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Use of the API-ZYM system in rapid identification of α and non-haemolytic streptococci

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SUMMARY The API-ZYM method of detecting enzymes was tested using 99 streptococci isolated from clinical material and 14 type species obtained from the National Collection of Type Cultures. We found the method easy and reliable. The results obtained indicate that this method could be a useful identification system in busy routine clinical laboratories.

The streptococci commonly known as the 'indifferent' streptococci, that is, those belonging to the 'viridans' group and the non-haemolytic organisms, include a number of species that differ greatly in their ability to cause specific diseases in man. *Streptococcus bovis*,[1] *Streptococcus mitior*, and *Streptococcus sanguis* are all associated with endocarditis,[1] while deep-seated abscesses often yield *Streptococcus milleri*.[1,2,3] These species can be identified by a whole battery of cultural and serological tests.[4] However, the tests as they are currently performed are slow, tedious, and time-consuming as well as utilising highly complex media. All of these factors combined occasionally lead to equivocal results being recorded. Because of this unreliability, it may be difficult to identify and subsequently classify the streptococci isolated from man, and these are most often dismissed with the general term 'viridans streptococci'.

Supplementary, or even entirely new, alternative means of identification are required. Such a new system has been successfully developed to detect rapidly specific enzymes from single substrates using a heavy inoculum of organisms or biological fluid.[5] This Auxotab system has been sucessfully applied in both animal tissues,[6] and biological fluids.[7,8] Both staphylococci[9] and Erwinia[10] have been identified using the above system. Recently, it has been modified into a commercially packaged system called API-ZYM (API Laboratory Products Ltd, Farnborough), utilising 19 substrates from which enzymes can be detected within 4 hours. Using this modified enzyme detection system, anaerobic[11] and aerobic bacteria, including a few streptococci,[12] have been identified.

This paper presents, in full, our initial results which were reported at the Second International Symposium on Rapid Methods, Cambridge, England, in 1976.[13]

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Material and methods

ORGANISMS

The following type strains were obtained from the National Collection of Type Cultures, Colindale, London. *Strep. faecalis* (NCTC 775, 2705, 5957); *Strep. durans* (NCTC 8174); *Strep. faecium* (NCTC 7380, 7366, 7176, 7182, 7379); *Strep. equinus* (NCTC 10389); *Strep. milleri* (NCTC 10708, 10709); *Strep. mutans* (NCTC 10449); and *Strep. sanguis* (NCTC 7864).

The remaining streptococci studied were those routinely sent to the Streptococcus Reference Laboratory for identification using the methods described by Parker and Ball.[1] The identity and numbers of streptococci used to test the API-ZYM system are shown in Table 1.

CULTURAL METHODS

The organisms to be tested were first grown on 5% horse blood agar to ensure purity of culture. Then a sweep inoculation was made into Todd Hewitt Broth, and this was incubated overnight at 37°C. After incubation the broths were centrifuged at 2000 rpm for 15 minutes, and the deposit was washed three times with physiological saline, before they were finally resuspended in saline to give approximately 10^6 cfu/ml. This suspension was used to inoculate the API-ZYM system.

IDENTIFICATION PROCEDURE

The API-ZYM system consists of a plastic gallery containing 20 cupules; 19 of these have substrates

Table 1 *Identity and number of streptococci used to test the API-ZYM system*

Strains		*No. tested (including NCTC strains)*
Strep.faecalis		21
Strep.durans		4
Strep.faecium		10
Strep.equinus		3
Strep.bovis		18
Strep.milleri		17
Strep.mutans		9
Strep.mitior Dex −	(8)	14
Strep.mitior Dex +	(6)	
Strep.sanguis Dex +, Aesc +, Arg +	(4)	17
Strep.sanguis Dex +, Aesc −, Arg +	(7)	
Strep.sanguis Miscellaneous	(6)	
Total		113

and buffer impregnated into inert supportive fabric, while cupule No. 1 contains no substrate and acts as a negative control. The substrates detect those enzymes listed in Table 2. Two drops of 0·02 ml prepared bacterial suspension were dropped into each cupule, and the gallery was fitted into a chamber previously moistened with distilled water. This was then incubated aerobically at 37°C for 4 hours. After incubation 0·04 ml of the API reagents A and B were added to each cupule and exposed for 30 seconds to a very strong light source (1000 Watt lamp). The resulting colours were recorded as intensities and could be read to give a semiquantitative notation (0 to 5) using a colour code supplied by the manufacturers. Each strain was tested at least twice with the API-ZYM to ensure reproducibility of results.

Results

The results of the API-ZYM system are summarised in Table 3. This presents all the reactions produced by the streptococci tested. From it emerges a distinct pattern of enzyme production which serves to differentiate the various strains. The colour intensity of 'key' reactions is reserved for those that attain a score of 3 or more on the colour chart provided. It can be seen that certain substrates are never metabolised, that is, 17 and 20 (see Table 2), while others are rarely utilised (5, 9, 13, 14, 15, and 19). Indeed, these substrates are totally redundant with regard to final identification, which is based on the remaining 11. Conversely, substrate 6 (leucine aminopeptidase) is always metabolised by streptococci and must surely form a 'key' test for this group of organisms. No other substrate provides such exclusive characterisation, with the exception of *Strep. mitior* Dextran + which alone uses substrate 18 (glucosaminidase).

The results presented in Table 3 also support the established biochemical divisions of certain controversial streptococci. For example, *Strep. mitior* can be divided into those organisms that produce dextran in sucrose broth or those that do not. The latter is typical of the classical *Strep. mitior*, while the former, although adhering to the other biochemical criteria, does produce dextran and is designated *Strep. mitior* Dextran +.[4] These two biochemically different organisms, *Strep. mitior* Dextran − and *Strep. mitior* Dextran +, can be recognised in the API-ZYM system; *Strep. mitior* Dextran − utilises substrates 6, 7, 8, 11, and 12 while *Strep. mitior* Dextran + utilises 2, 3, 6, 7, 8, 11, 12, and, uniquely, 18. Similarly, the classical biochemical divisions that exist between *Strep. sanguis* Aesculin −, Arginine hydrolysis +, and Dextran + (type specific strain) and *Strep. sanguis* Aesculin −, Arginine hydrolysis +, and Dextran +[1] are reinforced by the API-ZYM system (metabolism of substrates 2, 3, 6, 7, 8, and 11 and 2, 3, 6, 7, 8, 11, 12, and 16, respectively). However, the miscellaneous *Strep. sanguis* that do not strictly adhere to formal classification exhibit the same confusion even when the API-ZYM system is used.

Finally, Table 3 emphasised two important findings: firstly, that *Strep. mitior*, normally a very unreactive organism biochemically, metabolises such a vast array of substrates in the API-ZYM system; secondly, *Strep. milleri*, while providing the

Table 2 *Enzymes detected by the API-ZYM test*

Test	*Enzyme assayed for:*	*Test*	*Enzyme assayed for:*
1	Control	11	Phosphatase acid
2	Phosphatase alkaline	12	Phosphoamidase
3	Esterase (C 4)	13	α Galactosidase
4	Esterase lipase (C 8)	14	β Galactosidase
5	Lipase (C 14)	15	β Glucuronidase
6	Leucine aminopeptidase	16	α Glucosidase
7	Valine aminopeptidase	17	β Glucosidase
8	Cystine aminopeptidase	18	β Glucosaminidase
9	Trypsin	19	α Mannosidase
10	Chymotrypsin	20	α Fucosidase

Table 3 *Reaction of type strains with API-ZYM*

Organism	*No.*	*API-ZYM No.*																			
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
Strep.faecalis	21	—	*0	19	21		21		19	0	0	20	21				21		0		
			1	2	0	0	0	0	2	1	1	0	0	0	0	0	0	0	1	0	0
Strep.durans			0	4	4		4						4								
	4	—	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strep.faecium				10	10		10			0	10				0						
	10	—	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Strep.equinus							3	3	2			2									
	3	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strep.bovis			18	18			18	0	17		0			0	0		17		0		
	18	—	0	0	0	0	0	2	1	0	4	0	0	3	1	0	0	0	1	0	0
Strep.milleri		—	17	16			17	17	17			16	0	0			8				
	17	—	0	1	0	0	0	0	0	0	0	1	1	3	0	0	1	0	0	0	0
Strep.mutans			8	6	8	1	7	1			3	7	0		1		0		1		
	9	—	0	3	0	0	0	2	0	0	2	0	2	0	1	0	5	0	0	0	0
Strep.mitior Dex −			1	3	1	1	8	8	8		1	8	8		1	2	2				
	8	—	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strep.mitior Dex +			5	6		1	5	6	6		1	6	3	1	1	2	2		6		
	6	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strep.sanguis Dex +,			4	4			4	4	4			4		0							
Aesc +, Arg +	4	—	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Strep.sanguis Dex +,			7	7	1		7	6	7		1	7	7	0	0	1	7				
Aesc −, Arg +	7	—	0	0	2	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0
Strep.sanguis Miscellaneous			5	3	3		5	3	4		5	5	3	2	1	1	5		1	2	
	6	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*The first figure is the number of isolates with strong activity (3-5), the second number with weak activity (1-2).
0 alone indicates a completely negative reaction.

Table 4 *Summary of API-ZYM results*

Organism	*API-ZYM substrates/enzymes detected*																			
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
Strep.faecalis			*+	+		+		+			+	+				+				
Strep.durans			+	+		+						+								
Strep.faecium			+	+		+				+										
Strep.equinus						+	+	+			+									
Strep.bovis		+				+		+			+	+				+				
Strep.milleri		+				+	+	+			±					±				
Strep.mutans		+	+	+		+		+			+	+				±				
Strep.mitior Dex −						+	+	+			+	+								
Strep.mitior Dex +		+	+			+	+	+			+					+		+		
Strep.sanguis Dex +, Arg +, Aesc +		+	+			+	+	+			+									
Strep.sanguis Dex +, Arg +, Aesc −		+	+			+	+	+			±	+				+				

*Positive colour, intensity 3→5 on API-ZYM colour code.
± Half the numbers tested gave a positive reaction.

'key' reactions of 2, 6, 7, 8, and 11, also metabolises substrate 16 (glucosidase) in over half the numbers tested (9/17).

These reactions can be more clearly seen in Table 4, which presents the 'key' reactions as a code for each individual strain of streptococci investigated. A 'key' reaction is one that reacts strongly in the system (colour intensity 3 or greater) consistently with all strains tested. The code for each organism is then arranged using these 'key' reactions, as in Table 4.

If the code is strictly used, a high percentage of correct identification can be achieved (Table 5). Not surprisingly, those organisms that have a well-defined, conventional, biochemical classification identify most easily and most often using the API-ZYM, for example, *Strep. faecalis* (90% correct identification) and *Strep. faecium* (100% correct identification), while other streptococci reflect the

Table 5 *Percentage of correct identification using API-ZYM code*

Organism	*Code*	*No. tested*	*No. giving correct API code*	*% Correct*
Strep.faecalis	3, 4, 6, 8, 11, 12, 16	21	19	90
Strep.durans	3, 4, 6, 12	4	3	75
Strep.faecium	3, 4, 6, 10	10	10	100
Strep.equinus	6, 7, 8, 11, 12	3	2	66
Strep.bovis	2, 3, 6, 7, 8, 11, 12	18	17	94
Strep.milleri	2, 6, 7, 8, 11 (16)	17	16	94
Strep.mutans	2, 3, 4, 6, 12 (16)	9	6	67
Strep.mitior Dex −	6, 7, 8, 11, 12	8	5	62
Strep.mitior Dex +	2, 3, 6, 7, 8, 11, 12, 18	6	4	67
Strep.sanguis Dex +, Aesc +, Arg +	2, 3, 6, 7, 8, 11	4	4	100
Strep.sanguis Dex +, Aesc −, Arg +	2, 3, 6, 7, 8, 11, 12, 16	7	6	85
**Strep.sanguis* Miscellaneous	————————	6	——	——
Total number tested		113		

(16) Half of these organisms gave positive reactions.
*See text.

uncertainty of their biochemical biotypes with similar uncertain identification in the system, for example, *Strep. mitior* and *Strep. sanguis*.

Discussion

The results of this study indicate quite clearly that the API-ZYM system could be used as a simple, rapid, and reliable method to identify streptococci. The method is most useful when used on well-characterised strains such as the group D streptococci, where correct identification, as compared to the classical biochemical patterns, approximates nearly 90-95% accuracy. However, even the other less well-identified organisms, such as *Strep. mitior* Dextran +, give fairly respectable identification rates of 67%. This reflects a considerable saving in laboratory time, particularly when the API-ZYM method can be read with only 4 hours' incubation compared to periods of five days when using conventional biochemical methods. One of the most important viridans streptococci today is *Strep. milleri*. This organism has been established as a major cause of deep-seated abscesses and systemic infections by both Parker and Ball[1] and Poole and Wilson.[3,4] Identification using conventional methods is, as pointed out above, long and tedious. API-ZYM affords a quick and reliable method to identify the organism and thereby alert the clinician of the patient's potentially serious infection. It is interesting to note that approximately half of the *Strep. milleri* tested produced the enzyme α glucosidase. Whether this finding is significant would have to be investigated further.

In a previous study[14] we showed that viridans streptococci could be adequately identified in the routine laboratory using a variety of simple biochemical reactions. In the present one, the use of API-ZYM facilitates a quicker and easier method to achieve the same identification. But, even with this method, there are occasions when a more complete classification of the organism must be undertaken. When this occurs the full biochemical methods described by Waitkins *et al.*[14] would have to be used.

However, in the main we found that API-ZYM in our hands gave good, reliable, and accurate identification of potentially harmful streptococci. It would certainly enable the busy routine laboratory to pursue these organisms and achieve correct identification in at least 60%, and sometimes even 100%, of all streptococci investigated.

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