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Determination of Time and Temperature Correlation when Using API 20 E for the Identification of Yersinia ruckeri strains

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ÖZET

Yersinia ruckeri Suşlarının İdentifikasyonunda API 20 E Kullanıldığında Süre ve Sıcaklık İlişkisinin Saptanması

Bu çalışmada Yersinia ruckeri identifikasyonunda API 20 E kullanıldığında süre ve sıcaklık arasındaki ilişkinin araştırılması amaçlanmıştır. Farklı kaynaklardan izole edilen 15 suş 20, 22, 25 ˚C'de ve 24,48,72 saat süreyle inkübe edilmiştir. Dokuz farklı inkübasyon şekli denenmiş ve sonuçlar klasik yöntemle karşılaştırılmıştır. Sonuç olarak, API 20 E sistemin 20 ˚C'de 72 saat 22 ˚C'de 24-48 saat inkübasyonda en iyi sonuçları verdiği saptanmıştır.

Anahtar sözcükler : API 20 E, Yersinia ruckeri, identifikasyon

ABSTRACT

This study was performed for the determination of the time and temperature correlation when using API 20 E system for the identification of Yersinia ruckeri. 15 strains from different origins were incubated at 20, 22, 25 °C and for 24, 48, 72 hours. Totally nine different incubation options were checked and correlated with the conventional methods. As a result, API 20 E system could be used at 20°C for 72 hours; at 22 °C for 48 hours or at 25 °C for 24-48 hours to give the best results.

Keywords: API 20E, Yersinia ruckeri, identification

INTRODUCTION

The API 20 E is currently one of the most used miniaturized systems of rapid diagnosis of bacterial fish diseases. Different researches used different times and temperatures when using API 20 E system and find different results (Romalde&Toranzo 1991, Stevenson & Daly 1982, Davies & Frerichs 1989, Rintamaki, et al. 1986, Santos et al. 1993). The aim of this study was to determine the most suitible time and temperature for diagnosing Yerinia ruckeri.

MATERIAL AND METHODS

In this study 15 different Yersinia ruckeri isolates were used from different from different geographic origins (Tablo 1). All strains were initially subcultured on tryptone soya agar (STA) (Oxoid) at 22 °C to confirm purity and kept at 4 °C (Santos et al. 1993). Gram stained smears were prepared for microscopic examination after 24 hours cultures depending upon rate of growth (Romalde & Toranzo 1991). The time and temperatures of incubation were performed at 20 °C after 24, 48 and 72 hours, at 22 °C after 24, 48 and 72 hours, at 25 °C after 24, and 72 hours according to the manufacturer's instructions.

Table 1. The origins and sources of the isolates.

Strain number	Origin		Source
1	Egirdir/Turkey		Rainbow trout
2	38/85	Denmark	Eel
3	18/83	Itay	Rainbow trout
4	10/85	U.K.	Rainbow trout
5	107/10B	Denmark	Rainbow trout
6	951013-3/2	Denmark	Rainbow trout
7	951019/4/1	Denmark	Rainbow trout
8	850621-1/16B	Denmark	Rainbow trout
9	Aydin 1/	Turkey	Rainbow trout
10	Aydin 2/	Turkey	Rainbow trout
11	Egirdir/	Turkey	Rainbow trout
12	100/94	U.K	Rainbow trout
13	107/8C	Denmark	Rainbow trout
14	Aydin3/	Turkey	Rainbow trout
15	Aydin 4/	Turkey	Rainbow trout

RESULTS

The results of the incubation time and temperatures are shown at the tables 2, 3, 4, 5 and 6 below.

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Table 2. The conventional biochemical test results of the Y. ruckeri strains

Y.ruckeri strains	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Gram-satin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cytochrome oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O/F rcaction	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Methyl red test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges Proskauer reac.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine decarboxilase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ornithin decarboxilase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gas from glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
İndolc production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on Tween 80	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Tablo 3. 24 Hours incubation results of API 20 E system for *Y. ruckeri* strains

	ARG			LCD			CIT			VP			GEL			SOR		
24 hour		°C			°C			°C			°C			°C			°C	
	20	22	25	20	22	25	20	22	25	20	22	25	20	22	25	20	22	25
1	-	-	-	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-
2	-	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-
3	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-	+	+	+
4	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+		+
5	-	-	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	-
6	-	-	-	+	+	+	+	-	-	-		-	-	-	-	-	-	-
7	-	-	-	+	+	+	+	-	+	-	+	-	-	+	+	-	-	-
8	-	-	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+
9	-	-	-	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-
10	-	-	-	+	+	+	+	-	-	+	+	-	-	-	+	-	-	-
11	-	-	-	+	+	+	+	-	+	-	+	-	-	+	-	-	-	-
12	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-
13	-	-	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-
14	-	-	-	+	+	+	+	-	+	-	+	-	-	+	+	-	-	-
15	-	-	-	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-
		-			-			-			-			-			-	
		+			+			+			+			+			+	

ARG : Arginin dihydrolase , LCD : Lysine decarboxylase,
CIT : Citrat , VP : Voges-Proskauer GEL : Gelatinase SOR : Sorbitol fermentation

Tablo 4. 48 Hours incubation results of API 20 E system for *Y. ruckeri* strains

	ARG			LDC			CIT				VP			GEL			SOR	
48 hour		°C			°C			°C			°C			°C			°C	
	20	22	25	20	22	25	20	22	25	20	22	25	20	22	25	20	22	25
1	-	-	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	-
2	-	-	-	+	+	+	+	-	+	-	+	-	+	+	+	+	-	-
3	-	-	-	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+
4	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+
5	-	-	-	+	+	+	-	+	+	-	+	-	-	+	+	-	-	-
6	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
7	-	-	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	-
8	-	-	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+
9	-	-	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	-
10	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-
11	-	-	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	-
12	-	-	-	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-
13	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-
14	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-
15	-	-	-	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-
		-			-			-			-			-			-	
		+			+			+			+			+			+	

Tablo 5. 72 Hours incubation results of API 20 E system for *Y. ruckeri* strains

	ARG			LDC			CIT				VP			GEL			SOR	
48 hour		°C			°C			°C			°C			°C			°C	
	20	22	25	20	22	25	20	22	25	20	22	25	20	22	25	20	22	25
1	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
2	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
3	-	-	+	+	+	+	+	+	+	-	-	+	+	+	-	+	-	+
4	-	-	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+
5	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
6	-	-	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-
7	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
8	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
10	-	-	-	+	+	+	+	+	+	-	+	-	+	+	+	-	-	-
11	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-
12	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-
13	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-
14	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
15	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
		-			-			-			-			-			-	
		+			+			+			+			+			+	

Tablo 6. API 20 E system Which gave the same results of the *Y. ruckeri* strains for all incubation periods

	ONPG	ODC	H2S	URE	TDA	IND	GLU	MAN	INO	RHA	SAC	MEL	AMY	ARA	NO2
1	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
2	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
3	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
4	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
5	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
6	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
7	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
8	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
9	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
10	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
11	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
12	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
13	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
14	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
15	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+

DISCUSSION

In this study, the efficiency of the API 20 E rapid diagnostic kit was tested for 15 strains of Yersinia ruckeri at 20, 22 and 25 °C and for 24, 48 and 72 hours.

No previous results for this kit using 20 °C and 24 hours for Yersinia ruckeri was found and all the tests for this period and temperature in this present study gave only weak results, showing these levels to be of no practical use

With this kit overall the time periods H2S, URE, TDA, IND INO, RHA, SAC, MEL, AMY, ARA tests for Yersinia ruckeri were negative; ONPG, ODC, GLU, MAN, NO2 tests were positive and ADH, CIT, VIP, GEL, SOR tests gave mixed negative and positive results. The results for 20 °C and 48 hours agreed with those of Dwilow et al. (1987) for this kit except for the gelatine test, with which only 2 strains were found to give positive results at 48 hours, through this were 13 strains at 72 hours.
Davies & Frerichs (1989) found that tests at 22°C and 24 hours tended to be negative for CIT and GEL and our results obserwed the same tendering.

At 22 °C and 48 hours incubation gave positive results to GEL for 9 strains and after 48 hours, for 12 strains. These are similar results to those of Dear (1988) who also stated that CIT and GEL could give different results for the API test. Dear's resuts are similar toh those of Rintamaki et al. (1986) and Frerichs et al. (1985) and although Petrie et al. (1996). found ADH results negative, his other results agree with those of other authors.

At 22°C and 72 hours incubation all strains showed positive to CIT. At 48 hours, 22° C, 12 strains showed positive to GEL, but at 72 hours, this increased to 14, with only 1 strain then showing a negative while the GLU and MAN tests gave positives, the other sugars were negatives. These results agree with those of Davies&Frerichs (1989), but they also states GEL, CIT, VP, SOR, tests could give different results with Yersinia ruckeri strains.
At 25°C and 24 hours incubation 8 strains showed positive to CIT and 8 to GEL. SOR tests gave variable results and VP gave negatives to all the strains These results agree with the results of Stevenson & Daly (1982) who used the same incubation periods.

At 25°C and 48 hours incubation, more strais showed positive to GEL and CIT. SOR tests gave different results and VP was negative for all strains just as for 24 hours.

At 25°C and 72 hours incubation, all strains gave positive results with CIT. SOR tests again gave variable results, whilst two strains were negative to GEL, the rest showing positive.

The results of these three incubation periods at 25 °C, agree with those of Romalde & Toranzo (1991) for some of the strains they have used. However, they found different results, especially with LDC for some of the strains also used in this study. Romaldo & Toranzo (1991) also found positive results for Inositol and Amygdalin tets for some strains, but those tests gave no positive results in this study.
The conclusion is therefore, that for identification of Yersinia ruckeri strains with API, all the strains giving different biochemical reactions should be added to the AIPI profile index.

As 24 hour incubations at 20 °C and 22 °C gave only weak results and 72 hours at 22 °C gave only weak results and 72 hours at 22 °C and 25 °C gave mainly positives, these treatments must be ragarded as giving unreliable results. The best treatments as shown by this tduy are therefore 20 °C for 72 hours, 22 °C for 48 hours and 25 °C for 24-48 hours.

Species closely related to Yersinia ruckeri, such as Hafnia alvei which could give very similar API pofiles, further distinguishing tests (Xylose, Glucose, etc.) should be performed to avoid fro misidentification.

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