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REVIEW ARTICLE

Pseudomonas fluorescens: a potential food spoiler and challenges and advances in its detection

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Abstract

Purpose This review focuses on the spoilage strategies used by the *Pseudomonas fluorescens*, and in addition, it also discusses various diagnostic approaches used for its identification in food items. Some challenges faced and advances in the detection of *P. fluorescens* and also discussed in this review.

Methods An extensive literature search was performed with published work and data was analyzed in detail to meet the requirements of the objectives.

Results *P. fluorescens* are unicellular rods, with long straight or curved axis, but not helical, motility by one or more polar flagella, Gram-negative, non-spores former, stalks, or sheaths. *P. fluorescens* is represented by seven biotypes denoted by the letters A, B, C, D, E, F, and G. The microbe shows wide choice of growth temperature and causes contamination and spoilage in ordinary and refrigerated food items by its enzymes and pigment production. The biofilm formation by *P. fluorescens* poses another serious threat to the food industries.

Conclusion Molecular identification of *P. fluorescens* is generally done by 16S rRNA, intergenic spacer (ITS1) utilizing traditional polymerase chain reactions (PCR). Nowadays, qPCR and multiplex PCR are largely utilized in identification of *P. fluorescens* based on AprX gene (extracellular caseinolytic metalloprotease) in the milk and meat spoilage strains. The available methods still show some disadvantages with accuracy and specificity of detection. Rapid detection of *P. fluorescens* in food samples is the need of hour to improve the detection efficiency.

Keywords *Pseudomonas fluorescens* · Spoilage · Enzymes · Diagnosis · Advances

Introduction

In nineteenth Century, Dr. Migula, Professor at Karlsruhe Institute of Germany, first proposed the name Pseudomonas and it read as cells with polar organs of motility, with development of spores in a few categories (for instance: *Pseudomonas violacea*) (Palleroni 2010). In 1926, Den Dooren de Jong stressed on microbes of soil and featured the extreme adaptability of *Pseudomonas* (Palleroni 2010). It was represented by unicellular rods, with the long axis curved or straight, motility by one or more polar flagella, Gram-negative, non-spore forming, sheaths, or stalks (Stanier et al. 1966). The respiration is the only process involved in energy-yielding metabolism and all species utilize oxygen as a terminal oxidant, whereas some species use denitrification as an anaerobic respiratory system. All *Pseudomonas* spp. are chemoorganotrophs, while few are facultative chemolithotrophs which use H_2 as energy source. Pseudomonads has three subgeneric group: fluorescent group having species *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*; acidovorans group, with *P. acidovorans*, *P. testosterone*; and, alcaligenes group representing *P. alcaligenes*, *P. pseudoalcaligenes* sp.nov., *P. mutivorans* sp.nov, *P. stutzeri*, and *P. maltophilia* (Stanier et al. 1966). *Pseudomonas fluorescens* is represented by seven biotypes denoted by the letters A, B, C, D, E, F, and G (Stanier et al. 1966). Consequently, DNA/RNA hybridization confirmed the presence of five diverse rRNA groups (rRNA groups I–V) (Palleroni et al. 1972; Palleroni 1993; Kersters et al. 1996).

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Pseudomonas rRNA group I contained *P. aeruginosa*, all the fluorescent species (*P. fluorescens*, *P. putida*, *P. syringae*), and some non-fluorescent species (*P. stutzeri*, *P. alcaligenes*, *P. pseudoalcaligenes*, and *P. mendocina*) (Palleroni et al. 1973). Various types of spoilage caused by *P. fluorescens* and its spoilage agents are shown in Fig. 1. The various types of spoilage caused the *Pseudomonas fluorescens*, and various diagnostic approaches used for its identification are discussed in the following sections:

Pseudomonas fluorescens as a food spoiler

Pseudomonas fluorescens is a regular contaminant of ready to-eat foods. These microscopic organisms show wide choice of growth temperature, and contamination is a key issue in ordinary and refrigerated food items as shown in Table 1. A report from Italy also confirmed the presence of *P. fluorescens* in packed ready to-eat vegetables (Caldera and Franzetti 2014). Raw vegetables quality, processing, packaging system, and storage temperature are essential factors that influence the microbial composition in the final product. Pinkeye disease in potato tubers was caused by the pectic enzyme of *P. fluorescens* (Folsom and Friedman 1959; Huether and McIntyre 1969). Biotypes I and II of *P. fluorescens* were isolated from celery, cabbage, and chicory stored at 1° and 4 °C, and on the other hand, biotypes III and V were isolated from incompletely processed lettuce gathered from the processing plants (Brocklehurst and Lund 1981; Sellwood et al. 1981; Magnuson et al. 1990). Head spoil disease in broccoli had additionally confirmed the relationship of surfactant-positive strains of *P. fluorescens* biovar II, IV, which reduces the water surface tension and enables surfactant-deficient strains to colonize over water-soaked areas even in the absence of physical injury (Hildebrand 1989). Biosurfactants producing *P. fluorescens* are also responsible for the spoilage of aerobically stored chicken meat (Mellor et al. 2011). Different volatile compounds, for example, trimethylamine, methyl mercaptan, and dimethyl disulfide, were produced by *P. fluorescens* in sterile fish muscles under in vitro condition at 0 °C after 32 days of incubation (Miller et al. 1973). *P. fluorescens* also produced 2-butenal, methyl thiol n-butyrate, 3-octanol in spoiled chicken breast stored at 2 to 6 °C for 14 days (Pittard et al. 1982). Similarly, alcohols, aldehydes, esters, hydrocarbons, toluene, ketones, few sulfur-containing compounds were identified, when *P. fluorescens* biotype I was inoculated under in vitro conditions in beef stored at 6 °C at pH 5.5–5.7 (Edwards et al. 1987). *P. fluorescens* isolated from sea bream stored aerobically in Greece market at 0°, 10°, and 20 °C and modified-atmosphere packaging (MAP) conditions (40% CO_2–30% N_2–30% O_2) (Tryfinopoulou et al. 2002). An investigation in Belgium has confirmed

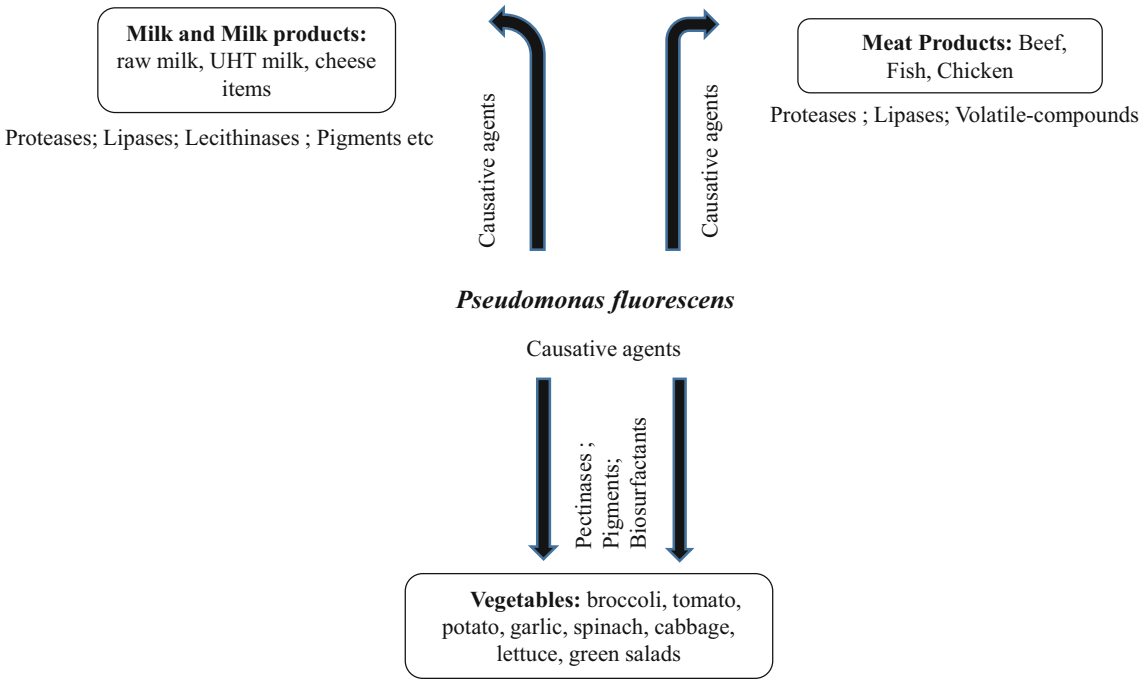

Fig. 1 Spoilage of different food items by *Pseudomnas fluorescens*

Table 1 Specific spoilage causes by *Pseudomonas fluorescens* in various food items

Strain types	Components	Food items	Alteration	References
Laboratory isolates	Lipases	Cheddar cheese	Rancidity	Law et al. (1976)
*Pseudomonas fluorescens*SIKW1	Lipases	Cow milk	Rancid flavor	Andersson et al. (1981)
Pseudomonas fluorescens AR11	Protease	UHT milk	Gelation	Law et al. (1977)
NS	Protease	Fresh cheese	Gelation and slimy texture due to the activity of enzymes	Samaržija et al. (2012)
NS	Protease	Soft cheese	Poor texture due to activity of enzymes	Samaržija et al. (2012)
Laboratory isolates	Pectin methylesterase	Potato	Tubers maceration	Huether and McIntyre (1969)
Laboratory isolates	Phospholipase C (lecithinase)	Milk	Bitter taste	Fox et al. (1976)
Pseudomonas fluorescens biotype I	Bluish-black pigment	Chicory	Vascular blackening	Sellwood et al. (1981)
Laboratory isolates	Surfactant	Broccoli	Rotting	Hildebrand (1989)
Laboratory isolates	Surfactant	Chicken meat	Emulsify fat	Mellor et al. (2011)
Laboratory isolates	Volatile compounds	Fish muscle	Off-odors	Miller et al. (1973)
Laboratory isolates	Volatile compounds	Chicken breast	Off-odors	Pittard et al. (1982)
Laboratory isolates	Volatile compounds	Beef	Off-odors	Edwards et al. 1987)
Pseudomonas fluorescens biotype IV	Blue pigment	Low-acid cheese	Discoloration	Martin et al. (2011)
Laboratory isolates	Bacterial load(reducing O_2 partial pressure)	Beef	Discoloration	Chan et al. (1998)
Laboratory isolates	Volatile compounds	Sea bream fillets	Off-odors, discoloration	Parlapani et al. (2015)
Pseudomonas fluorescens biotype B1-B6	Protease, Lecithinase	Pasteurized milk	–	Bedeltavana et al. (2010)
Pseudomonas fluorescens NCIMB 702085, 701274 (Referred strains)	Protease	UHT skimmed milk	–	Mlipano et al. (2018)
Laboratory isolates	Volatile compounds	Refrigerated chicken	Off-odors	Freeman et al. (1976)
Pseudomonas fluorescens BJ-10	Protease	Raw milk	Gelation	Zhang and Lv (2014)
Laboratory isolates	Blue pigmentation	Mozzerll cheese	Discoloration	Fasolato et al. (2018)

NS not specified

the growth of *P. fluorescens* in tofu packed in modified conditions and showed that some strain can survive under 100% CO_2 (Stoops et al. 2012). The postpasteurization contamination (PPC) of high temperature, short time-pasteurized fluid milk with *P. fluorescens* continues to be an issue with processor defects like lower flavor scores, coagulation, and fruity fermented milk (Reichler et al. 2018).

P. fluorescens having protease, lecithinase, and lipase activity were isolated from the raw and pasteurized milk in four dairy processing plants in New York (USA), from chicken in Santiago (Chile) and cheese from El-Menofia (Egypt) (Dogan and Boor 2003; Hammad 2015; Morales et al. 2016). *P. fluorescens* acts as most common contaminants in Italian bulk milk tank and shows lipolytic, proteolytic, and lecithinase activity (Decimo et al. 2014). A research report from Brazil additionally confirmed the deterioration of goat milk by *P. fluorescens* that was strongly associated with its proteolytic activity at different temperatures (Scatamburlo et al. 2015). A study in Iraq revealed the presence of *P. fluorescens* in raw cow and buffalo's milk due to protease activity (Al-Rodhan and Nasear 2016). *P. fluorescens* biotypes I, II, and III were likewise identified due to proteolytic and lypolytic activities in the milk, minced beef, chicken, and fish sold at the different sale points in Izmir, Turkey (Keskin and Ekmekçi 2007). *P. fluorescens* isolated from the pork meat in Budapest, Hungary, showed quite intense proteolytic activity as compared to lipase (Márta 2012).

Mystery of blue cheese

P. fluorescens can induce troublesome changes in food items by producing pigment molecule (Andreani et al. 2014). A notable model is blue mozzarella cheese events that happened in Italy, when shoppers observed blue stains on mozzarella cheese in the wake of opening the bundles (RASFF 2010). About 70,000 mozzarella cheese chunks were tested and *P. fluorescens* group was found associated with this specific spoilage event (Andreani et al. 2015). Substance responsible for blue color in contaminated mozzarella cheese was indigoidine compound produced by *P. fluorescens* (Caputo et al. 2015). Later on, some reports confirmed that the blue shade created by *P. fluorescens* strains was not because of indigo or indigoidine; however, it was a substantial particle, and an indigo-derivative (Andreani et al. 2015). Besides, in the ongoing finding, additionally it was observed that blue shade is most probably an indigoid molecule (Fasolato et al. 2018). A recent study reported that the genes involved in blue pigment production possibly play role in tryptophan biosynthetic pathway and also provide antioxidant protection (Andreani et al. 2019).

Enzymes of *P. fluorescens*

Lipases, i.e., triacylglycerol hydrolases, act on food fat molecules and cause the release of unsaturated glycerol and fatty acids (Andreani 2016). Free short-chain unsaturated fats give unpleasant flavors, stated to be rancid, whereas medium-chain unsaturated fats are associated with bitter, foamy, or unclean flavors (Samaržija et al. 2012). Various report stated that lipolytic activity is more noteworthy at refrigeration temperatures (Woods et al. 2001; Rajmohan et al. 2002). In cheese, lipases get absorbed within fat globules and remain in the cheese, inciting decay impacts amid ripening of hard and semi-hard cheese (Samaržija et al. 2012). Presence of different lipases in spoiled food improves the heat stability of lipase (Teh et al. 2014). Few strains of *P. fluorescens* cause rancidity in cheddar cheese due to lipase that retained 20–25% of their lipolytic activity at 100 °C for 10 min (Law et al. 1976). Extracellular lipase enzymes from *P. fluorescens* responsible for the off flavoring of the milk are treated at ultra-high-temperature (UHT) (Andersson et al. 1981). In meat, lipases break down glycerides forming free fatty acids and produce off-flavor, frequently referred to as rancidity (Huis 1996). Novel lipase from *P. fluorescens* C9 strain, in which *lipA* gene was reported, strongly suggested the presence of second lipase in this strain (Dieckelmann et al. 1998). On the other hand, in *P. fluorescens* B52, genes encoding for thermostable lipase (lipA) and protease (aprX) situated within same operon, differed by associated genes with discharge of the protease (extracellular) and gene expression of both hydrolases, are interlinked (Woods et al. 2001; McCarthy et al. 2004). In another report, *P. fluorescens* grown in refrigerated raw milk with lipase enzyme had high activity at 25 °C and a broad pH optimum extending from 7.0 to 10 (Martins et al. 2015).

Gelation of UHT milk by proteinases of *Pseudomonas fluorescens* strain isolated from raw milk depends on its amount before heat treatment (Law et al. 1977). Shelf-life of UHT milk is much lower than processed raw milk stored at 6 °C (Griffiths et al. 1988). The proteinase produced in the milk leads to extensive breakdown of k-casein to para-k-casein, an event similarly to the action of rennet. Additionally, modification of milk casein is encouraged because of the activity of milk proteinase (endogenous), particularly in plasmin (Datta and Deeth 2001).

Production of proteinases destabilizes the casein and influences the cheese yield (Mitchell and Marshall 1989). In this process specifically, plasminogen and plasmin are liberated from casein micelles, altering the cheese yield and influencing the texture and flavor of the final product (Samaržija et al. 2012). A recent study based on the phenotypic examination of 87 *P. fluorescens* species revealed that each strain (94%) could stimulate proteolysis on nutrient agar plates with 2% UHT milk at 22 °C, while around 72% could initiate this at refrigeration temperatures, showing high predominance of

Table 2 Common molecular approaches used in *Pseudomonas fluorescens* detection

Strains used	Target site	Primers	Probe	Amplification conditions	References
Laboratory isolates	16S rRNA DNA 16S–23S intergenic spacer (ITS1) 16S rRNA gene partial region	16SF 5′ AGAGTTTGATCCTGGCTCAG-3′ 16SR 5′-CTACGGCTACCTTGTTACGA-3′) (16F945 5′-GGGCCCGCACAAGCGGTGG-3′; 23R458 5′-CTTTCCCTCACGGTAC-3′) (16SPSEfluF 5′-TGCATTCAAAACTG ACTG-3′; 16SPSER 5′AATCACAC CGTGGTAACCG-3′)	NA	2 min at 94 °C; 5 cycles consisting of 94 °C for 45 s, 55 °C for 1 min, 72 °C for 2 min; 35 cycles consisting of 92 °C for 45 s, 60 °C for 45 s, 72 °C for 2 min; final extension of 72 °C for 2 min and final cooling at 4 °C 5 min at 94 °C; 30 cycles consisting of 94 °C for 1 min, 72 °C for 2 min; final extension of 72 °C for 2 min	Franzetti and Scarpellini (2007)
Laboratory isolates	16S rDNA	forward 5′-CTACGGGAGGCAGCAGTGG-3′ and reverse 5′-TCGGTAACGTCAAA ACAGCAAAGT-3′	NA	NS	Márta (2012)
Laboratory isolates	16S rDNA metalloprotease gene (aprX)	F:5′-TGCATTCAAAACTGACTG-3 R:5′-AATCACACCGTGGTAACCG-3′ SM2F (5′-AAA-TCG-ATA-GCT-TCA-GCC-AT-3′), SM3R (5′-TTG-AGG-TTG-ATC-TTC-TGG-TT-3′)	NA	2 min at 94 °C for 1 cycles, 35 cycles of 45 s at 94 °C, 45 s at 59 °C, 2 min at 68 °C with 1 final extension of 5 min at 72 °C and cooling at 4 °C	Al-Rodhan and Nasear (2016)
Laboratory isolates	16S DNA 16S rRNA gene partial region alkaline protease gene AprX	PA-GS-F (GACGGGTGAGTAATGCCTA) and PA-GS-R (CACTGGTGTTCCTTCCTATA) 16SPS EfluF (TG-CATTCAAAACTGACTG) and 16SPSER (AATCA-CACCGTGG TAACCG) FP *apr* I (TAYGGBTTCAAYTCCAAYAC) and RF *apr* II (VGCGATSGAMACRTTRCC)	NA	NS	Hammad (2015)
Referred strains	alkaline protease gene aprX	F/5′-ACCGAGAACACCAGCTTGTC-3′ R/5′-CTCACGGTCAATGGCAAAC-3′	5′-CTCACGGTCAATGG CAAACTTTTTTTTTTTTT TTTTTTTT-3′	Denaturation at 94 °C for 5 min followed by 35 cycles of amplification at 94 °C for 20 s, annealing at 58 °C for 20 s, extension at 72 °C for 20 s final extension at 72 °C for 7 min	Chiang et al. (2012)
Laboratory isolates	partial sequence *rpo*B gene aprX	PSF (5′-AGTT CA-TGG-ACC-AGA-ACA-ACC-3′) as forward/rpoB-PTR (5′-CCT-TGA-CGG-TGA-ACT-CGT-TTC-3′) as reverse SM2F (5′-AAA-TCG-ATA-GCT-TCA-GCC-AT-3′)/SM3R (5′-TTG-AGG-TTG-ATC-TTC-TGG-TT-3′	NA	Denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and elongation at 72 °C for 1 min, final extension at 72 °C for 10 min Initial denaturation at 95 °C fpr 5 min followed by 30 cycles with denaturation for 30 s at 95 °C, annealing for 30 s at 60 °C, extension for 1 min at 72 °C and final elongation at 72 °C for 8 min	Decimo et al. (2014)
Laboratory isolates	16S rRNA	PA-GS-F (5′-GACGGGTGAGTAATGCCTA-3′) and PA-GS-R (5′-CACTGGTGTTCCTT CCTATA-3′)	NA	95 °C denaturation for 5 min, 10 cycles at 94 °C for 15 s, annealing at 53 °C for 30 s and elongation at 72 °C for 45 s 25 cycles repeated increasing elongation step at 72 °C by 5 s every cycle and final extension at 72 °C for 10 min	Ardura et al. (2013)
Referred strains	16S rRNA DNA 16S–23S intergenic spacer (ITS1)	16SF 5′ AGAGTTTGATCCTGGCTCAG-3′ 16SR 5′-CTACGGCTACCTTGTTACGA-3′) (16F945 5′-GGGCCCGCACAAGCGGTGG-3′; 23R458 5′-CTTTCCCTCACGGTAC-3′)	NA	2 min at 94 °C; 5 cycles consisting of 94 °C for 45 s, 55 °C for 1 min, 72 °C for 2 min; 35 cycles consisting of 92 °C for 45 s, 60 °C for 45 s, 72 °C for 12 min; final extension of 72 °C for 2 min and final cooling at 4 °C	Scarpellini et al. (2004)

Table 2 (continued)

Strains used	Target site	Primers	Probe	Amplification conditions	References
	16S rRNA gene partial region	(16SPSEfluF 5′-TGCATTCAAAACTG ACTG-3′; 16SPSER 5′AATCACAC CGTGGTAACCG-3′)		5 min at 94 °C; 30 cycles consisting of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min; final extension of 72 °C for 2 min	
Laboratory isolates	*odc* (ornithine decarboxylase)	PUT2-F ATHWGNTWYGGNAAYACNATHAARAA PUT2-R GCNARNCCNCCRAAYTTNCCDATRTC	NA	10 min enzyme activation at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 53 °C and 2 min at 72 °C and final extension 20 min at 72 °C	De las Rivas et al. (2006)
Laboratory isolates	alkaline protease gene AprX	FP *apr* I (TAYGGBTTCAAYTCCAAYAC) and RF *apr* II (VGCGATSGAMACRTTRCC)	NA	1 min denaturation at 94 °C, 30 s annealing at 55 °C and a 30 s extension at 72 °C	Martins et al. (2005)
Laboratory isolates	16S rRNA gene partial region	16SPS EfluF (TG-CATTCAAAACTGACTG) and 16SPSER (AATCA-CACCGTGG TAACCG)	NA	NS	Caldera and Franzetti (2014)
Laboratory isolates	16S rRNA gene partial region	16SPS EfluF (TG-CATTCAAAACTGACTG) and 16SPSER (AATCA-CACCGTGG TAACCG)	NA	1 min at 94 °C; 30 cycles consisting of 56 °C for30 s, 72 °C for 1 min; a final extension of 72 °C for 7 min; final cooling at 4 °C	Morales et al. (2016)

NA not applicable, *NS* not specified

proteolytic strains inside the *P. fluorescens* group (Andreani et al. 2014). The most common family of thermostable proteases within the genus *Pseudomonas* is serralysin protease family, a much conserved protein group from the AprX with an alkaline zinc metalloprotease family with molecular masses in the range of 39.2 and 45.3 kDa (Dufour et al. 2008; Marchand et al. 2009; Teh et al. 2014). Extracellular protease from the *P. fluorescens* CY091 with sub-atomic weight of 50 kDa retained 20% of its activity even after heating at boiling temperature for 10 min, revealing its high resistance to heat inactivation (Liao and McCallus 1998). Another zinc-metalloacid protease produced by *Pseudomonas fluorescens* RO98 was isolated from raw milk with molecular weight of 52 kDa and demonstrated its activity between 15 and 55 °C and pH 4.5–9.0 (Koka and Weimer 2000). In a few *Pseudomonas* spp. strains, AprX has been identified as the only protease associated with food decay (Woods et al. 2001). Proteases are chiefly synthesized at the end of the exponential stage, when thickness of cell is high, featuring the contribution of quorum sensing mechanism in spoilage activity (Liu et al. 2007; Pinto et al. 2010; Bai and Rai 2011).

Pectic lyase is additionally produced by soft rot causing strains of *P. fluorescens* (Liao 1989). Yield of pectin lyase produced by *P. fluorescens* W51 was progressively increased when glycerol was utilized as a sole source of carbon, whereas thermal stability of pectate lyase produced by *P. fluorescens* CY091 expanded when $CaCl_2$ or positively charged molecules, for example, polylysine was used at 48 °C in the culture medium (Schlemmer et al. 1987; Liao et al. 1997). Another critical class of extracellular enzymes present in spoiled food is constituted by lecithinases and different phospholipases that disturbs the fat globules of milk and makes fat substances accessible for further lipase action (Samaržija et al. 2012). The most common enzyme of this family is phospholipase C (lecithinase) which is produced by most of the pseudomonads (Fox et al. 1976).

Biofilm formation by *P. fluorescens*

P. fluorescens has been regarded as the predominant and most harmful microbiota during the cold storage of raw milk (Machado et al. 2015; Von-Neubeck et al. 2015; Vithanage et al. 2016). During storage of the dairy product, not only the *P. fluorescens* heat-resistant enzymes will remain active after heat sterilization, causing milk bitterness, sediment, and gelation, but they also form biofilm on the surface of equipments and tools in dairy production line (Shpigel et al. 2015; Stoeckel et al. 2016). Once the biofilm is formed by the *Pseudomonas* bacteria, it is hard to remove it by the hygienic treatments during the processing of raw milk (Ksontini et al. 2013). The biofilm formation will not only cause the pipelines corrosion, but also provides appropriate substratum for the growth of other bacteria including pathogens, which may

Table 3 Some advances in detection of *Pseudomonas fluorescens* causing food spoilage

Method	Strains used	Study sample	Target/marker used	References
TaqMan Assay	Referred strains	Food equipment	*cpn60* gene	Saha et al. (2012)
Raman spectroscopy using chemometric analysis	Referred strains	Water	Fluorescens	Yilmaz et al. (2015)
LAMP	*Pseudomonas fluorescens* 38	Raw cow milk	Conserved lipase gene sequence	Xin et al. (2017)
Multiplex PCR	Laboratory isolates	Raw milk	Biofilm formation gene *AdnA*	Xu et al. (2017)
Oligotyping	Laboratory isolates	Meat and dairy	V1–V3 regions of 16S rRNA gene	Stellato et al. (2017)

threaten the health of consumers (Costerton 1999; Aswathanarayan and Vittal 2014). The availability of nutrient in the environment will also affect biofilm growth and *P. fluorescens* can form biofilms under any nutrient concentration that allows growth (Teh et al. 2014). Biofilm formation by *P. fluorescens* strains was temperature dependent, and lower incubation temperature (± 10 °C) favored the formation of biofilm after 48 h (Rossi et al. 2016).

Methods used for diagnosis of *P. fluorescens*

Isolation of *P. fluorescens* on all universal (supplement agar) medium and in specific media is a normal practice in food industries. Toward the end of nineteenth century, ELISA-based methodologies have given effectively recognizable proof of microscopic organisms from different foods, particularly the meat items. Various ELISA-based techniques were developed for the identification of *P. fluorescens* using meat surface inhibition ELISA with affectability level as low as 3×10^5 microscopic organisms for each milliliter (Eriksson et al. 1995). Polyclonal antibodies against F protein of *P. fluorescens* cell envelope were produced and were dependent on indirect ELISA approach for the detection of refrigerated microscopic organisms at an affectability of 10^4–10^5 cfu cm^{-2} (González et al. 1996). Another indirect ELISA kit was developed against the live cells of *P. fluorescens* in the refrigerated meat with sensitivity level of 10^4 cfu cm^{-2} (Gutierrez et al. 1997).

Molecular identification of the *P. fluorescens* and its biotype dependent on 16S rRNA and intergenic spacer (ITS1) utilizing traditional polymerase chain reactions (PCR) were extensively developed (Scarpellini et al. 2004; Franzetti and Scarpellini 2007; Márta 2012; Ardura et al. 2013; Caldera and Franzetti 2014; Hammad 2015; Al-Rodhan and Nasear 2016; Morales et al. 2016). *P. fluorescens* enzyme-specific gene identification approaches are also regularly used in its identification as shown in Table 2 (Martins et al. 2005; Decimo et al. 2014; Hammad 2015; Al-Rodhan and Nasear 2016). PCR-denaturing gradient gel electrophoresis (DGGE) was additionally used to examine the V3 and V6-V8 areas of 16S rRNA quality; however, this strategy was not a valid proof to distinguish *Pseudomonas* in the biological meat community (Jiang et al. 2011). Nowadays, qPCR and multiplex PCR techniques are commonly utilized to identify the *P. fluorescens* on the basis of AprX gene coding for extracellular caseinolytic metalloprotease in the meat and milk spoilage strains (Dufour et al. 2008; Chiang et al. 2012). Enhanced multiplex PCR was also developed to identify the food microorganisms producing biogenic amines as presence of *P. fluorescens odc* gene is normally recognized to produce ornithine decarboxylase (De las Rivas et al. 2005, 2006).

Challenges and advances in diagnosis of *P. fluorescens*

Immunological approaches using ELISA for the detection of *P. fluorescens* in meat products were well designed but could not get commercialized as most of the experiments were conducted under in vitro conditions. On the other hands, the test showed the variations in their sensitivity level, and this was one of the challenges in diagnosis of the potential food spoiler (Eriksson et al. 1995; González et al. 1996; Gutierrez et al. 1997).

Molecular identification methods based on 16S rRNA gene sequencing provide low resolution and cannot discriminate *Pseudomonas* at the species level (Ait Tayeb et al. 2005). Although methods based on *rpoB* gene sequencing are widely used in identification of *P. fluorescens* strains (Ait Tayeb et al. 2005; Machado et al. 2015), markers targeting protein-coding sequences have also been used to improve the resolution of molecular detection methods for example gene *aprX*; coding the alkaline protease was used for the identification of *P. fluorescens* in dairy products, but presence of same gene in other species of *Pseudomonas* makes it difficult to distinguish between them and poses another challenge in its accurate identification (Martins et al. 2005; Decimo et al. 2014).

To overcome the challenges faced in accurate identification of *P. fluorescens* from spoiled food items, some advanced methods have been used as shown in Table 3. The taxonomic

resolution of 16S rRNA gene-based study is generally limited to the genus level, and the common use of operational taxonomic units (OTUs) based on 97% sequence similarity cut-off often results in phylogenetically mixed units (Koeppel and Wu 2013). These approaches in some cases fail to resolve ecologically meaningful differences between closely related organisms in complex environments (Eren et al. 2014, 2015). An alternative approach used to overcome this problem is using oligotyping, which decomposes a given taxon, or 97% OTU, into high-resolution units ('oligotypes') by only using the most information-rich nucleotide positions identified by Shannon entropy calculations (Eren et al. 2013; Schmidt et al. 2014). This approach was successfully used in meat and dairy processing environment for the isolation of *P. fluorescens* oligotypes (Stellato et al. 2017). Another study reported the use of multiplex PCR for the detection of *P. fluorescens* showing the ability of biofilm formation with a detection limit of target strain to 10^2 cfu/ml (Xu et al. 2017). AdnA protein, a transcriptional activator related to biofilms formation in *P. fluorescens*, is very important for its spreading and survival in soil (Marshall et al. 2001).

In one of the studies, a loop-mediated isothermal amplification (LAMP) assay was developed to detect the *P. fluorescens* in raw milk (cow), as most of the frequently reported heat-resistant lipase-producing bacterial species with detection limit of 4.8×10^2 cfu/reaction of the template DNA and 7.4×10^1 cfu/reaction of *P. fluorescens* led to contamination of pasteurized cow milk (Xin et al. 2017). LAMP assay cannot distinguish between DNA from viable cells and to that from dead cells (Chen et al. 2011; Wan et al. 2012). This serves as an advantage to accurately assess the potential contamination of heat-resistant lipase produces in milk as these microorganisms remain active in contaminated dairy products even after *P. fluorescens* has been destroyed. Therefore, LAMP detection method is more accurate than culture-dependent method.

Raman spectroscopy, an alternative cultivation-free verification method with 15-min analysis time, was developed to detect the *P. fluorescens* in water samples. According to this test, test colonies were screened under UV light at 365 nm, and fluorescent and nonfluorescent colonies were specifically marked (Yilmaz et al. 2015). In another study, TaqMan assay was developed that showed results better than 16S rRNA for the identification and enumeration of closely related species and strains with a sensitivity 10 cfu/ml. The assay was also successful in determining the concentration of the test preparation within 2 h (Saha et al. 2012).

Conclusion

It is observed that the rates of *P. fluorescens* are steadily growing and making them perfect spoiler of food items. There is dire need for improvements in accurate detection of this organism. There is still scope for improvement in the presently available method of *P. fluorescens* detection. Some biosensors have already been developed for detecting food-related diseases, e.g., ultrasensitive transglutaminase-based nano-sensor used for early diagnosis of celiac diseases in human (Gupta et al. 2017). Further, thought is required for more capability in *Pseudomonas* recognition techniques with new developments in accuracy and specificity to meet the future demand. New approaches using biosensors with high specificity and sensitivity can be developed for robust identification of *P. fluorescens* present in food and plant samples.

Compliance with ethical standards

Ethical approval No studies with humans/animals have been performed by any of the authors for the purpose of this review article.

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was taken from all the authors.

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