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Growth Rate and Histamine Production of *Klebsiella* sp. CK02 Isolated from Skipjack Tuna Compared with *Morganella morganii* ATCC 25830 at Various Incubation Temperatures

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Abstract

One of an important quality parameter in tuna is the level of histamine content. The contamination of histamine in tuna is mainly due to the activity of histidine decarboxylase produced by the bacteria. A rapid growth of histamine producing bacteria is correlated with the practice of temperature abuse during handling. This study aimed to develop predictive growth modeling of two histamine-producing bacteria in the function of temperature. The growth and histamine production of *Klebsiella* sp. CK02 and *Morganella morganii* ATCC 25830 at various temperatures were measured in tryptic soy broth histidine (TSBH) and tuna fish infusion broth (TFIB) growth media. Broths were incubated at 4°C and 15°C for 7 days, and at 30°C and 40°C for 24 hours. The Baranyi and Roberts model was used with DMFit to determine primary growth kinectics, and the Ratkowsky square root model to describe bacterial growth rate as a function of temperature. Histamine production was enumerated by the apparent yield factor ($pY_{his/CFU}$) value. Growth rate increased with temperature, with a maximum rate at 40°C for *Klebsiella* sp. CK02 (0.740 log CFU/h) and *M. morganii* (0.578 log CFU/h). The T_{min} for *Klebsiella* sp. CK02 in TFIB was -8.9°C, indicating better survival in low storage temperature, compare to *M. morganii* ATCC 25830. In addition, *Klebsiella* sp. CK02 produced a lower $pY_{his/CFU}$ at 15 and 30°C compared to *M. morganii* ATCC 25830.

Keywords: growth rate, temperature, histamine, *Klebsiella* sp. CK02, *M. morganii* ATCC 25830

1. Introduction

Scombridae and *Scomberesocidae* fish commonly have a high concentration of the amino acid histidine (Rawles, Flick, & Martin, 1996). During fish spoilage, bacteria can produce decarboxylase, an enzyme that converts free histidine and other amino acids into histamine and other biogenic amines, two substances frequently used as fish quality indicators (Lehane & Olley, 2000), as well as food safety indicators due to the toxic effects of histamine (Sumner, Ross, & Ababouch, 2004). Histamine-related toxication, known as histamine fish poisoning (HFP), is often associated with consumption of seafood (Rawles et al., 1996). Histamine production in fish is mainly caused by improper handling temperature, which causes the growth of histidine decarboxylase (HDC)-producing bacteria or histamine-producing bacteria (HPB) (Lehane & Olley, 2000). Sumner et al. (2004) reported that common HPB are from the Enterobacteriaceae family, such as *Hafnia, Klebsiella*, and *Morganella*, as well as from the Bacillaceae family, with varied abilities of histamine production among species. *Enterobacter aerogenes, Morganella morganii, Photobacterium damselae, Raoultella planticola,* and *R. ornithinolytica* can produce >1,000 ppm histamine, whereas *Citrobacter freundii, Escherichia coli, Hafnia alvei,* and *Vibrio alginolyticus* generate low histamine levels of <500 ppm, under similar culture conditions (Björnsdóttir-Butler, Bolton, Jaykus, McClellan-Green, & Green, 2010). Among HPB species, *H. alvei, Klebsiella pneumoniae,* and *M. morganii* have been isolated from fish samples that allegedly caused scombroid poisoning (Rawles et al., 1996). Moreover,

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M. psychrotolerance and *P. phosphoreum* that were most frequently detected in fresh yellowfin tuna fillets showed growth and histamine production at 4°C (Trevisani, Cecchini, Fedrizzi, Corradini, Mancusi, & Tothill, 2019). The ability of *M. psychrotolerance* in producing histamine at low temperature shows that there is still a risk of histamine poisoning in fish during low temperature handling (Wang, Yamaki, Kawai, & Yamazaki, 2020).

Low temperature handling plays a critically important role in fish quality control, particularly in preventing histamine production. Models to predict HPB growth (Jorgensen, Huss, & Dalgaard, 2000; Emborg & Dalgaard, 2008) may be used to predict the relationship between temperature, other environmental parameters and HPB growth, as well as histamine production. Such models may help the fishery industry and food safety supervisory agencies to estimate shelf-life, optimize storage conditions, and analyze histamine exposure assessment (Emborg, 2007).

HPB strains used in histamine prediction models are generally isolated from subtropical fish. Jorgensen et al. (2000) developed a biogenic amine prediction model, including histamine, using bacteria isolated from cold-smoked salmon in Norway, while Emborg & Dalgaard (2008) constructed a growth model using *M. morganii* and *M. psychrotolerans* isolated from yellowfin tuna in Denmark. Previously, we isolated HPB strain *Klebsiella* sp. CK02 from the skipjack tuna caught in the tropical area of Sadeng Port of Yogyakarta, which showed high histamine production. However, characteristic of its growth kinetics and histamine production rate remain unknown.

Studies on histamine production by *Klebsiella* spp. are merely observing the histamine concentration over time overlooking its relation to temperature. An earlier study by Taylor, Guthertz, Leatherwood, & Lieber (1979) reported *K. pneumoniae* incubated in tuna fish infusion broth (TFIB) for 7 h produced 19,900 nmol histamine/ml (i.e. 442 mg histamine/100 g tuna) at 32°C. The highest concentration of histamine produced by *K. oxytoca* was 1,415 ppm after 18 h at 37°C (López-Sabater, Rodríguez-Jerez, Hernández-Herrero, & Mora-Ventura, 1996). Later research by Özoğul (2004) demonstrated that the highest biogenic amine production from *K. pneumoniae* NCMIB 673 was 3,416 mg/L histamine at 37°C, in histidine decarboxylase broth (HDB) incubated for 2 d.

Several ASEAN countries are prominent tuna producers in the world. Located in tropical area, they face challenging environmental conditions of high and fluctuating temperatures during cold chain handling of fish, which can promote microbial growth. Therefore, this study was conducted to quantitatively assess the effect of temperature on growth and histamine production of *Klebsiella* sp. CK02 compared with *M. morganii* ATCC 25830, a known HPB. The resulting data and predictive model may be used to manage microbial risks of tropical fishery products.

2. Materials and Methods

2.1. Bacterial Culture

Klebsiella sp. CK02 was obtained from the culture collection of Fishery Product Quality and Safety Laboratory, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, and was isolated from skipjack tuna in Sadeng Port, Yogyakarta. Culti-Loops™ *Morganella morganii subsp. morganii* ATCC™ 25830™ was purchased from Thermo Scientific™ (Germany). Both bacteria tested positive for the histamine-producing gene (hdc), based on the method of Takahashi, Kimura, Yoshikawa, & Fujii (2003).

2.2. Media

Tryptic soy broth (TSB; Oxoid) was used to prepare the inoculum. Tryptic soy broth histidine (TSBH) and tuna fish infusion broth (TFIB) were prepared to analyze bacterial growth and histamine production, according to Taylor & Woychik (1982) and Taylor et al. (1979), respectively. TSBH was prepared from 3% TSB fortifed with 1% histidine (Merck, Germany). TFIB was prepared by homogenization of skipjack tuna in twice its amount of water (w/w), followed by steaming at 100°C for 1 h. After cooling, the boiled fish was filtered using Whatman filter paper No.1, and then 1% glucose was added and the media was sterilized. Tryptic soy agar (TSA; Oxoid) was used for bacterial enumeration.

2.3. Analysis of Bacterial Growth and Histamine Production

Klebsiella sp. CK02 and *M. morganii* ATCC 25830 inocula were prepared by inoculation of TSB, followed by 24 h incubation at 37°C. One loop of each species was inoculated in 10 ml TSBH and 10 ml TFIB, followed by incubation at 4, 15, 30 and 40°C. Samples incubated at 4 and 15°C were observed every 24 h for 168 h, whereas those incubated at 30 and 40°C were observed every 3 h for 24 h. At each sampling time, bacterial and histamine concentration were recorded. All treatments were conducted in duplicate.

For bacterial enumeration on TSA, 0.1 ml of bacteria grown in TSBH and TFIB was serially diluted in 0.9 ml Butterfield Phosphate Buffer (BPB), plated on TSA,

and incubated at 37°C for 24 h. Counts were converted to log CFU/ml.

Histamine concentration in TSBH and TFIB media was determined by thin-layer chromatography (TLC) silica gel plates (60F254, Merck, Germany) based on the method of Bajc & Gaènik (2009). Approximately 1.0 µl sample or histamine standard solution was spotted on plates. Compound separation was performed in a chamber using methanol:ammonia (20:1, v/v) as the mobile phase. Plates were removed from the chamber, dried in an oven, and then sprayed with ninhydrin solution (300 mg ninhydrin in 100 ml *n*-butanol, containing 3 ml glacial acetic acid) for spot visualization. TLC plates were scanned, and spots processed using Photoshop to measure brightness. A mathematical equation produced from brightness level versus histamine standard concentrations (100–3,000 ppm) was used to convert sample brightness to histamine concentration (ppm).

2.4. Data Analysis

Bacterial growth data were plotted with incubation time to obtain growth curves for *Klebsiella* sp. CK02 and *M. morganii* using MS Excel, and then fitted with the Baranyi & Roberts (1995) primary model, using DMFit (http://www.combase.cc), to measure bacterial growth kinetic parameters of lag time, growth rate (μ_{max}) and maximum population density (N_{max}). The secondary square root model of Ratkowsky, Olley, McMeekin, & Ball (1982) was used to estimate μ_{max} as a function of temperature as follows:

$$\mu_{max} = [b(T - T_{min})]^2$$

where μ_{max} is the maximum growth rate (log CFU/h), T the temperature (°C), T_{min} the extrapolated minimum growth temperature, and b is a regression coefficient.

To measure accuracy of the Ratkowsky square root model, root mean square error (RMSE) was calculated:

$$RMSE_{model} = \sqrt{\frac{\sum_{i=t}^{n}(observed_i - fitted_i)^2}{n}}$$

with observed as the observed value, fitted as the prediction value, and n as the data number. Bacterial histamine production was calculated using the apparent yield factor ($pY_{his/CFU}$), according to Jorgensen et al. (2000):

$$pY_{his/cfu} = -\log\left(\frac{His_{final} - His_{initial}}{N_{final} - N_{initial}}\right)$$

with His_{final} and $His_{initial}$ as the final and initial histamine concentration (ppm), respectively, and, N_{final} and $N_{initial}$ as the final and initial bacterial number (CFU/ml), respectively. Jorgensen et al. (2000) used $pY_{his/CFU}$ to measure bacterial histamine production, with lower $pY_{his/CFU}$ indicating higher production ability.

3. Results and Discussion

3.1. Effect of Temperature on Growth Rate of *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830

Both *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830 grew from 4 to 40°C (Figure 1). In general, there was no significant lag phase. For *Klebsiella* sp. CK02, growth rates (μ_{max}) in TSBH ranged from 0.008 to 0.740 log CFU/h at 4 and 40°C, respectively (Table 1). In TFIB, μ_{max} were 1.5 and 2.7 times lower at 30 and 40°C, respectively. Compared to *Klebsiella* sp. CK02, μ_{max} for *M. morganii* ATCC 25830 were more similar between TSBH and TFIB. However, μ_{max} were 1.24 and 1.06 times higher in TFIB than TSBH at 30 and 40°C, respectively. Overall, N_{max} ranged from 7.2 to 8.4 log CFU/ml, with the exception of *Klebsiella* sp. CK02 where N_{max} was 5.5 log CFU/ml.

Within the genus of *Klebsiella*, *K. pneumoniae* and *K. oxytoca* are well-known for producing significant levels of histamine in fish (Taylor et al., 1979; López-Sabater et al., 1996). In present study, skipjack isolate *Klebsiella* sp. CK02 shows a significant increase in cell number at 15, 30, and 40°C (Figure 1a and 1b). Similar increase in cell number at the same temperature range has also been reported in *Klebsiella pneumoniae*. Behling & Taylor (1982) showed that during the initial 6 h at 37°C, bacterial numbers increased from 7 to 9 log CFU/ml, with lower numbers at lower temperatures (30 and 15°C). Interestingly, it was also reported that despite faster growth at 37°C, the maximum density at 30°C was higher. Similar results were also observed in this study, in which the maximum (N_{max}) and the final bacterial number (N_{final}) of *Klebsiella* sp. CK02 at 30°C were higher than those at 40°C in both media (Table 1). López-Sabater, Rodriguez-Jerez, Hernández-Herrero, & Mora-Ventura (1994) also reported that the *K. pneumoniae* number incubated at 37°C for 18 h was 8.34 log CFU/ml, whereas Taylor et al. (1979) reported an increase in bacterial number of 1.74 log CFU/ml at 32°C for 7 h.

Klebsiella sp. CK02 μ_{max} in TSBH medium tended to be higher than in TFIB at all temperatures (Table 1). As previously reported by Chen, Wei, Koburger, & Marshall (1989) regarding the growth of *K. pneumoniae* and other HPB in four types of media with various L-histidine and glucose levels, the histidine level clearly affected bacterial growth, whereas glucose levels provided inconsistent pattern in bacterial growth. Significant growth was observed for *K. pneumoniae, M. morganii,* and *K. oxytoca* in media with high histidine levels, but *H. alvei* showed a higher μ_{max} in low histidine medium (Chen et al., 1989).

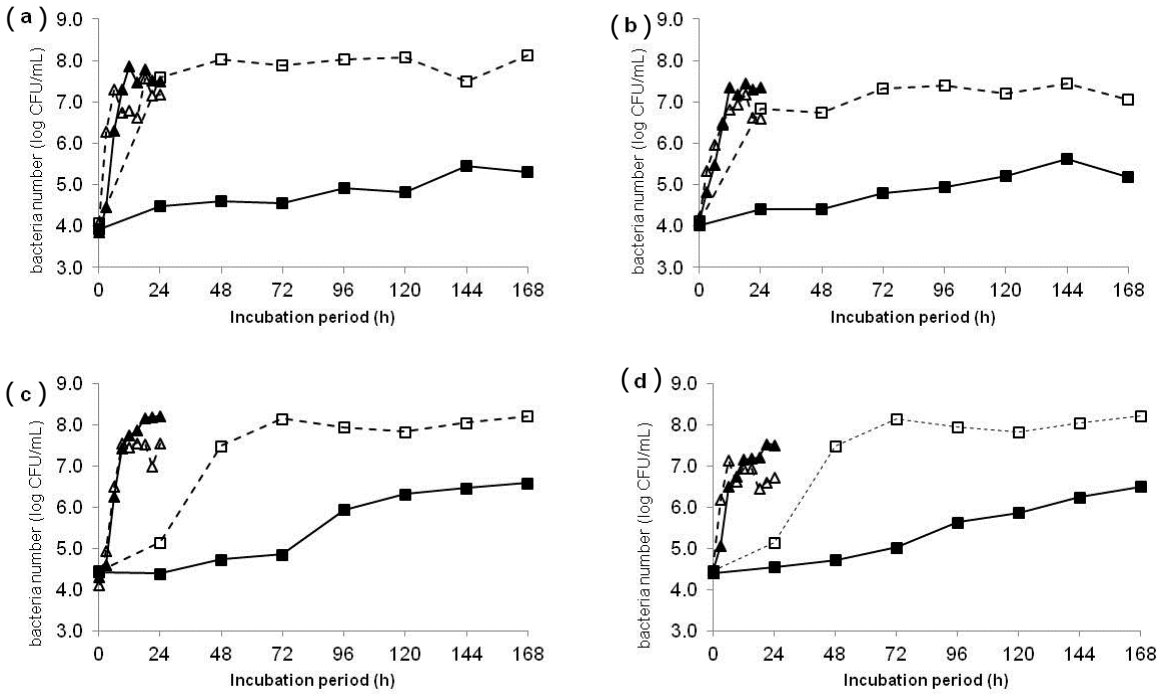

Figure 1. *Klebsiella* sp. CK02 (K) and *Morganella morganii* ATCC 25830 (M) growth rate at 40°C (△), 30°C (▲), 15°C (□), and 4°C (■). (a) K in TSBH; (b) K in TFIB; (c) M in TSBH; (d) M in TFIB.

Table 1. *Klebsiella* sp. CK02 and *Morganella morganii* ATCC 25830 growth rate at various incubation temperatures and media obtained using DMFit primary model

Bacteria	Temp. (°C)	Medium	μ_{max} (log CFU/h)[a]	$N_{initial}$ (log CFU/ml)[b]	N_{final} (log CFU/ml)[b]	N_{max} (log CFU/ml)[b]
Klebsiella sp. CK02	4	TSBH	0.008	4.1	5.4	5.5
		TFIB	0.008	4.1	5.6	5.7
	15	TSBH	0.157	4.1	7.9	8.3
		TFIB	0.124	4.1	7.2	7.5
	30	TSBH	0.43	3.6	7.6	8
		TFIB	0.274	4	7.4	7.4
	40	TSBH	0.74	4.1	7	7.6
		TFIB	0.269	4.3	6.8	7.2
M. morganii ATCC 25830	4	TSBH	0.022	4.2	6.6	7.6
		TFIB	0.018	4.4	6.5	6.9
	15	TSBH	0.089	4.3	8	8.3
		TFIB	0.08	4.2	8	8.4
	30	TSBH	0.359	4	8.1	8.3
		TFIB	0.444	4.5	7.4	7.6
	40	TSBH	0.544	4.1	7.4	7.7
		TFIB	0.578	4.5	6.8	7.3

Note :

[a] Mean of maximum growth rate from duplicate; [b] Initial, final, and maximum bacteria number from duplicate

Table 2. Ratkowsky square root model for *Klebsiella* sp. CK02 and *Morganella morganii* ATCC 25830 growth rate in temperature function

Bacteria	Medium	T_{min} (°C)	b	RMSE	Prediction Model
Klebsiella sp. CK02	TSBH	-1.7	0.0209	0.021	$\mu_{max} = [0.0209 (T+1.722)]^2$
	TFIB	-8.9	0.0119	0.051	$\mu_{max} = [0.0119 (T+8.889)]^2$
M. morganii ATCC 25830	TSBH	-4.1	0.0169	0.017	$\mu_{max} = [0.0169 (T+4.053)]^2$
	TFIB	-2.5	0.0184	0.048	$\mu_{max} = [0.0184 (T+2.483)]^2$
M. morganii DSM 14850[a)]	Canned Tuna	0.3	0.0331		
M. psychrotolerans JB T-11[a)]	Canned Tuna	-7.5	0.021		

[a)] Emborg & Dalgaard (2008)

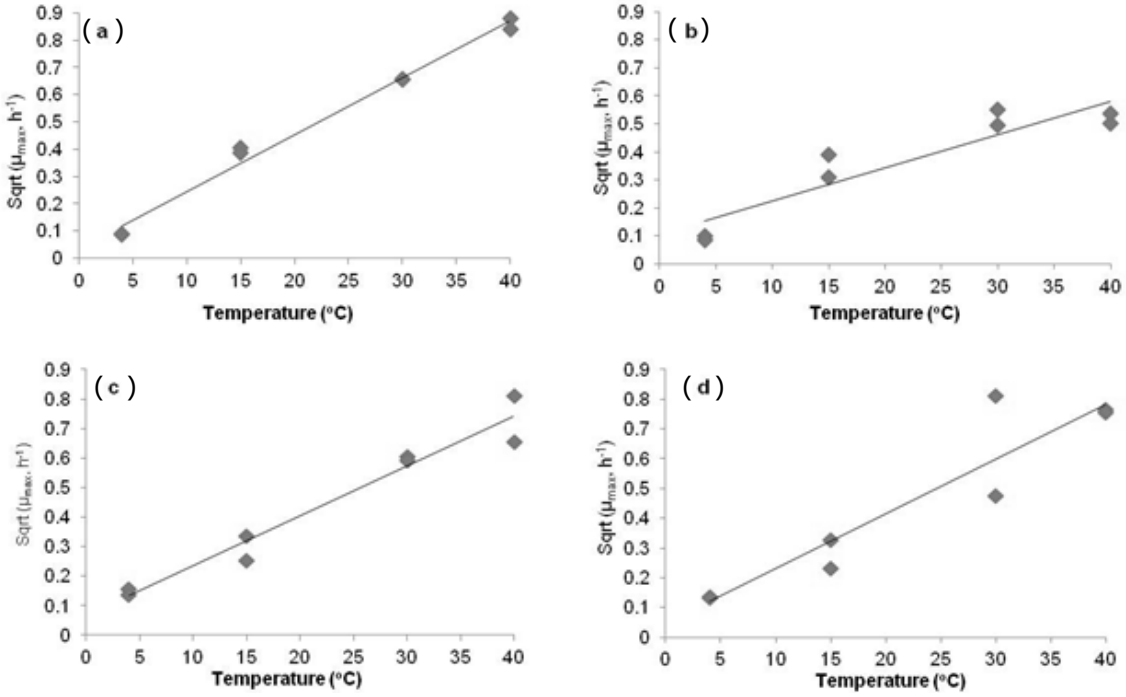

Figure 2. Correlation of temperature and growth rate of *Klebsiella* sp. CK02 (K) and *Morganella morganii* ATCC 25830 (M) in the Ratkowsky square root model. (a) K in TSBH; (b) K in TFIB; (c) M in TSBH; (d) M in TFIB

In contrast to *Klebsiella* sp. CK02 μ_{max}, that of *M. morganii* ATCC 25830 in TFIB was higher than in TSBH, especially as temperature increased (Table 1). Kim, Ben-Gigirey, Barros-Velázquez, Price, & An (2000) reported higher *M. morganii* μ_{max} in TFIB at 37°C than at 15 and 25°C, with a high N_{max} of 8 log CFU/ml on day-1, whereas the highest cell numbers at 25°C and 15°C were observed on day-2 and day-3, respectively. Another study by Torres, Roeckel, & Cristina (2002) regarding *M. morganii* growth in TSBH at 10, 15, 20 and 30°C reported that μ_{max} increased with increasing temperature. At 30°C, μ_{max} reached the highest level of 0.8 log CFU/h.

Cell numbers of *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830 showed a significant increase at 15°C in both media, but the rate appeared to be slower than that at 30°C and 40°C (Figure 1). The primary model analysis using DMFit also showed that the μ_{max} of *Klebsiella* sp. CK02 tended to be higher at 15°C than that of *M. morganii* ATCC 25830 in both TSBH and TFIB media (Table 1). Behling & Taylor (1982) also reported that *K. pneumoniae* exhibited

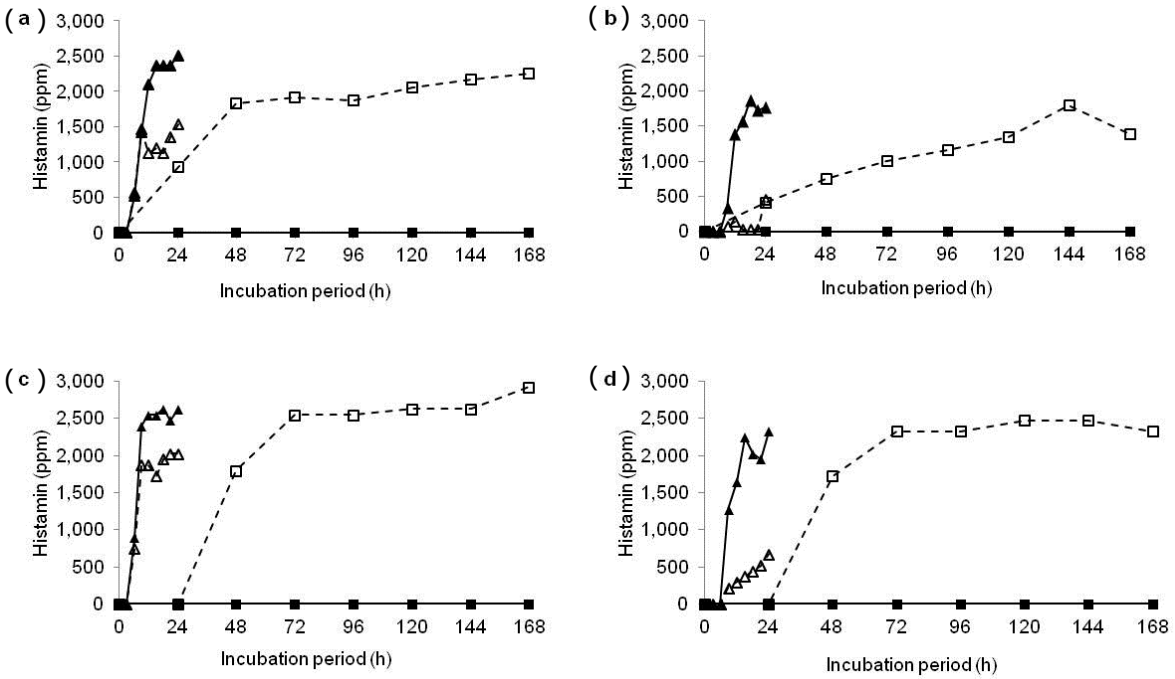

Figure 3. Histamine formation of *Klebsiella* sp. CK02 (K) and *Morganella morganii* ATCC 25830 (M) at 40°C (△), 30°C (▲), 15°C (□), and 4°C (■). (a) K in TSBH; (b) K in TFIB; (c) M in TSBH; (d) M in TFIB

Table 3. Apparent yield factor of histamine produced by *Klebsiella* sp. CK02 and *Morganella morganii* ATCC 25830 compared to other bacteria

Bacteria	Temp (°C)	Time (h)	Medium	$pY_{his/CFU}$ [log (µg/CFU)]
Klebsiella sp. CK02	15	168	TSBH	4.8
	15	168	TFIB	4.1
	30	24	TSBH	4.3
	30	24	TFIB	4.1
	40	24	TSBH	4.8
	40	24	TFIB	4
M. morganii ATCC 25830	15	168	TSBH	4.9
	15	168	TFIB	5.1
	30	24	TSBH	4.7
	30	24	TFIB	4.3
	40	24	TSBH	4.3
	40	24	TFIB	4.2
K. pneumoniae [a)]	10	48	TFIB	5.8
	20	24	TFIB	5.9
K. oxytoca [a)]	8	84	Tuna	4.3
	20	18	Tuna	4.8
M. morganii [a)]	10	168	TFIB	5.8
	12	240	TSBH	4.7
	20	96	TFIB	5.9
	20	120	TSBH	5.2

Note: [a)] Emborg (2007) as calculated from other researches on several histamine-producing bacteria

increasing μ_{max} at 15°C from 7 to approximately 9 log CFU/ml during 72 h of incubation. Similarly, Kim et al. (2000) also reported that *M. morganii* in TFIB at 15°C increased from 1.5 to 6.5 log CFU/ml at 48 h, and then entered the stationary phase at 8 log CFU/ml. Conversely, Torres et al. (2002) reported a lower μ_{max} for *M. morganii* at 15°C (0.2 log CFU/h) than at 20 and 30°C.

At 4°C, *M. morganii* ATCC 25830 showed a higher μ_{max} than *Klebsiella* sp. CK02 (Table 1). This was different to that reported by Kim et al. (2000), who observed no noticeable μ_{max} of *M. morganii* OSL36 at 4°C for up to 5 days. Emborg & Dalgaard (2008) reported that the minimum growth temperature for a mixture of *M. morganii* strains was 2.8°C.

Growth rates were modelled as a function of temperature using the Ratkowsky square root model. The models for *Klebsiella* sp. CK02 were y = 0.0209x + 0.036 in TSBH (R^2=0.9887) and y = 0.0119x + 0.1059 in TFIB (R^2 = 0.8525) (Table 2, Figure 2). For *M. morganii* ATCC 25830 they were y = 0.0169x + 0.0685 in TSBH (R^2 = 0.9579) and y = 0.0184x + 0.0457 in TFIB (R^2= 0.8787) (Table 2, Figure 2). The predicted T_{min} of *Klebsiella* sp. CK02 in TFIB was lowest compared to other treatments (Table 2).

The Ratkowsky square root model was used to describe the relationship between temperature and sqrt μ_{max} (Ratkowsky et al. 1982). The obtained mathematical model indicated a higher sqrt μ_{max} with increasing temperature (Figure 2). Emborg & Dalgaard (2008) also reported that *M. morganii* showed a higher sqrt μ_{max} with increasing temperature up to 40°C, but it decreased at 45°C; however, the sqrt μ_{max} for *Klebsiella* spp. have not yet been reported. The Ratkowsky square root model for *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830 μ_{max} in temperature function (Table 2) represented T_{min}, the theoretical minimum temperature for bacterial growth. A lower T_{min} represents a theoretical lower temperature at which bacterial growth ceases. *Klebsiella* sp. CK02 grown in TFIB showed the lowest T_{min} (Table 2), which indicates *Klebsiella* sp. CK02 ability to grow at low temperature. Behling and Taylor (1982) reported *K. pneumoniae* grew from 7 to 8 log CFU/ml at 7°C at 72 h, whereas no noticeable growth was detected at 0°C and -3°C. In TFIB, *Klebsiella* sp. CK02 exhibited higher ability to grow at low temperature than *M. morganii* ATCC 25830. The T_{min} of *M. morganii* ATCC 25830 reflects its ability to grow at low temperatures, but the value obtained in this study was lower than that of *M. morganii* DSM 14850 reported by Emborg and Dalgaard (2008). They reported the T_{min} of *Morganella* varied based on type and strain, with *M. psychrotolerans* tending to persist at low temperatures compared with *M. morganii* (Table 2).

3.2. Effect of Temperature on Histamine Production by *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830

The histamine production rates of *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830 in TSBH and TFIB were relatively high at 40, 30 and 15°C (Figure 3). However, at 4°C, no histamine production was detected, although growth was observed at a relatively low rate of 0.008–0.022 log CFU/h, with an associated cell number increase of 1.3–2.4 log CFU/ml until N_{max} (Table 1). In TSBH, the highest histamine production by *Klebsiella* sp. CK02 was observed at 30°C, ranging from 519 ppm at 6 h to 2,098 ppm at 12 h (Figure 3a), with a lower histamine production of 1,120 ppm at 40°C during 12 h of incubation. Histamine production was also observed at 15°C but at a longer time period than that at 30°C and 40°C, which was 2,060 ppm after 120 h. A similar histamine production pattern was also observed in TFIB, at a lower rate than that in TSBH. After 12 h, the histamine production in TFIB was significantly higher at 30°C than at 40°C, i.e., 1,384 versus 143 ppm, respectively (Figure 3b). At 15°C, 1,345 ppm of histamine was produced after 120 h.

The histamine production pattern of *M. morganii* ATCC 25830 was similar to that of *Klebsiella* sp. CK02, with a higher production rate at 30°C than at 40°C. After 12 h of incubation in TSBH, 2,549 ppm of histamine was produced at 30°C, which was significantly higher than 1,872 ppm at 40°C (Figure 3c). Similarly, during the same incubation period in TFIB, 1,647 and 293 ppm of histamine were produced at 30 and 40°C, respectively (Figure 3d). Histamine production at 15°C started at 48 h, with levels of 1,797 ppm in TSBH (Figure 3c) and 1,722 ppm in TFIB (Figure 3d).

The higher histamine production by *K. pneumoniae* and *M. morganii* at 30°C than at higher temperatures were reported. Behling & Taylor (1982) showed a maximum histamine level of 40.8 µmoles/ml, or approximately 800 ppm, was produced by *K. pneumoniae* in TFIB after 72 h at 30°C, which was much higher than at 37°C (i.e. 33 µmoles/ml or approximately 660 ppm). Taylor et al. (1979) reported that *K. pneumoniae* incubated at 32°C in TFIB produced 4,420 ppm histamine at 7 h. Histamine production by *K. pneumoniae* at low temperature was reported by Behling & Taylor (1982). At 7°C, 14 µmoles/ml (approximately 280 ppm histamine) was produced after 72 h, whereas at 0°C and -3°C, no histamine was detected. In *M. morganii,* Kim et al. (2000) reported that after 24 h, 2,000 ppm of histamine was produced in TFIB at 25°C, which was double the value at 37°C. Behling & Taylor (1982) also reported that

after 72 h of incubation, the optimum temperature for histamine production was 30°C for *M. morganii* 110SC2, *K. pneumoniae,* and *H. alvei,* and 37°C for *E. coli* and *C. freundii.* Özoðul (2004) reported that the highest production of biogenic amines after 2 d of incubation of *M. morganii* and *K. pneumoniae* in HDB at 37°C was 4,038 and 3,416 mg/L of histamine, respectively. *M. morganii* also reportedly produced 2,000 ppm of histamine at 15°C in TFIB after 48 h of incubation (Kim et al. 2000).

The results of this study indicate that the highest histamine production does not necessarily occur at the temperature associated with the highest μ_{max}. Specifically, *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830 showed the highest histamine production at 30°C (Figure 3), whereas the highest μ_{max} was at 40°C (Table 1). A similar result was also reported for *Photobacterium phosphoreum* YS4-7, psychrotrophic bacteria isolated from Japanese fish, *Iwashi maruboshi,* which showed the highest μ_{max} at 27°C, but significantly higher histamine production at 20°C (Kanki, Yoda, Ishibasi, & Tsukamoto, 2004). This result was probably caused by the difference in the optimum temperature for HDC activity and bacterial growth. Kanki, Yoda, Tsukamoto, & Baba (2007) reported varied optimum temperature and temperature stability of recombinant HDC activity from several HPB strains. This study also demonstrated higher histamine production by *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830 in TSBH than in TFIB, which was probably caused by differences in histidine availability. Histidine acts as a substrate for HDC enzyme, as well as an inducer. The higher concentration of histidine in the medium increases the production of bacterial histamine as reported by Chen et al. (1989) in *K. pneumonia* and *M. morganii that* produced higher histamine levels in medium containing 2.7% L-histidine than in 1% L-histidine.

In TLC analysis, histamine production by *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830 in TSBH and TFIB at 4°C was not detected, arguably due to the low production below 50 ppm and the low sensitivity of the TLC method. Moreover, the absence of an apparent increase in histamine production until the final observation stage at 4°C was probably due to the low HDC activity at low temperature. Kim et al. (2000) reported that histamine production by *M. morganii* OSL 36 at 4°C for up to 2 weeks can be negligible, although there was only 5 d of observation. Kanki et al. (2004) reported that *M. morganii* JCM 1672 incubated in TSBH at 20°C for 72 h produced 6,000 ppm of histamine, but it significantly decreased at 12°C, with no histamine being detected at 4°C. Kanki et al. (2007) reported 90 % of histidine decarboxylase enzyme activity of *M. morganii* JCM 1672 at 30°C, which significantly decreased to 10 % at 5°C.

Histamine production by bacteria can also be measured by the apparent yield factor ($pY_{his/CFU}$), which represents the minimum bacterial number needed to produce detectable histamine. A lower $pY_{his/CFU}$ value indicates higher histamine production (Jorgensen et al., 2000). The $pY_{his/CFU}$ of *Klebsiella* sp. CK02 at 15–40°C in TSBH and TFIB ranged at 4.0–4.8 log (µg/CFU) (Table 3). The highest $pY_{his/CFU}$ of *Klebsiella* sp. CK02 of 4.0 log (µg/CFU) was observed in TFIB at 40°C, whereas that of *M. morganii* ATCC 25830 was slightly lower at 4.2–5.1 log (µg/CFU). The results of $pY_{his/CFU}$ value obtained from *Klebsiella* sp. CK02 and *M. morganii* ATCC 25830 were comparable with those of *K. pneumoniae, K. oxytoca,* and *M. morganii* reported by Emborg (2007). Prominently, *Klebsiella* sp. CK02 was able to produce higher histamine level in TFIB at 15°C compare to *M. morganii* ATCC 25830, as shown by a lower $pY_{his/CFU}$ (Table 3). This result indicates a higher possibility of histamine production when *Klebsiella* sp. contaminated fish experienced temperature abuse during handling.

4. Conclusion

This study demonstrated that temperature affected growth rate and histamine production of *Klebsiella* sp. CK02, with the highest μ_{max} obtained at different temperatures than for optimum histamine production. Furthermore, histamine production by *Klebsiella* sp. CK02 varied at different temperatures and media. *Klebsiella* sp. CK02 has the potential to grow and produce histamine from 15–40°C and exhibited growth potential at lower temperature. Moreover, this study shows that *Klebsiella* sp. CK02 had a higher ability compare to *M. morganii*, a well-known histamine producing bacteria, to grow and produce histamine at 15°C in TFIB, a medium which nutrient content close to fish tissue. At temperature fluctuations during fish handling, *Klebsiella* sp. CK02 is able to grow despite sometimes insignificant histamine production. These findings indicate the importance of maintaining low temperature and short periods when handling fresh skipjack tuna to control growth of HPB and production of histamine. Further development of models is needed to predict the recommended temperature and the handling time period for quality and safety assurance of tropical fresh skipjack tuna.

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References

Bajc, Z. & Gaènik, K. (2009). Densitometric TLC analysis of histamine in fish and fishery products. *J. Planar. Chromatogr. – Mod. TLC, 22*(1), 15–17. doi:10.1556/JPC.22.2009.1.3.

Baranyi, J. & Roberts, T.A. (1995). Mathematics of predictive food microbiology. *Int. J. Food Microbiol., 26*(2), 199–218. doi:10.1016/0168-1605(94)00121-L.

Behling, A.R & Taylor, S.L. (1982). Bacterial histamine production as a function of temperature and time of incubation. *J. Food Sci., 47*(4), 1311–1314. doi:10.1111/j.1365-2621.1982.tb07675.x.

Björnsdóttir-Butler, K., Bolton, G.E., Jaykus, L.-A., McClellan-Green, P.D., & Green, D.P. (2010). Development of molecular-based methods for determination of high histamine producing bacteria in fish. *Int. J. Food Microbiol., 139*(3), 161–167. doi:10.1016/J.IJFOODMICRO.2010.03.017.

Chen, C.-M., Wei, C.I., Koburger, J.A., & Marshall, M.R. (1989). Comparison of four agar media for detection of histamine-producing bacteria in tuna. *J. Food Prot., 52*(11), 808–813. doi:10.4315/0362-028X-52.11.808.

Emborg, J. (2007). *Morganella psychrotolerans* - Identification, histamine formation and importance for histamine fish poisoning [PhD Thesis]. [Denmark]: Danish Institute for Fisheries Research.

Emborg, J. & Dalgaard, P. (2008). Growth, inactivation and histamine formation of *Morganella psychrotolerans* and *Morganella morganii* — development and evaluation of predictive models. *Int. J. Food Microbiol., 128*(2), 234–243. doi:10.1016/J.IJFOODMICRO.2008.08.015.

Jorgensen, L.V., Huss, H.H., & Dalgaard, P. (2000). The effect of biogenic amine production by single bacterial cultures and metabiosis on cold-smoked salmon. *J. Appl. Microbiol., 89*(6), 920–934. doi:10.1046/j.1365-2672.2000.01196.x.

Kanki, M., Yoda, T., Ishibashi, M., & Tsukamoto, T. (2004). *Photobacterium phosphoreum* caused a histamine fish poisoning incident. *Int. J. Food Microbiol., 92*(1), 79–87. doi:10.1016/J.IJFOODMICRO.2003.08.019.

Kanki, M., Yoda, T., Tsukamoto, T., & Baba, E. (2007). Histidine decarboxylases and their role in accumulation of histamine in tuna and dried saury. *Appl. Environ. Microbiol., 73*(5), 1467–73. doi:10.1128/AEM.01907-06.

Kim, S.-H., Ben-Gigirey, B., Barros-Velázquez, J., Price, R.J., & An, H. (2000). Histamine and biogenic amine production by *Morganella morganii* isolated from temperature-abused albacore. *J. Food Prot., 63*(2), 244–251. doi:10.4315/0362-028X-63.2.244.

Lehane, L. & Olley, J. (2000). Histamine fish poisoning revisited. I*nt. J. Food Microbiol., 58*(1–2), 1–37. doi:10.1016/S0168-1605(00)00296-8.

López-Sabater, E.I., Rodríguez-Jerez, J., Hernández-Herrero, M., & Mora-Ventura, M.T. (1996). Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. *Int. J. Food Microbiol., 28*(3), 411–418. doi:10.1016/0168-1605(94)00007-7.

López-Sabater, E.I., Rodriguez-Jerez, J., Hernández-Herrero, M., & Mora-Ventura, M.T. (1994). Evaluation of histidine decarboxylase activity of bacteria isolated from sardine (*Sardina pilchardus*) by an enzymic method. *Lett. Appl. Microbiol. 19*(2), 70–75. doi:10.1111/j.1472-765X.1994.tb00908.x.

Özoðul, F. (2004). Production of biogenic amines by *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* using a rapid HPLC method. *Eur. Food. Res. Technol., 219*(5), 465–469. doi:10.1007/s00217-004-0988-0.

Ratkowsky, D.A., Olley, J., McMeekin, T.A., & Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol., 149*(1), 1–5.

Rawles, D.D., Flick, G.J., & Martin, R.E. (1996). Biogenic amines in fish and shellfish. *Adv. Food Nutr. Res., 39*, 329–365. doi:10.1016/S1043-4526(08)60076-5.

Sumner, J.L., Ross, T., & Ababouch, L. (2004). *Application of risk assessment in the fish industry*. Fisheries Technical paper 442. Rome: FAO.

Takahashi, H., Kimura, B., Yoshikawa, M., & Fujii, T. (2003). Cloning and sequencing of the histidine decarboxylase genes of gram-negative, histamine-producing bacteria and their application in detection and identification of these organisms in fish. *Appl. Environ. Microbiol., 69*(5), 2568–2579. doi:10.1128/AEM.69.5.2568-2579.2003.

Taylor, S.L., Guthertz, L.S., Leatherwood, M., & Lieber, E.R. (1979). Histamine production by *Klebsiella pneumoniae* and an incident of scombroid fish poisoning. *Appl. Environ. Microbiol., 37*(2), 274–8.

Taylor, S.L. & Woychik, N.A. (1982). Simple medium for assessing quantitative production of histamine by Enterobacteriaceae. *J. Food Prot., 45*, 747–751.

Torres, S., Roeckel, M., & Cristina, M. M. (2002). Histamine formation by *Morganella morganii* isolated from *Trachurus murphii* (Chilean Mackarel). *Lat. Am. Appl. Res., 32*, 209–214.

Trevisani, M., Cecchini, M., Fedrizzi, G., Corradini, A., Mancusi, R., & Tothill, I. E. (2019). Biosensing the histamin producing potential of bacteria in tuna. *Front. Microbiol*., *1844*(10), 1–11.

Wang, D., Yamaki, S., Kawai, Y., & Yamazaki, K. (2020). Histamine production behaviors of a psychrotolerant histamine producer, *Morganella psychrotolerans*, in various environmental conditions. *Curr. Microbiol., 77*, 460–467.