram negative rods, with the remaining 2 colonies being gramme positive chain of rods. In TCBS media, none of the isolates could grow. The results are presented in Figure 1a to 1f. All of the isolates produced catalase. With the exception of one isolate, all were oxidase producers. 51 of the 53 colonies could develop in the PIA but not the H2s producers. All of the isolates grew on SAA media but did not produce amylase, indicating that they were Aeromonas sp. Only 3.7% of the isolates grew on pseudomonas agar (CFC), and 3.7% of the culturable flora grew on STAA, and they are gramme positive rods in single or chains. In the H&L test, all 53 isolates were non-fermentative; however, nine of these isolates were non-fermentative with an alkaline top.

The study identified that dominant gram-negative bacteria are belonged to non-Enterobacteriaceae, probably 97% related to the *Shewanella* sp., having their ability to grow in PIA and 3.7% representing the *Brochothrix* sp., with oxidase negative and growth at STAA. True reflection of the aerobic plate count for the indication of spoilage of fish stored under chilled or iced conditions are not the total mesophilic aerobic bacterial count, and it is a reflection of psychrotrophic aerobic plate count. The acceptance limit for psychrotrophic count is over *6 log cfu/g* for the spoiled fish (Mol et al., 2007). *Pseudomonas marinoglutinosa, Aeromonas hydrophila* isolated from unirradiated spoiling Indian mackerel (*Rastrelliger kanagurta*) (Alur et al., 1989). Majority of the studies were carried out based on the Total mesophilic aerobic count or it is compared with the Total aerobic count (Psychrotrophic). This study finds its relevance, how much percentage of bacteria isolated from Psychrotrophic plate count get reflected in the media recommended for the other spoilage specific organism.

To conclude, the total psychrotrophic aerobic plate count is the best reflection of the spoilage and 6log cfu/g is the acceptance limit for consumption. The psychrotrophic count reflects 97% of the PIA and other 3.7%.

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