Growth behavior comparison of *Listeria monocytogenes* between Type strains and beef isolates in raw beef

So-Yeon Lee¹ · Ki-Hyun Kwon² · Changhoon Chai³ · Se-Wook Oh¹

Received: 1 August 2017 / Revised: 27 October 2017 / Accepted: 9 November 2017 / Published online: 30 November 2017
© The Korean Society of Food Science and Technology and Springer Science+Business Media B.V., part of Springer Nature 2017

Abstract This study was conducted to compare the growth parameters of *Listeria monocytogenes* between beef isolates and Type strains in raw beef. Beef was artificially inoculated with 3 Log CFU/g levels and growth was measured during storage at various temperatures (5–25 °C) using conventional plating methods. The R^2 value for lag time (λ) and specific growth rate (μ) were determined using modified-Gompertz model, which were greater than 0.98 at all storage temperature except at 5 °C. B_f , A_f , and RMSE showed acceptable ranges, showed that the models are suitable for the modeling the growth of *L. monocytogenes*. At all temperatures, the λ of *L. monocytogenes* beef isolates was shorter than that of the *L. monocytogenes* Type strains, and the μ of beef isolates was higher than that of Type strains. These results showed that growth pattern prediction of beef inoculated with *L. monocytogenes* beef isolates gives more actual results than with Type strains.

Keywords Beef · Predictive modeling · Isolates · Growth parameter · *Listeria monocytogenes*

Introduction

Beef is easily contaminated by microorganisms [1] owing to its rich nutrients content, that includes high quality proteins, essential amino acids, lipids, minerals, and vitamins. In addition, the intrinsic characteristics of beef, such as pH and water activity, are also responsible for general spoilage caused by pathogenic bacteria [2]. In beef, microbial contamination can occur during slaughtering, packaging, processing, distribution, and storage; therefore, hygiene and safety measures of beef are primary concerns.

Listeria monocytogenes is a Gram-positive and facultative anaerobic bacteria. Listeriosis caused by *L. monocytogenes* can be critical and fatal especially to the vulnerable groups—the elderly, pregnant women, neonates, and immunocompromised adults [3]. It is considered important because of its severity and high death rates [2]. Raw beef is refrigerated at a temperature of 2–4 °C to control and prevent microbial growth [4]. However, *L. monocytogenes* can grow at a temperature as low as – 0.4 °C [5], and can survive mild food preservation treatment [6].

The traditional microbiological analysis methods are time-consuming and rapid enumeration method is expensive and also requires pre-enrichment steps for the detection of food-borne pathogens [7]. The prediction of microbial growth curve for food-borne pathogen in food products can be used for faster microbiological analysis, which is less expensive and can serve as an alternative method to these traditional microbiological analysis methods [8]. It can estimate the changes in microbial population in food products in a real time situation [9]. Predictive microbiology in food products is considered to be a useful tool for product development, risk assessment, Hazard Analysis and Critical Control Points (HACCP) system, and educational purpose [10].

✉ Se-Wook Oh
swoh@kookmin.ac.kr

¹ Department of Food and Nutrition, Kookmin University, 77, Jeongneung-ro, Seoungbuk-gu, Seoul 136-702, Republic of Korea

² Korea Food Research Institute, Bundang-gu, Gyeonggi-do, Republic of Korea

³ Division of Applied Science, Kangwon National University, Chuncheon, Republic of Korea

The predictive model of *L. monocytogenes* in various food samples has been studied. And many researchers used the food isolates for growth modelling [11–13]. But, no studies have compared the growth pattern between Type cultures and food isolates in beef. Therefore, the purpose of this study was to compare the growth curve characteristics between *L. monocytogenes* isolates and Type cultures by predictive modeling in artificially inoculated beef.

Materials and methods

Isolation of *L. monocytogenes* presumptive from raw beef

Raw beef in dices (approximately 1 cm × 1 cm × 1 cm) were purchased from retail markets located in northern Seoul, Korea in July 2014. Totally 300 samples were gathered and microbiological analysis was conducted. Beef sample (25 g) was mixed with 225 ml of UVM-modified *Listeria* enrichment broth (UVM; Oxoid Ltd., Basingstoke, Hampshire, UK) and homogenized using a stomacher (AES Stomacher; AES Chemunex, France). After incubation at 30 °C for 24 h, 100 µL of cultured media was inoculated into 10 mL of Fraser *Listeria* broth (Oxoid Ltd., Basingstoke, Hampshire, UK), and cultured at 37 °C for 24 h. Following incubation, the cultured Fraser broth was streaked onto Oxford agar (Oxoid) and incubated at 30 °C for 24–48 h. From the Oxford agar, black colonies with halo were selected and confirmed by PCR and 16S rRNA gene sequencing.

Preparation of *L. monocytogenes* culture cocktail

Three of *L. monocytogenes* Type strains (ATCC 7644, 19113 and 19115; all human origin) and five of *L. monocytogenes* beef isolates were used in this study. All the strains were maintained in stock culture at – 80 °C in TSB containing 25% glycerol. For activation, 0.1 mL of the stock culture was inoculated into 10 mL of TSB and incubated at 30 °C for 24–48 h and centrifuged at 2200×g for 25 min at 20 °C. The final pellets were resuspended in 0.85% saline and combined to construct a culture cocktail for artificial inoculation in raw beef.

Identification of presumptive *L. monocytogenes*

PCR analysis was performed to identify the presumptive *L. monocytogenes* with strain specific primer for detecting *hlyA* gene. The sequences of the primers used were 5'-GCA GTT GCA AGC GCT TGG AGT GAA-3', and 5'-GCA ACG TAT CCT CCA GAG TGA TCG-3', and the expected size of the amplicon was 456 bp [14].

After confirmation of *L. monocytogenes* presumptive by PCR analysis, 16 s rRNA identification was conducted. The sequences of the universal primers were 5'-AGA GTT TGA TCC TGG CTC AG-3' for 27F, and 5'-GGT TAC CTT GTT ACG ACT T-3' for 1492R. The sequencing of 16 s rDNA was performed using an ABI PRISM 3730XL DNA analyzer (Applied Biosystems). Sequence similarity was searched using GenBank BLASTn (<http://www.ncbi.nlm.nih.gov/BLAST/>), and assembled by Phrap (<http://www.phrap.org/>).

Artificial inoculation and bacterial enumeration for growth modeling

Cocktail of *L. monocytogenes* Type culture and isolates were inoculated onto the raw beef (10 g) surface. The initial microbial loads of *L. monocytogenes* were 3 log CFU/g. The inoculated samples were aerobically stored at 5, 10, 15, 20, and 25 °C. After storage, 90 mL of saline solution was added to a 10 g sample, homogenized using a stomacher, and plated onto Oxford agar. After the incubation at 30 °C for 24–48 h, black colonies on the plates were enumerated.

Application of mathematical model for *L. monocytogenes* growth

Calculations for the primary model at different temperature (5, 10, 15, 20, and 25 °C) for lag time (LT, λ), specific growth rate (SGR, μ), initial population (N_0), and maximum population (N_{max}) values were carried out by applying the modified-Gompertz equation (Eq. 1) using a GraphPad Prism software v. 4.03 (GraphPad Software, San Diego, CA, USA). The λ , μ , and N_{max} at 5, 10, 15, 20, and 25 °C were obtained from a modified-Gompertz equation regressed into exponential decay (Eq. 2), and exponential growth (Eq. 3), respectively using a SigmaPlot software v. 12.0 (Systat Software Inc., Richmond, CA, USA).

$$N = N_0 + A \cdot \exp(-\exp((\mu \cdot e \cdot (\lambda - t)/A + 1))) \quad (1)$$

where N is the bacterial population at time (t) and A is N_{max}/N_0 .

$$\lambda = \lambda_0 + a_\lambda \cdot \exp(-b_\lambda \cdot T) \quad (2)$$

where λ_0 , a_λ , and b_λ are the regression constants.

$$\mu = \mu_0 + a_\mu \cdot \exp(b_\mu \cdot T) \quad (3)$$

where μ_0 , a_μ , and b_μ are the regression constants.

Validation of predictive models

The validation of the predictive models were evaluated by determination (R^2), Bias factor (B_f), accuracy factor (A_f), and root mean square error (RMSE).

$$R^2 = 1 - (\sum e_i^2 / \sum (y_i - \bar{y})^2) \quad (4)$$

where e_i is error of predictive data, y_i is the predictive data, and $\bar{y}$ is the average of predictive data.

$$B_f = 10^{(\sum \log(pred/obs)/n)} \quad (5)$$

$$A_f = 10^{(\sum |\log(pred/obs)|/n)} \quad (6)$$

$$RMSE = \sqrt{(\sum (obs - pred)^2)/n} \quad (7)$$

where obs is the observed value, $pred$ is the predicted value, and n repetition number of the observed data.

Results and discussion

Identification of presumptive *L. monocytogenes*

A total of five presumptive colonies were isolated from the Oxford agar based on the distinctive black color formation. Totally 300 different samples were analyzed and all presumptive colonies were obtained from two different retail market with same food distribution company. It might be due to the poor sanitation of retail markets itself or contamination of raw material.

Fig. 1 PCR amplification of *L. monocytogenes* isolates from raw beef. (A) 100 bp DNA ladder (TAKARA, Bio Inc., Shiga, Japan); (B) *L. monocytogenes* ATCC 7644; (C) strain B-1 isolates; (D) strain B-2 isolates; (E) strain B-3 isolates; (F) strain B-4 isolates; (G), strain B-5 isolates

Figure 1 shows results of PCR amplification patterns with *L. monocytogenes* presumptive from raw beef. All isolated strains were amplified *hlyA* gene fragment (456 bp).

Secondary confirmation was performed using 16 s rRNA identification. There are several advantage to analyzing 16 s rRNA identification of bacteria in comparison with biochemical methods. The 16 s rRNA gene sequence is specific characters at the species level [15]. The closest relative species were determined by comparing with sequence database provided by the GenBank. All strains showed similarity with *L. monocytogenes* 07PF0776 (99% sequence similarity). The strains gathered by selective media were identified as *L. monocytogenes*, which differs from other studies. Barros et al. [16] reported that they isolated five different *Listeria* species from meat, consisting *L. monocytogenes* (13.2%), *L. innocua* (77.4%), *L. seeligeri* (1.3%), *L. welshimeri* (7.5%), and *L. grayi* (0.6%). Cocolin et al. [17] reported that *L. monocytogenes* 5UD and *L. monocytogenes* 9UD were isolated from sliced S. Daniele ham and De-boned S. Daniele ham, respectively. There are differences in isolated bacterial strain due to different food sample sources.

Primary models for *L. monocytogenes*

The predictive model can describe the *L. monocytogenes* growth pattern at various temperatures. Lopez [18] reported that the Gompertz model described microbial growth practically.

Figure 2 showed growth patterns of *L. monocytogenes* Type culture and beef isolates on raw beef at various temperatures (5–25 °C). It was reported that the growth of bacteria in food is influenced by storage temperature [19]. In this study, *L. monocytogenes* grew well at all conditions except at 5 °C temperature. At 5 °C, bacterial growth was observed but there was no clear lag phase and stationary phase. *L. monocytogenes* can growth under refrigerated condition, but as storage temperature decreased, the growth rate also decreased [12]. The N_{max} of *L. monocytogenes* Type culture was between 7.12 and 7.76, and that of *L. monocytogenes* beef isolates was between 7.53 and 7.80. The load of background microbiota was regarded as important factor influencing the N_{max} [20]. The samples used in this study were not sterilized. So various background microbiota might interrupted the growth of *L. monocytogenes*.

At higher temperature, shorter λ and higher μ were calculated (Table 1). At all temperatures, the λ of *L. monocytogenes* Type strains was longer than that of beef isolates. However, the μ of Type strains was lower than that of beef isolates. The λ of *L. monocytogenes* Type strains in raw beef were predicted to be 44.99, 23.44, 8.74,

Fig. 2 Gompertz model for *L. monocytogenes* type culture (filled circle) and *L. monocytogenes* isolates from beef in raw beef (filled triangle) at 25 °C (A), 20 °C (B), 15 °C (C), 10 °C (D), and 5 °C (E)

and 5.81 h and that of beef isolates were calculated as 32.07, 8.02, 4.52, and 1.77 h at 10, 15, 20, and 25 °C, respectively. The μ of *L. monocytogenes* Type strains stored at 10, 15, 20, and 25 °C were estimated to be 0.01, 0.02, 0.03, and 0.09 and that of beef isolates were predicted as 0.02, 0.02, 0.04, and 0.18, respectively. Compared to our result, different λ and μ values at 15 °C were reported [21]. They reported that the λ of 2-strain cocktail of *L. monocytogenes* containing *L. monocytogenes* ATCC 15313 and

isolated *L. monocytogenes* L13-2 was 7 h and μ was 0.47 on raw pork meat at 15 °C. The different results are due to using different food and bacterial strains. Among strains, the differences in λ were significantly greater than in μ . Under lower temperature, the difference of λ and μ was more [22].

Table 2 showed the growth parameters of modified-Gompertz model to estimate the growth of *L. monocytogenes* Type strains and beef isolates. The primary model

Table 1 Estimated values of lag time (λ) and specific growth rate (μ) of *L. monocytogenes* Type strains and *L. monocytogenes* beef isolates by modified-Gompertz model in artificially inoculated raw beef

Temperature (°C)	<i>L. monocytogenes</i> Type strains		<i>L. monocytogenes</i> isolates	
	Lag time, λ (h)	Specific growth rate, μ (log CFU/g/h)	Lag time, λ (h)	Specific growth rate, μ (log CFU/g/h)
5	N.A ^a	N.A	N.A	N.A
10	44.99	0.01	32.07	0.02
15	23.44	0.02	8.02	0.02
20	8.74	0.03	4.52	0.04
25	5.81	0.09	1.77	0.18

^aNot available**Table 2** Validation of primary model to predict the growth of *L. monocytogenes* Type strains and *L. monocytogenes* beef isolates in raw beef

Temperature (°C)	<i>L. monocytogenes</i> Type strains			<i>L. monocytogenes</i> isolates		
	R ^{2a}	RMSE ^b	B _f ^c	R ²	RMSE	B _f
5	N.A ^d	N.A	N.A	N.A	N.A	N.A
10	0.99	0.12	1.00	0.99	0.19	1.01
15	0.99	0.20	1.00	1.00	0.04	1.00
20	0.99	0.17	1.00	0.98	0.25	1.00
25	0.99	0.18	1.01	0.99	0.20	0.99

^aDetermination coefficient^bRoot-mean-square error^cBias factor

with the modified-Gompertz model at all condition fit well with high R² value (R² = 0.98–1.00). In addition, RMSE was close to 0 (RMSE = 0.04–0.25) and bias was close to 1 (bias = 0.99–1.01). The evaluation of RMSE also provided goodness of fit, with value of RMSE near 0 [23]. Therefore, these results indicated that the model provide a good description of the growth behavior.

Secondary models for *L. monocytogenes*

The λ and μ calculated using the primary predictive model of *L. monocytogenes* in raw beef were applied to secondary model as a function of temperature using exponential equation. These results showed that λ is inversely proportional to μ and is influenced by temperature change. Secondary model was developed to define the primary model parameters that were affected by temperature, including λ and μ [24].

Table 3 showed the evaluations of secondary model of *L. monocytogenes* Type strains. For the λ and μ equation, R² values were both 1.00. B_f and A_f of λ equation were 0.99 and 1.12, respectively. In addition, in the μ equation, B_f and A_f were 1.02 and 1.12, respectively. These results showed that both B_f and A_f values of λ and μ equation were close to 1.00. The bias and accuracy factor equal to 1.0 indicated a perfect agreement between predicted and

observed values [25]. The R² values of secondary model of *L. monocytogenes* beef isolates were both 1.00. In the λ equation, B_f and A_f were 1.03 and 1.18, respectively. For μ equation, B_f and A_f were 1.00 and 1.03, respectively. As mentioned above, both B_f and A_f values of λ and μ equation were also close to 1.00. Hence, it was found that the predictions were similar to the experimental results.

Under higher temperature, the difference of λ and μ between *L. monocytogenes* Type strains and beef isolates was more. Therefore, the growth rate of *L. monocytogenes* beef isolates is probably much higher than Type strains with increases in the storage temperature.

The Type strains used in this study is widely used to construct culture cocktail in many studies [26–28]. But these strains were not isolated from beef, all Type strains used in this study were isolated from human. So if when these strains were used to construct culture cocktail for measuring growth pattern by artificial inoculation, it might not represent the actual growth pattern in raw beef.

In conclusion, based on the differences of growth parameters it was suggested that more realistic information on *L. monocytogenes* growth behaviors in beef can be obtained by using beef isolates, and can be used in subsequent quantitative microbial risk assessments for compliance with food safety criteria.

Table 3 Evaluation of exponential equation to predict the growth of *L. monocytogenes* Type strains and *L. monocytogenes* beef isolates in raw beef

Cultures	Variables	Exponential equation	Regression analysis		
			R ^{2a}	B _f ^b	A _f ^c
<i>L. monocytogenes</i> Type strains	λ (h)	$\lambda = -1.0385 + 182.9051 \cdot \exp^{-0.1374 \cdot T}$	1.00	0.99	1.12
	μ (log CFU/g/h)	$\mu = 0.0111 + 0.00009059 \cdot \exp^{0.2707 \cdot T}$	1.00	1.02	1.12
<i>L. monocytogenes</i> isolates	λ (h)	$\lambda = 2.2416 + 709.2921 \cdot \exp^{-0.3170 \cdot T}$	1.00	1.03	1.18
	μ (log CFU/g/h)	$\mu = 0.0186 + 0.0000061977 \cdot \exp^{0.4067 \cdot T}$	1.00	1.00	1.03

^aDetermination coefficient^bBias factor^cAccurate factor

Acknowledgements This study was supported by a program of High Value-added Food Technology Development of Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Grant No. 313032-03-2-HD020).

Author's contribution So-Yeon Lee: Execution of experiment, and preparation of manuscript; Gi-Hyeon Gwon: Discussion of the results; Changhoon Chai: Discussion of results relating to shelf life; Se-Wook Oh: Advice for the study.

Compliance with ethical standards

Competing interest The authors declare that have no competing interests.

References

- Hygreeva D, Pandey M, Radhakrishna K. Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. *Meat Sci.* 98: 47–57 (2014)
- Mataragas M, Skandamis P, Drosinos E. Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. *Int. J. Food Microbiol.* 126: 1–12 (2008)
- Jalali M, Abedi D. Prevalence of *Listeria* species in food products in Isfahan, Iran. *Int. J. Food Microbiol.* 122: 336–340 (2008)
- Petrou S, Tsiraki M, Giatrakou V, Savvaidis I. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *Int. J. Food Microbiol.* 156: 264–271 (2012)
- Walker S, Archer P, Banks JG. Growth of *Listeria monocytogenes* at refrigeration temperatures. *J. Appl. Microbiol.* 68: 157–162 (1990)
- Linton R, Webster J, Pierson M, Bishop J, Hackney C. The effect of sublethal heat shock and growth atmosphere on the heat resistance of *Listeria monocytogenes* Scott A. *J. Food Prot.* 55: 84–87 (1992)
- Velugoti PR, Bohra LK, Juneja VK, Huang L, Wesseling AL, Subbiah J, Thippareddi H. Dynamic model for predicting growth of *Salmonella* spp. in ground sterile pork. *Food Microbiol.* 28: 796–803 (2011)
- García-Gimeno RM, Zurera-Cosano G. Determination of ready-to-eat vegetable salad shelf-life. *Int. J. Food Microbiol.* 36: 31–38 (1997)
- Ross T, Dalgaard P, Tienungoon S. Predictive modelling of the growth and survival of *Listeria* in fishery products. *Int. J. Food Microbiol.* 62: 231–245 (2000)
- Devlieghere F, Van Belle B, Debevere J. Shelf life of modified atmosphere packed cooked meat products: a predictive model. *Int. J. Food Microbiol.* 46: 57–70 (1999)
- Danyluk MD, Friedrich LM, Schaffner DW. Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. *Food Microbiol.* 38: 52–55 (2014)
- Hong CH, Sim WC, Chun SJ, Kim YS, Oh DH, Ha SD, Choi WS, Bahk GJ. Predictive Growth Model of Native Isolated *Listeria monocytogenes* on raw pork as a Function of Temperature and Time. *Kor. J. Food Sci. Technol.* 37: 850–855 (2005)
- Tiwari U, Walsh D, Rivas L, Jordan K, Duffy G. Modelling the interaction of storage temperature, pH, and water activity on the growth behaviour of *Listeria monocytogenes* in raw and pasteurised semi-soft rind washed milk cheese during storage following ripening. *Food control.* 42: 248–256 (2014)
- Paziak-Domańska B, Bogusławska E, Więkowska-Szakiel M, Kotłowski R, Różalska B, Chmiela M, Kur J, Dąbrowski W, Rudnicka W. Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. *FEMS Microbiol. Lett.* 171: 209–214 (1999)
- Kolbert CP, Persing DH. Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. *Curr. Opin. Microbiol.* 2: 299–305 (1999)
- Barros MA, Nero LA, Silva LC, d'Ovidio L, Monteiro FA, Tamanini R, Fagnani R, Hofer E, Beloti V. *Listeria monocytogenes*: Occurrence in beef and identification of the main contamination points in processing plants. *Meat Sci.* 76: 591–596 (2007)
- Cocolin L, Stella S, Nappi R, Bozzetta E, Cantoni C, Comi G. Analysis of PCR-based methods for characterization of *Listeria monocytogenes* strains isolated from different sources. *Int. J. Food Microbiol.* 103: 167–178 (2005)
- López S, Prieto M, Dijkstra J, Dhanoa M, France J. Statistical evaluation of mathematical models for microbial growth. *Int. J. Food Microbiol.* 96: 289–300 (2004)
- Voyer R, McKellar R. MKES Tools: A microbial kinetics expert system for developing and assessing food production systems. *J. Ind. Microbiol.* 12: 256–262 (1993)

20. Lebert I, Robles-Olvera V, Lebert A. Application of polynomial models to predict growth of mixed cultures of *Pseudomonas* spp. and *Listeria* in meat. *Int. J. Food Microbiol.* 61: 27–39 (2000)
21. Lee YJ, Jung BS, Yoon HJ, Kim KT, Paik HD, Lee JY. Predictive model for the growth kinetics of *Listeria monocytogenes* in raw pork meat as a function of temperature. *Food Control.* 44: 16–21 (2014)
22. Begot C, Lebert I, Lebert A. Variability of the response of 66 *Listeria monocytogenes* and *Listeria innocua* strains to different growth conditions. *Food Microbiol.* 14: 403–412 (1997)
23. Baranyi J, Ross T, McMeekin T, Roberts T. Effects of parameterization on the performance of empirical models used in predictive microbiology. *Food Microbiol.* 13: 83–91 (1996)
24. Lee YJ, Jung BS, Kim KT, Paik HD. Predictive model for the growth kinetics of *Staphylococcus aureus* in raw pork developed using Integrated Pathogen Modeling Program (IPMP) 2013. *Meat Sci.* 107: 20–25 (2015)
25. Zurera-Cosano G, Garcia-Gimeno R, Rodriguez-Perez M, Herivas-Martinez C. Validating an artificial neural network model of *Leuconostoc mesenteroides* in vacuum packaged sliced cooked meat products for shelf-life estimation. *Eur. Food Res. Technol.* 221: 717–724 (2005)
26. Beltrame CA, Kubiak GB, Rottava I, Toniazzi G, Cansian RL, Lerin LA, Oliveira D, Treichel H. Comparative lethality kinetic curves and predictive models of F-value for *Listeria monocytogenes* using different sanitizers. *Food Sci. Nutr.* 1: 27–31 (2013)
27. Guillier L, Augustin J-C. Modelling the individual cell lag time distributions of *Listeria monocytogenes* as a function of the physiological state and the growth conditions. *Int. J. Food Microbiol.* 111: 241–251 (2006)
28. Walter EH, Kabuki DY, Esper LM, Sant'Ana AS, Kuaye AY. Modelling the growth of *Listeria monocytogenes* in fresh green coconut (*Cocos nucifera* L.) water. *Food Microbiol.* 26: 653–657 (2009)