Chapter 3

RESULTS

1. Cloning and characterization for *H. brasiliensis hmgs2*

The *H. brasiliensis hmgs1* cDNA was used as a probe to screen the latex cDNA library for a new cDNA encoding HMG-CoA synthase. Five positive clones were obtained from primary screening (Figure 17 A). Each positive clone was again screened with the *hmgs1* probe in the secondary screening. Some of those positive clones obtained from primary screening show several positive plaques (Figure 17 B and C). Five positive clones which showed strong dark spots from the secondary screening were chosen to perform the teriary screening. All chosen clones produced a positive signal on the X-ray film for all positions of plaque clear zone in the plate (Figure 17 D). Therefore, they were all subjected to *in vivo* excision using the ExAssist interference-resistant helper phage in bacteria SOLR cells.

After they were subcloned into bacteria XL1-Blue MRF' cells, five positive clones underwent digestion reaction with *Xba*I/*Xho*I, and the pattern of digested plasmid DNA is shown Figure 18. Only four clones exhibited complete digestion with the two endonulease restriction enzymes. Agarose gel electrophoresis analysis of the digestion products indicated; insert DNA fragments of approximately 1.8 kb from three clones, which were likely to contain a DNA sequence similar to *hmgs1*. One clone showed an approximate 1.4 kb DNA band that might be a different gene, and another clone which was not cut with *Xba*I and *Xho*I.

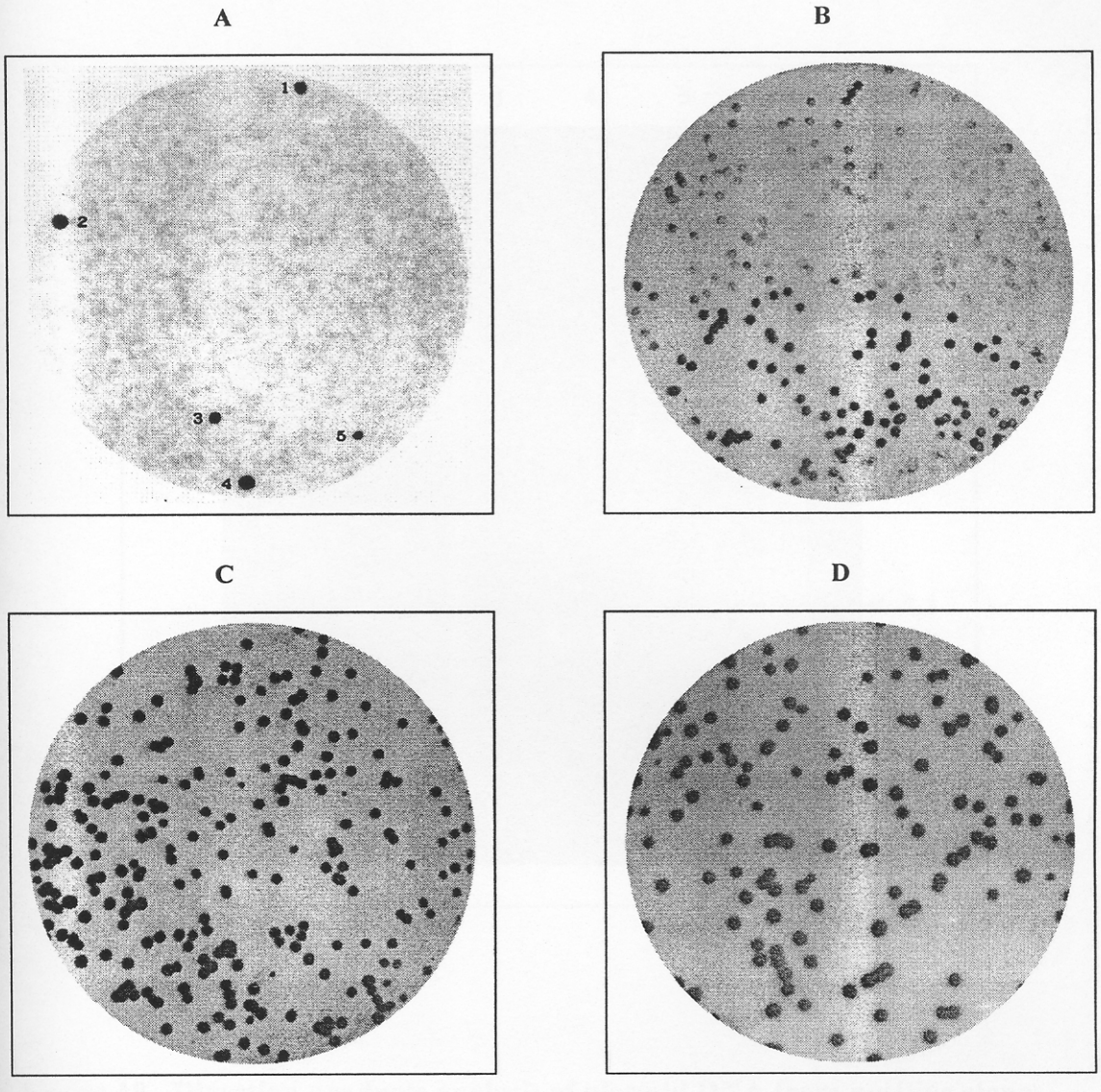


Figure 17. Plaque hybridization of latex cDNA library screening by using *hmgs1* as a probe.

A: The five positive plaques in the primary screening

B and C: The positive plaques in the secondary screening

D: The positive plaques in the tertiary screening

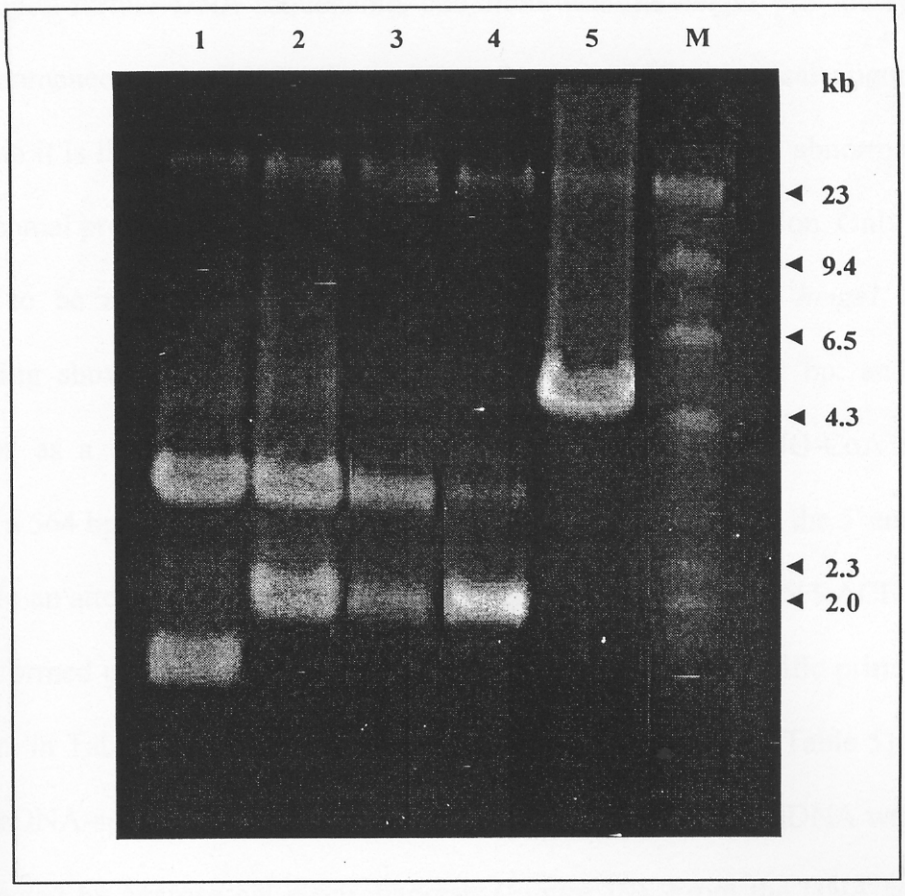


Figure 18. Digestion products analysis of plasmid DNA from positive clones

Five positive clones obtained from the secondary screening were digested by *Xba*I and *Xho*I and separated on a 1% agarose gel with ethidium bromide staining.

Lane 1-4: Four positive clones digested with restriction enzymes.

Lane 5: Clone which was undigestible with restriction enzymes.

Lane M: 1 kb DNA marker

Upon further DNA sequencing, one clone was the *hmgs1* previously reported by Suwanmanee et al. (2002). Two clones showed 99.8% identical alignment with *hmgs1*, so it is likely that they are *hmgs1*. Another clone has some abnormality in the chromosomal process in case of inversion on a chromosomal variation. Only one clone seemed to be a new *hmgs* gene. It shows 93% identity with *hmgs1* and DNA sequencing showed that this clone contained an insert of 1,352 bp; and this was classified as a new partial *H. brasiliensis* cDNA encoding HMG-CoA synthase2, missing a 564 bp segment including part of the noncoding region at the 5' end.

In an attempt to isolate the full-length cDNA of *hmgs2*, the 5' RACE procedure was performed using 5' GeneRacer Nested Primer and a gene-specific primer (GSP1), as shown in Table 5 for the first PCR. The ForHS2 and RevHS2 (Table 5) were used as new cDNA-specific primers in the second PCR and full-length cDNA was obtained and detected by agarose gel electrophoresis (Figure 19). From the DNA sequencing, the full-length cDNA encoding HMG-CoA synthase 2 in *H. brasiliensis*, called *hmgs2*, is 1,916 bp in length, with an open reading frame (ORF) of 1,392 bp between bases 317 and 1,708. The coding sequence is flanked by 5' and 3' untranslated regions (5' UTR, and 3' UTR) with 316 and 213 bp, respectively. A putative polyadedenylation site (AAUAAA) was shown at position 1,861 (GenBank accession number AY534617) and the detail comparison bewteen *hmgs*1 and *hmg*2 is shown in Table 8 and Figure 20. The amino acid sequence deduced from the *hmgs2* cDNA reveals 464 residues with a predicted molecular mass of 51.27 kDa and an isoelectric point of 6.02. Comparison of *hmgs1* with *hmgs2* showed 92% nucleotide sequence identity and 94% polypeptide sequence identity. In the deduced amino acid sequence, there are 28 residues different in HMG-CoA synthase2 which are shown in Table 9.

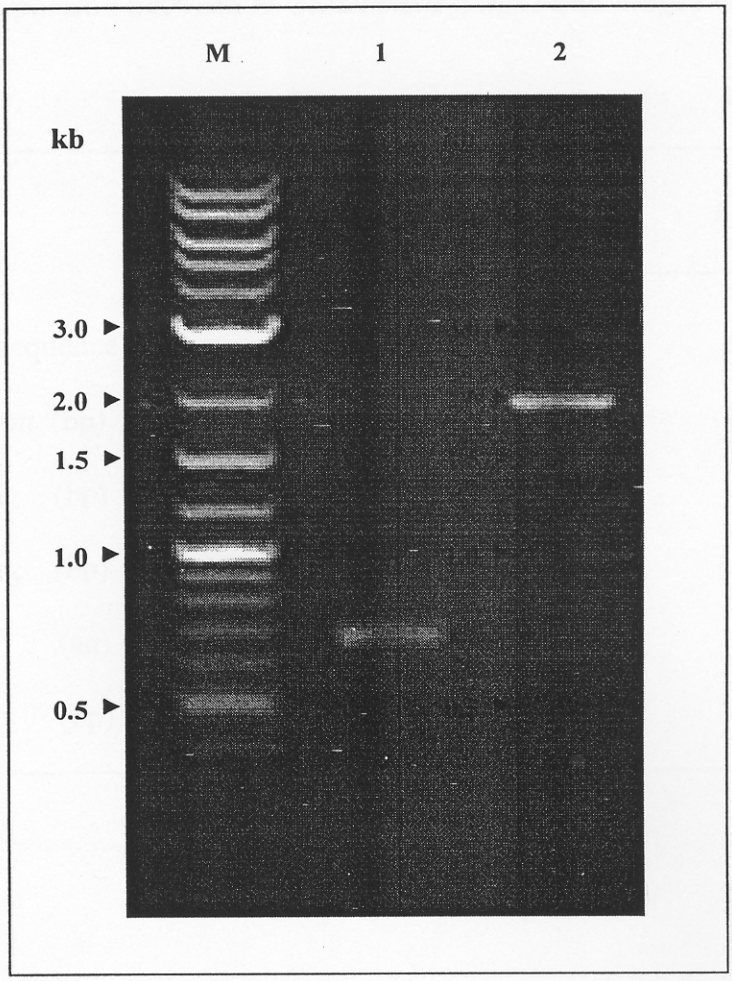


Figure 19. ***H. brasiliensis hmgs2*** **cDNA after 5' RACE on the 1% agarose gel electrophoresis.**

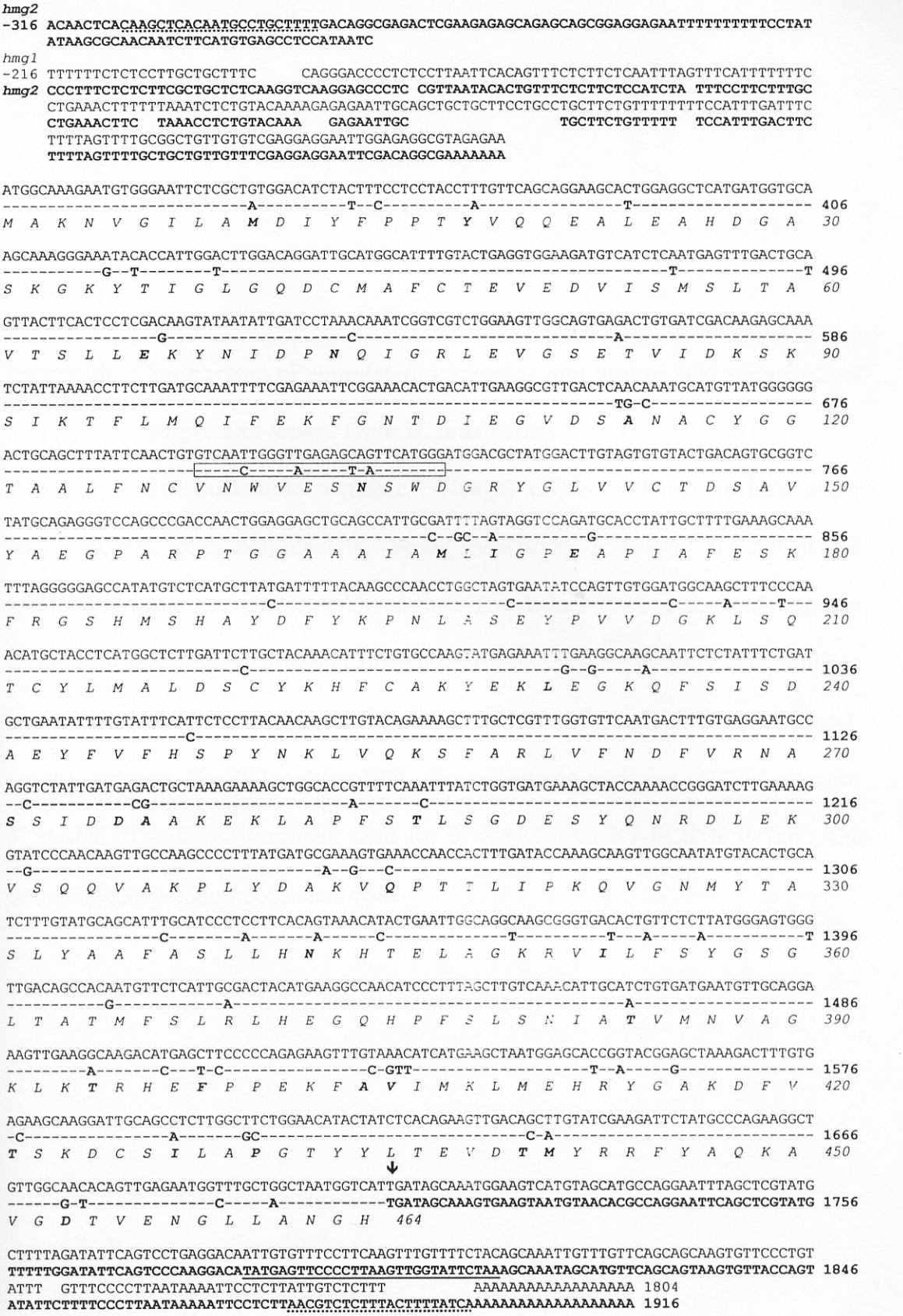
Lane M: 2 log DNA marker

Lane 1: First PCR of 5' RACE (650 bp)

Lane 2: Second PCR of full-length *hmgs2* cDNA (1,916 bp)

Table 8. Comparison between *H. brasiliensis hmgs1* and *hmgs2* cDNA

	hmgs1	*hmgs2*
Nucleotide sequence (bp)	1,804	**1,916**
Coding region (bp)	1,392	1,392
5' noncoding (bp)	216	**316**
3' noncoding (bp)	196	**206**
Amino acid (aa)	464	464
Poly $^{+}$A (bp)	18	**19**


hmg2
-316 ACAACTCACAAGCTCACAATGCCTGCTTTTGACAGGCGAGACTCGAAGAGAGCAGAGCAGCGGAGGAGAATTTTTTTTTTCCTAT
ATAAGCGCAACAATCTTCATGTGAGCCTCCATAATC
hmg1
-216 TTTTTTCTCTCCTTGCTGCTTTC CAGGGACCCCTCTCCTTAATTCACAGTTTCTCTTCTCAATTTAGTTTCATTTTTTTC
hmg2 CCCTTTCTCTCTTCGCTGCTCTCAAGGTCAAGGAGCCCTC CGTTAATACACTGTTTCTCTTCTCCATCTA TTTCCTTCTTTGC
CTGAAACTTTTTTAAATCTCTGTACAAAAGAGAGAATTGCAGCTGCTGCTTCCTGCCTGCTTCTGTTTTTTTTCCATTTGATTTC
CTGAAACTTC TAAACCTCTGTACAAA GAGAATTGC TGCTTCTGTTTTT TCCATTTGACTTC
TTTTAGTTTTGCGGCTGTTGTGTCGAGGAGGAATTGGAGAGGCGTAGAGAA
TTTTAGTTTTGCTGCTGTTGTTTCGAGGAGGAATTCGACAGGCGAAAAAAA
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676
120
766
150
856
180
946
210
1036
240
1126
270
1216
300
1306
330
1396
360
1486
390
1576
420
1666
450
1756
464
1804
1846
1916

Figure 20. Comparison of completed nucleotide and amino acid sequences of *hmgs1* and *hmgs2* from *H. brasiliensis*.

The nucleotide sequence of *hmgs2* cDNA is shown in bold under *hmgs1* cDNA. Only non-identical coding nucleotides in *hmgs2* are shown and identical residues are represented by dashes. The deduced amino acids of *hmgs2* is shown and the residues different from *hmgs1* are indicated by italic and boldface type. The fragments underlined by the bold dashed lines correspond to the *hmgs2*-specific primers, ForHS2 and RevHS2. The underlined fragment is GSP1 used for 5' RACE. The GSP2 fragment used for 5' RACE and RT-PCR is in box. The translation stop codon is denoted by↓.

Table 9. Changes in amino acids sequences between HMGS 1 and HMGS 2

Letters in italic indicate base changes

No.	Changing amino acid position	HMGS1		HMGS2	
		Codon (5'→3')	Amino acid	Codon (5'→3')	Amino acid
1	10	*G*TG	Val	*A*TG	Met
2	18	T*T*T	Phe	T*A*T	Tyr
3	66	GA*C*	Asp	GA*G*	Glu
4	73	AA*A*	Lys	AA*C*	Asn
5	114	*AC*A	The	*GC*C	Ala
6	134	A*G*T	Ser	A*A*T	Asn
7	167	AT*T*	Ile	AT*G*	Met
8	169	*G*TA	Val	*A*TA	Ile
9	172	GA*T*	Asp	GA*G*	Glu
10	231	TT*T*	Phe	TT*G*	Leu
11	271	AG*G*	Arg	AG*C*	Ser
12	275	GA*G*	Glu	GA*C*	Asp
13	276	*A*CT	The	*G*CT	Ala
14	286	A*A*T	Asn	ACT	Thr
15	315	*A*AA	Lys	CAA	Gln
16	342	A*G*T	Ser	A*A*T	Asn
17	353	AC*A*	The	A*T*A	Ile
18	384	*T*CT	Ser	*A*CT	Thr
19	394	*G*CA	Ala	*A*CA	Thr
20	398	C*T*T	Leu	*T*TC	Phe
21	404	G*T*A	Val	GCA	Ala
22	405	*AA*C	Asn	*GT*T	Val
23	421	AGA	Arg	AC*A*	Thr
24	427	CTC	Leu	*A*TC	Ile
25	430	*T*CT	Ser	*C*CT	Pro
26	440	AG*C*	Ser	AC*C*	Thr
27	441	*T*TG	Leu	*A*TG	Met
28	453	*AA*C	Asn	*GA*T	Asp

2. The expression of *hmgs2* is tissue specific.

Information regarding the tissue specific expression of *H. brasiliensis hmgs2* mRNA was determined by semiquantitative RT-PCR. The 18S ribosomal RNA, an endogenous sequence that is normally present in a constant amount in series of samples, was used as a control for amount of total RNA in the RT-PCR. The total RNA isolated from rubber latex, petiole, and leaf of mature tree was checked for integrity by agarose gel electrophoresis and two bands of 28S and 18S ribosomal RNA appeared (Figure 21). The RT-PCR was performed in two steps, first the cDNA of rubber latex, petiole, and leaf were synthesized for use as template for amplification of the *hmgs2* gene. Then, the PCR reaction was performed using specific fragments designed as primers for the *hmgs2* cDNA; ForHS2 at the 5' noncoding region and GSP2 at the 3' coding region to amplify a region extending from bases -308 to 409 of 717 bp in length of *hmgs2* cDNA with 18S ribosomal RNA used as calibration standard as shown in Figure 22. The *hmgs2* mRNA is differently expressed in latex, petiole, and leaf. It is well expressed in laticifer and petiole, whereas it is poorly expressed in leaf, with calculated relative intensity with the standard gene of 1.17%, 0.48%, and 0.96% in latex, leaf, and petiole, respectively.

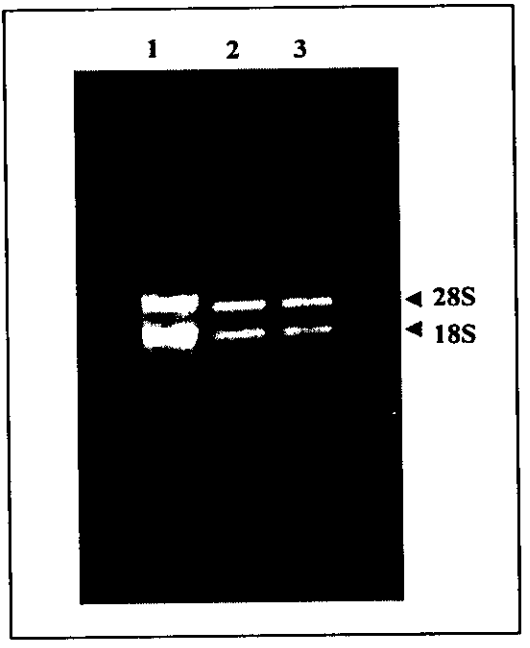


Figure 21. Total RNA isolated from various tissues was separated on 1% agarose gel with ethidium bromide staining.

Lane 1: Latex (10 μg)

Lane 2: Petiole (4 μg)

Lane 3: Leaf (4 μg)

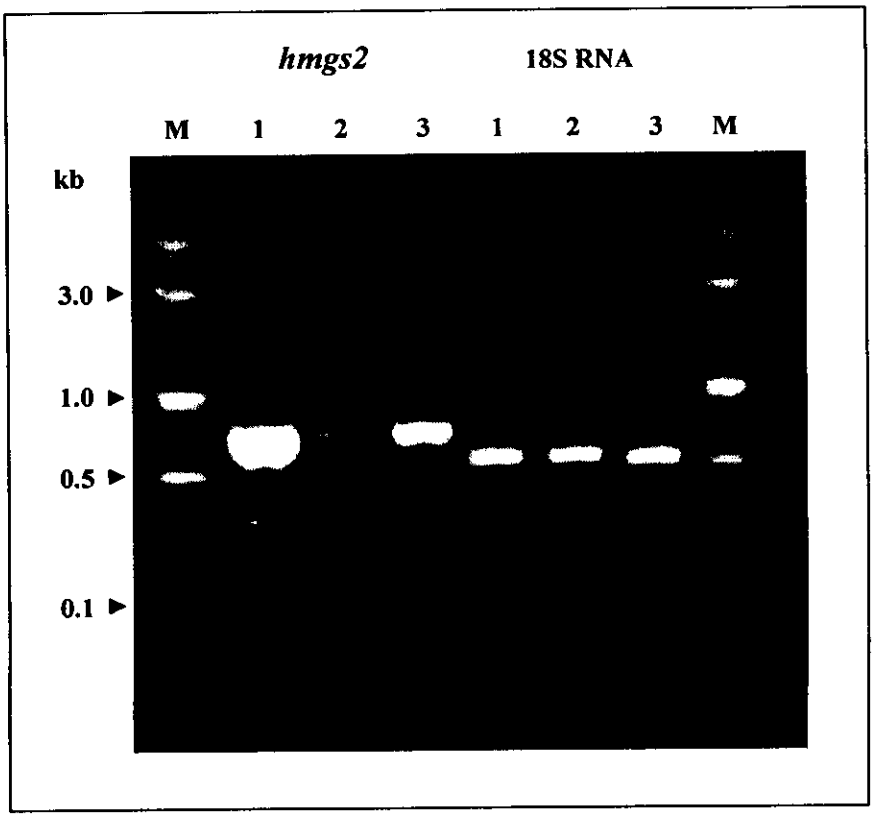


Figure 22. Differential expression of hmgs2 mRNA in different
Jatrophas tissues.

CR products (717 bp) of semiquantitative RT-PCR performed by
A and the *hmgs2* specific primers were separated on 1% agarose
ed with ethidium bromide stain. The PCR products for 18S RNA

ne 1: Latex

ne 2: Leaf

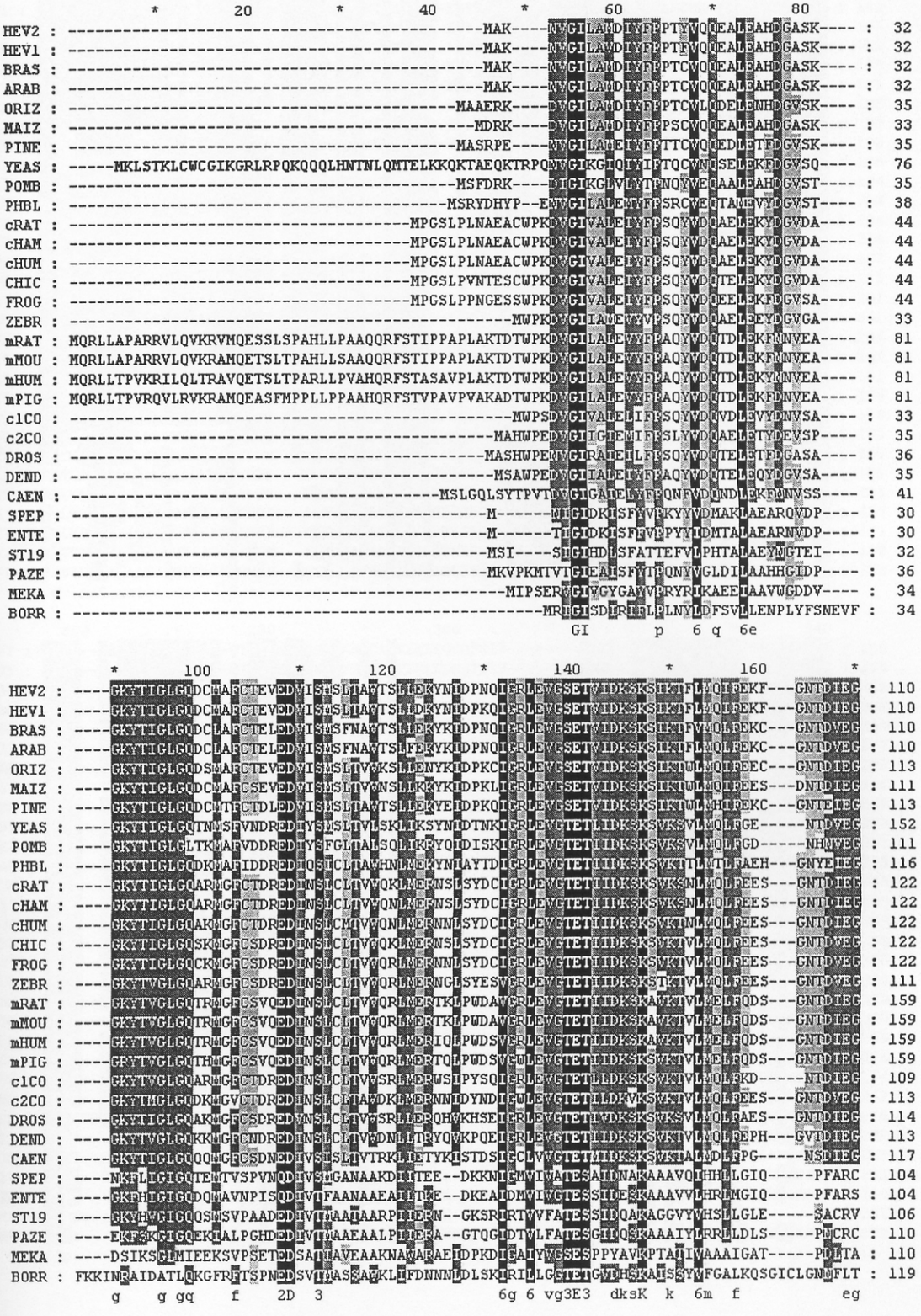
ne 3: Petiole

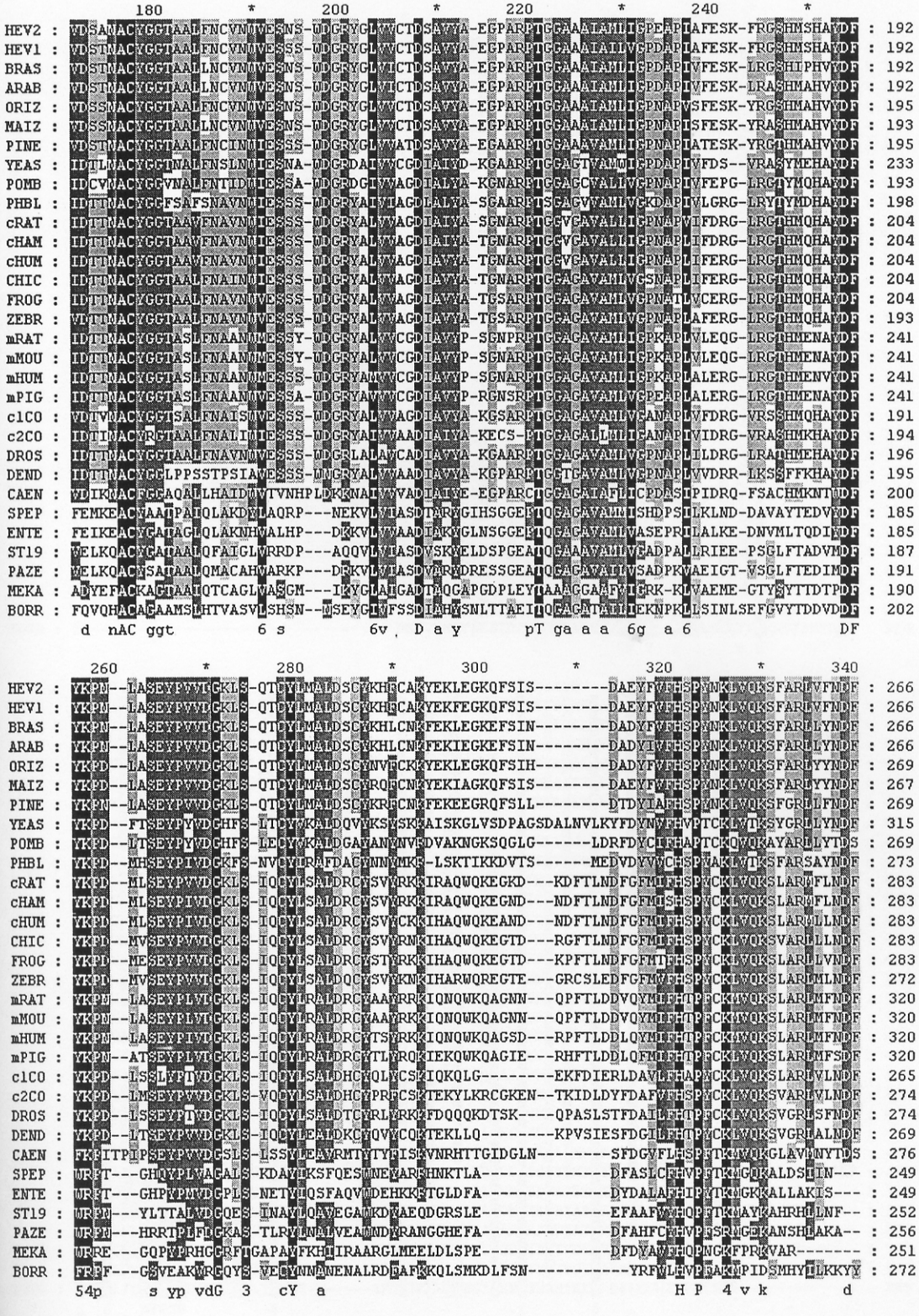
3. Sequence alignment and phylogenetic tree of HMG-CoA synthase and ACP synthase III

The *Hevea brasiliensis* HMG-CoA synthase amino acid sequence was used as query for searching in GenBank nr protein database (NCBI) with BLASTP to find all known HMG-CoA synthase sequences from other organisms and any possible related proteins. Many HMG-CoA synthase sequences and related sequences were found in GenBank; approximately 55 sequences were HMG-CoA synthases. The highest scores of similarity occurred in plants, and declined for animals and fungi and were lowest for bacteria. There were also low scoring sequences from potential HMG-CoA synthase relatives; near the end of the long list of approximately 25 sequences. Most of these sequences were 3-oxoacyl-acyl carrier protein synthase or β-ketoacyl-acyl carrier protein (ACP) synthases from bacteria. It was interesting that searching with Blast using HMG-CoA synthase as a query, ACP synthases III were obtained. The relationship between HMG-CoA synthase and ACP synthases III has not previously been explored. In exploring further the relationship between HMG-CoA synthase and ACP synthase III, a multiple alignment was made by the progressive alignment procedure of Feng and Doolittle (1990).

In the first multiple alignment, only *H. brasiliensis* HMG-CoA synthase amino acid sequence was compared with 30 sequences of other HMG-CoA synthase by using the ClustalX 1.81 program and the sequences showed all the conserved regions that exist in the HMG-CoA synthase proteins which were represented by GeneDoc program in the conservation mode (Figure 23). The amino acid sequences of HMG-CoA synthase in various species; from plants (*Arabidopsis thaliana*, *Brassica juncea*, *Pinus sylvestris*, *Oryza sativa*, and *Zea mays*), mammals (rat, mouse, hamster,

pig, and human mitochondrial forms, and rat, hamster, and human cytosolic forms), avian (chicken), insects (*Blattela germanica* cytoplasm1 and 2), *Drosophila melanogaster* and *Dendroctonus jeffreyi*), yeast (*Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*), amphibian (*Xenopus laevis*; African clawed frog), fish (*Danio rerio*; zebra fish), worm (*Caenorharbditis elegans*), and 7 bacteria sequences (*Staphylococcous epidermidis, Streptomyces SP.CL190, Enterococcous faeclais, Pycomyces blakesleeanus, Paracoccous zeaxanthinifaciens, Methanopyrus kandleri AV19,* and *Borrelia burgdorferi*).




180 * 200 * 220 * 240 *
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HEV1 : VDSTNACYGGTAALFNCVNDVESSS-WDGRYGLVVCTDSAVYA-EGPARPTGGAAAIALIVGPDAPLAFESK-FRGSHMSHAMDF : 192
BRAS : VDSTNACYGGTAALLNCVNDVESNS-WDGRYGLVICTDSAVYA-EGPARPTGGAAALAMLIGPDAPIVFESK-LRGSHIPHVMDF : 192
ARAB : VDSTNACYGGTAALLNCVNDVESNS-WDGRYGLVICTDSAVYA-EGPARPTGGAAALAMLIGPDAPIVFESK-LRASHMAHVMDF : 192
ORIZ : VDSSNACYGGTAALFNCVNDVESNS-WDGRYGLVVCTDSAVYA-EGPARPTGGAAAIAMLIGPNAPVSFESK-YRGSHMAHVMDF : 195
MAIZ : VDSSNACYGGTAALLNCVNDVESNS-WDGRYGLVVCTDSAVYA-EGPARPTGGAAALAMLIGPNAPISFESK-YRASHMAHVMDF : 193
PINE : VDSTNACYGGTAALFNCINDIESSS-WDGRYGLVVATDSAVYA-EGAARPTGGAAAVAMLIGPNAPIATESK-YRGTHMAHVMDF : 195
YEAS : IDTLNACYGGTNALFNSLNDIESNA-WDGRDAIVVCGDTAIYD-KGAARPTGGAGTVAMQIGPDAPIVFDS--VRASYMEHAMDF : 233
POMB : IDCVNACYGGVNALFNTIDDIESSA-WDGRDGIVVAGDTALYA-KGNARPTGGAGCVALIVGPNAPIVFEPG-LRGTYMQHAMDF : 193
PHBL : IDTTNACYGGFSAFSNAVNDIESSS-WDGRYAIVVAGDTALYA-SGAARPTSGAGVVAMLVGKDAPIVLGRG-LRYTYMDHAMDF : 198
cRAT : IDTTNACYGGTAAVFNAVNDIESSS-WDGRYALVVAGDTAIYA-SGNARPTGGVGAVALLIGPNAPVIFDRG-LRGTHMQHAMDF : 204
cHAM : IDTTNACYGGTAAVFNAVNDIESSS-WDGRYALVVAGDTAIYA-TGNARPTGGVGAVALLIGPNAPLIFDRG-LRGTHMQHAMDF : 204
cHUM : IDTTNACYGGTAAVFNAVNDIESSS-WDGRYALVVAGDTAVYA-TGNARPTGGVGAVALLIGPNAPLIFERG-LRGTHMQHAMDF : 204
CHIC : IDTTNACYGGTAALFNAINDIESSS-WDGRYALVVAGDTAVYA-TGNARPTGGAGAVAMLVGSNAPLIFERG-LRGTHMQHAMDF : 204
FROG : IDTTNACYGGTAALFNAVNDVESSS-WDGRYALVVAGDTAVYA-TGSARPTGGAGAVAMLVGPNATLVCERG-LRGTHMQHAMDF : 204
ZEBR : VDTTNACYGGTAALFNAVNDVESSS-WDGRYALVVAGDTAVYA-TGSARPTGGAGAVAMLVGPNAPLAFERG-LRGTHMQHAMDF : 193
mRAT : IDTTNACYGGTASLFNAANDVESSY-WDGRYALVVCGDTAVYP-SGNPRPTGGAGAVAMLIGPKAPLVLEQG-LRGTHMENAMDF : 241
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mHUM : IDTTNACYGGTASLFNAANDVESSS-WDGRYAMVVCGDTAVYP-SGNARPTGGAGAVAMLIGPKAPLALERG-LRGTHMENVMDF : 241
mPIG : IDTTNACYGGTASLFNAANDVESSA-WDGRYAVVVCGDTAVYP-RGNSRPTGGAGAVAMLVGPEAPLALERG-LRGTHMENAMDF : 241
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c2CO : IDTINACYRGTAALFNALIDIESSS-WDGRYAIVVAADTAIYA-KECS-PTGGAGALLMLIGANAPIVIDRG-VRASHMKHAMDF : 194
DROS : IDTTNACYGGTAALFNAVNDVESSS-WDGRLALAVCADTAVYA-KGAARPTGGAGAVAMLVGPNAPLLLDRG-LRATHMEHAMDF : 196
DEND : IDTTNACYGGLPPSSTPSIAVESSS-WDGRYALVVAADTAVYA-KGPARPTGGTGAVAMLVGPNAPLVVDRR-LKSSFFKHAMDF : 195
CAEN : VDIKNACFGGAQALLHAIDWVTVNHPLDKKNAIVVVADIAIYE-EGPARCTGGAGALALICPDASIPIDRQ-FSACHMKNTMDF : 200
SPEP : FEMKEACVAAQPATQLAKDVLAQRP---NEKVLVIASDTRRYGIHSGGEPTQGAGAVAMMISHDPSILKLND-DAVAYTEDVFDF : 185
ENTE : FEIKEACVGATAGLQLAKNHVALHP---DKKVLVVAADIAKYGLNSGGEPTQGAGAVAMLVASEPRILALKE-DNVMLTQDIFDF : 185
ST19 : VELKQACYGATAALQFAIGLVRRDP---AQQVLVIASDVSKYELDSPGEATQGAAAVAMLVSADPALLRIEE-PSGLFTADVMDF : 187
PAZE : VELKQACYSATAALQMACAHVARKP---DFKVLVIASDVRRYDRESSGEATQGAGAVAILVSADPKVAEIGT-VSGLFTEDIMDF : 191
MEKA : ADYEFACKAGTAATQTCAGLVASGM---IKYGIAIGADTAQGAPGDPLEYTAAAGGAAFVIERK-KLVAEME-GTYSYTTDTPDF : 190
BORR : FQVQHACAGAAMSLHTVASVLSHSN--MSEYGLVFSSDIDHYSNLTTAEIIQFAGATALIIEKNPKLLSINLSEFGVYTDDVDDF : 202
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HEV1 : YKPN--LASEYPVVDGKLS-QTQYLMALDSCVKHFCAKYEKFEGKQFSIS--------DAEYFVFHSPYNKLVQKSFARLVFNDF : 266
BRAS : YKPN--LASEYPVVDGKLS-QTQYLMALDSCVKHLCNKFEKLEGKEFSIN--------DADYFVFHSPYNKLVQKSFARLLYNDF : 266
ARAB : YKPN--LASEYPVVDGKLS-QTQYLMALDSCVKHLCNKFEKIEGKEFSIN--------DADYIVFHSPYNKLVQKSFARLLYNDF : 266
ORIZ : YKPD--LASEYPVVDGKLS-QTQYLMALDSCVNVFCKKYEKLEGKQFSIH--------DADYFVFHSPYNKLVQKSFARLYYNDF : 269
MAIZ : YKPD--LASEYPVVDGKLS-QTQYLMALDSCVRQFCNKYEKIAGKQFSIS--------DAEYFVFHSPYNKLVQKSFARLYYNDF : 267
PINE : YKPN--LASEYPVVDGKLS-QTQYLMALDSCVKRFCNKFEKEEGRQFSLL--------DTDYIAFHSPYNKLVQKSFGRLLFNDF : 269
YEAS : YKPD--FTSEYPVVDGHFS-LTQYVKALDQVVKSYSKEAISKGLVSDPAGSDALNVLKYFDYNVFHVPTCKLVTKSYGRLLYNDF : 315
POMB : YKPD--LTSEYPVVDGHFS-LEQYVKALDGAVANYNVFDVAKNGKSQGLG------LDRFDYCIFHAPTCKQVQKAYARLLYTDS : 269
PHBL : YKPD--MHSEYPIVDGKFS-NVQYLRALDACVMNYMKF-LSKTIKKDVTS------MEDVDYVVCHSPYAKLVTKSFARSAYNDF : 273
cRAT : YKPD--MLSEYPVVDGKLS-IQQYLSALDRCVSVYRKKIRAQWQKEGKD---KDFTLNDFGFMIFHSPYCKLVQKSLARMFLNDF : 283
cHAM : YKPD--MLSEYPIVDGKLS-IQQYLSALDRCVSVYRKKIRAQWQKEGND---NDFTLNDFGFMIFHSPYCKLVQKSLARMFLNDF : 283
cHUM : YKPD--MLSEYPIVDGKLS-IQQYLSALDRCVSVYCKKIHAQWQKEAND---NDFTLNDFGFMIFHSPYCKLVQKSLARMLLNDF : 283
CHIC : YKPD--MVSEYPVVDGKLS-IQQYLSALDRCVSVYRMKIHAQWQKEGTD---RGFTLNDFGFMIFHSPYCKLVQKSVARLLLNDF : 283
FROG : YKPD--MESEYPVVDGKLS-IQQYLSALDRCVSTYRKKIHAQWQKEGTD---KPFTLNDFGFNIFHSPYCKLVQKSLARLLVNDF : 283
ZEBR : YKPD--MVSEYPVVDGKLS-IQQYLSALDQCVSVYKNKIHARWQREGTE---GRCSLEDFGFMVFHSPYCKLVQKSLARLMLNDF : 272
mRAT : YKPD--LASEYPLVDGKLS-IQQYLRALDRCVAAYRRKIQNQWKQAGNN---QPFTLDDVQYMIFHTPFCKMVQKSLARLMFNDF : 320
mMOU : YKPN--LASEYPLVDGKLS-IQQYLRALDRCVAAYRKKIQNQWKQAGNN---QPFTLDDVQYMIFHTPFCKMVQKSLARLMFNDF : 320
mHUM : YKPN--LASEYPIVDGKLS-IQQYLRALDRCVTSYRKKIQNQWKQAGSD---RPFTLDDLQYMIFHTPFCKMVQKSLARLMFNDF : 320
mPIG : YKPN--ATSEYPLVDGKLS-IQQYLRALDRCVTLYRQKIEKQWKQAGIE---RHFTLDDLQFMIFHTPFCKLVQKSLARLMFSDF : 320
c1CO : YKPD--LSSLYPIVDGKLS-IQQYLSALDHCVQLYCSKIQKQLG--------EKFDIERLDAVLFHAPYCKLVQKSLARLVLNDF : 265
c2CO : YKPD--LMSEYPVVDGKLS-VQQYLSALDHCVPRFCSKTEKYLKRCGKEN--TKIDLDYFDAFVFHSPYCKLVQKSVARLVLNDF : 274
DROS : YKPD--LSSEYPIVDGKLS-IQQYLSALDTCVRLYRKKFDQQQKDTSK----QPASLSTFDAILFHTPFCKLVQKSVGRLSFNDF : 274
DEND : YKPD--LTSEYPVVDGKLS-IQQYLEALDKCVQVYCQKTEKLLQ--------KPVSIESFDGILFHTPYCKLVQKSVGRLALNDF : 269
CAEN : FKSITPIPSEYPVVDGSLS-LSSYLEAVRMIYTYFISKVNRHTTGIDGLN--------SFDGVFLHSPYTKMVQKGLAVLNYTDS : 276
SPEP : URFT---GHQYPLVAGALS-KDAVLKSFQESLNEYARKHNKTLA--------------DFASLCFHVPYTKMGQKALDSLIN--- : 249
ENTE : URFT---GHPVPLVDGPLS-NETYLQSFAQVLDEHKKKSTGLDFA--------------DYDALAFHIPYTKMGKKALLAKIS--- : 249
ST19 : URPN---YLTTALVDGQES-INAVLQAVEGALKDVAEQDGRSLE--------------EFAAFVYHQPYTKMAYKAHRHLLNF-- : 252
PAZE : URPN---HRRTPLFLQKAS-TLRVLNALVEAMNDYRANGGHEFA--------------DFAHFCFHVPYSRMGDKANSHLAKA-- : 256
MEKA : URRE---GQPVPRHGGRFTGAPAVFKHLIRAARGLMEELDLSPE--------------DFDYAVFHQPNGKFPRKVAR------- : 251
BORR : FRPF---GQVEAKYRGQYS-VEQYNNANENALRDLAFKKQLSMKDLFSN----------YRFWLHVPSAKMPIDSMHYILKKYY : 272
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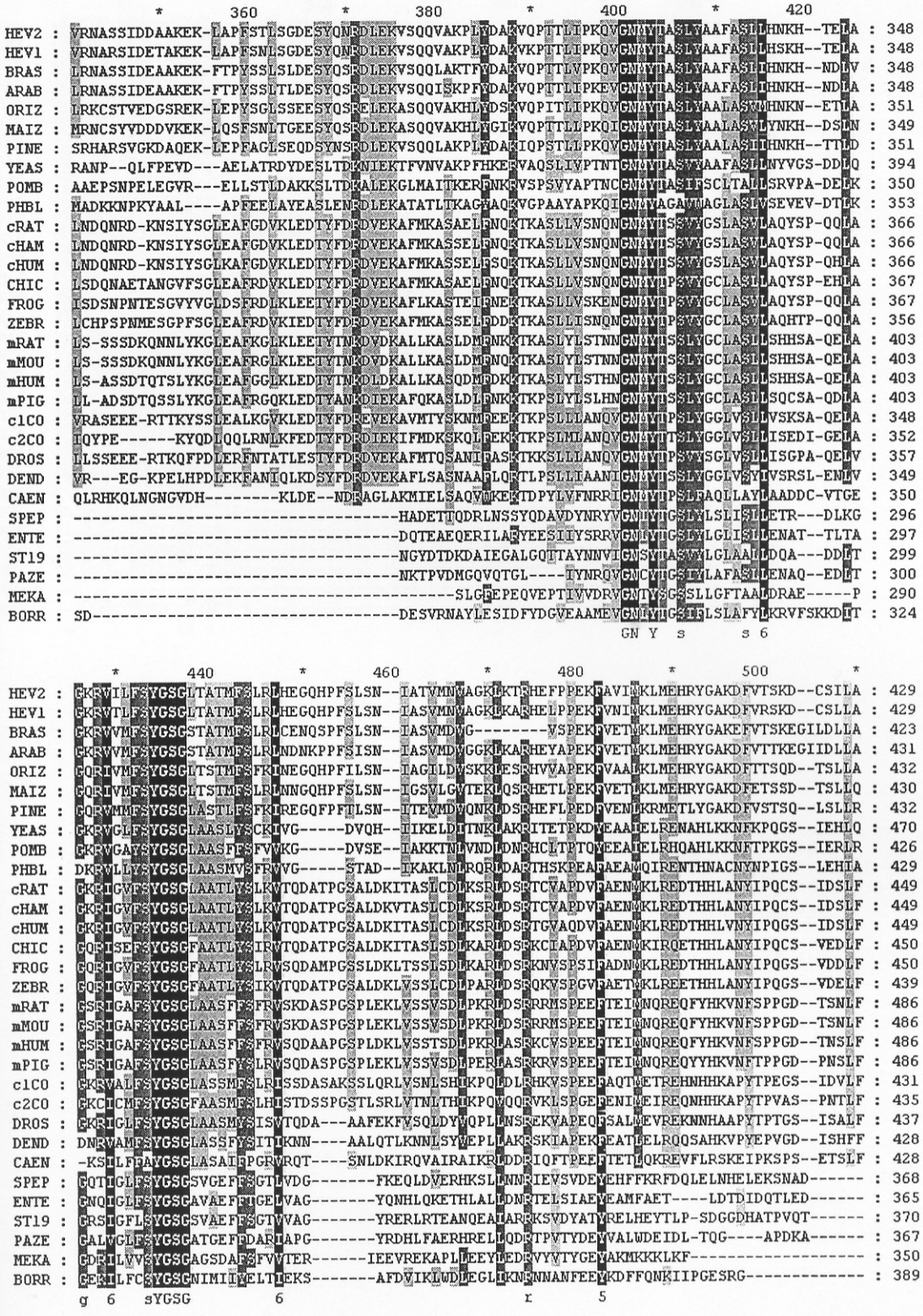
HEV2
HEV1
BRAS
ARAB
ORIZ
MAIZ
PINE
YEAS
POMB
PHBL
cRAT
cHAM
cHUM
CHIC
FROG
ZEBR
mRAT
mMOU
mHUM
mPIG
c1C0
c2C0
DROS
DEND
CAEN
SPEP
ENTE
ST19
PAZE
MEKA
BORR

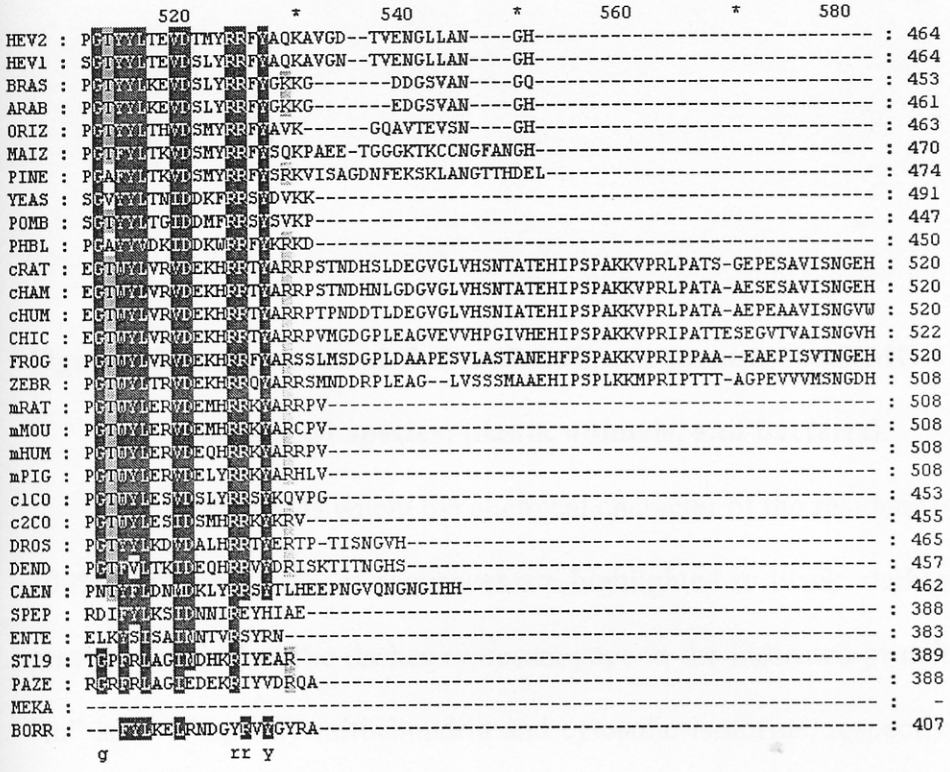
520 * 540 * 560 * 580
HEV2 : PGTYYLTEWDTMYRRFYAQKAVGD--TVENGLLAN----GH---------- : 464
HEV1 : SGTYYLTEWDSLYRRFYAQKAVGN--TVENGLLAN----GH---------- : 464
BRAS : PGTYYLKEWDSLYRRFYGKKG-------DDGSVAN----GQ---------- : 453
ARAB : PGTYYLKEWDSLYRRFYGKKG-------EDGSVAN----GH---------- : 461
ORIZ : PGTYYLTHWDSMYRRFYAVK------GQAVTEVSN----GH---------- : 463
MAIZ : PGTFYLTKWDSMYRRFYSQKPAEE-TGGGKTKCCNGFANGH---------- : 470
PINE : PGAFYLTKWDSMYRRFYSRKVISAGDNFEKSKLANGTTHDEL---------- : 474
YEAS : SGVYYLTNLDDKFRRSYDVKK---------- : 491
POMB : SGTYYLTGLDDMFRRSYSVKP---------- : 447
PHBL : PGAFYWDKLDDKWRRFYKRKD---------- : 450
cRAT : EGTVYLVRWDEKHRRTYARRPSTNDHSLDEGVGLVHSNTATEHIPSPAKKVPRLPATS-GEPESAVISNGEH : 520
cHAM : EGTVYLVRWDEKHRRTYARRPSTNDHNLGDGVGLVHSNTATEHIPSPAKKVPRLPATA-AESESAVISNGEH : 520
cHUM : EGTVYLVRWDEKHRRTYARRPTPNDDTLDEGVGLVHSNIATEHIPSPAKKVPRLPATA-AEPEAAVISNGVW : 520
CHIC : EGTVYLVRWDEKHRRTYARRPVMGDGPLEAGVEVVHPGIVHEHIPSPAKKVPRIPATTESEGVTVAISNGVH : 522
FROG : PGTVYLVRWDEKHRRFYARSSLMSDGPLDAAPESVLASTANEHFPSPAKKVPRIPPAA--EAEPISVTNGEH : 520
ZEBR : PGTVYLTRWDEKHRRQYARRSMNDDRPLEAG--LVSSSMAAEHIPSPLKKMPRIPTTT-AGPEVVVMSNGDH : 508
mRAT : PGTVYLERWDEMHRRKYARRPV---------- : 508
mMOU : PGTVYLERWDEMHRRKYARCPV---------- : 508
mHUM : PGTVYLERWDEQHRRKYARRPV---------- : 508
mPIG : PGTVYLERWDELYRRKYARHLV---------- : 508
c1CO : PGTVYLESWDSLYRRSFKQVPG---------- : 453
c2CO : PGTVYLESIDSMHRRKYKRV---------- : 455
DROS : PGTFYLKDWDALHRRTYERTP-TISNGVH---------- : 465
DEND : PGTRVLTKLDEQHRPVYDRISKTITNGHS---------- : 457
CAEN : PNTFTLDNMDKLYRPSYTLHEEPNGVQNGNGIHH---------- : 462
SPEP : RDIFYLKSIDNNIREYHIAE---------- : 388
ENTE : ELKGSISAINNTVESYRN---------- : 383
ST19 : TGPQRLAGLDDHKRIYEAR---------- : 389
PAZE : RGRQRLAGLEDEKRIYVDRQA---------- : 388
MEKA : ---------- : -
BORR : ---FYLKEARNDGYEVVGYRA---------- : 407
g rr y

Figure 23. Alignment of deduced amino sequences for HMG-CoA synthase among 31 species; plants, animals, and bacterial.

Positions where the chemical character of the residues is conserved in 100%, 80%, and 60% of sequences are highlighted in black, dark gray, and light gray, respectively. The dashes represent gaps in the indicated proteins. The letters "m" and "c" specify mitochondria and cytosolic isoforms, respectively. GenBank accession numbers are shown in ( ) and see others in Figure 25. HEV1 and 2: *H. brasiliensis*1 and 2, respectively, BRAS: *B. juncea*, ARAB: *A. thaliana*, ORIZ: *O.sative*, MAIZ: *Zea mays* (AY104370), PINE: *P. sylvestris*, YEAS: *S. cerevisiae* (NP_013580), POMB: *S. pombe*, ZEBR: *D. rerio* (NP_957379), RAT: *R. norvegicus*, MOU: *M. musculus*, HUM: *H. sapiens*, PIG: *S. scrofa*, HAM: *C. griseus*, CHIC: *G. gallus*, c1CO and c2CO: *B. germanica*1 and 2, respectively, FROG: *X. laevis*, DROS: *D. melanogaster*, DEND: *D. jeffreyi*, CAEN: *C. elegans*, SPEP: *S. epidermidis* (AAG02433), ENTE: *E. faecalis* (AAG02438), ST19: *S. SP. CL190* (BAB07795), PHBL: *P. blakesleeanus* (CAC18553), PAZE: *P. zeaxanthinifaciens* (CAD24420), MEKA: *M. kandleri AV19* (NP_614662), BORR: *B. burgdorferi*, (NP_212817).

Then all amino acid sequences of HMG-CoA synthase were compared to determine the percent identity between them by using the progressive alignment program. A multiple comparison obtained by careful "cropping" of the amino acid sequences to about the same length showed the percent identity of the HMG-CoA synthase groups, (Table 10). HMG-CoA synthase 1 and 2 from *Hevea brasiliensis* showed high percent identity at 94%. The percent identities of mitochondrial and cytosolic HMG-CoA synthase in the same species ranges from 62% to 68%, for example, the percent identity between rat cytosolic and mitochondrial HMG-CoA synthase is 65%, whereas mitochondrial or cytosolic isoform in the different species showed higher identity, such as is 91% between human and rat mitochondrial isoform. Comparison between plants and vertebrates showed 47-53 % percent identities and the identities decreased to 43-44% and 39-40%, in yeast and worm, respectively. Low percent identities ranging from 23-31% are present in comparison among HMG-CoA synthase from bactaria and archaea to HMG-CoA synthase sequences from other species.

When 8 amino acid sequences of 3-oxoacy-acyl carrier protein synthase III from bacteria were added and aligned with the group of HMG-CoA synthases. The multiple alignments were also carefully examined by shortenig the HMG-CoA synthase and ACP synthase III sequences. This cropping modification was important and necessary for proper multiple alignment of the sequences, since the sequences have different lengths. On the average, HMG-CoA synthases are 460 amino acids long, whereas ACP synthase III are only 330 amino acids long. In comparisons, sequences of approximately 300 amino acids long were compared by the progressive alignment procedure of Feng and Doolittle (1990).

The alignment produced for HMG-CoA synthases and ACP synthases is well aligned; there are not many gaps, and several important residues are completely conserved throughout all species including cysteine-117, histidine-247, asparagines-326, glycine-358, and glycine-360 (Figure 24). The Cys^{117} and His^{247} (the residue numbering corresponds to *H. brasiliensis* HMG-CoA synthase) were known to be active residues in both kinds of proteins. Surprisingly, there is an Asn at residue 326, which is known to play a catalytic role in ACP synthase III, but no studies of this residue on any HMG-CoA synthase have been reported. The percent identities resulting from the alignments of HMG-CoA synthase and ACP synthase sequences were calculated and shown in Table 10. In comparison between amino acid sequences of HMG-CoA synthase and ACP synthase III, the overall percent identities ranged from 55% between *M. kandleri* HMG-CoA synthase and *A. pernix* ACP synthase III which are both from archaea, to a low of 18% observed between HMG-CoA synthase from frog (*X. laevis*), zebra fish (*D. rerio), A. thaliana, P. sylvestris, O. sativa*, bacteria (*S. epidermidis*) and ACP synthase III from *M. tuberculosis. Hevea brasiliensis* HMG-CoA synthase1 and 2 and ACP synthase III show 20% identity for *M. tuberculosis.*

The result from the multiple alignment was used to construct a phylogenetic tree. The phylogenetic tree can be divided into two major groups of a gene tree and a species tree. For HMG-CoA synthase, the average branch lengths from the divergence point to the extant species appear not to differ significantly between various species; this is also the case with the lineage in the ACP synthase III. All the ACP synthase III sequences were not grouped together in their own cluster. The ACP synthase III from archaebacteria is located close to HMG-CoA synthase rather than to the eubacterium ACP synthase III (Figure 25).

Table 10. Percent identities of amino acid sequences in HMG-CoA synthase and ACP synthase III.

The amino acid sequences of HMG-CoA synthase among mammals, plants, insects, chicken, frog, fish, yeast, worm, and bacteria. Letter "c" and "m" specify cytosolic and mitochondria isoform, respectively, including ACP synthase III in *A. pernix* (AERO) and *M. tuberculosis* (MYCO). For abbreviation, see in Figure 23.

	cRAT	cHUM	CHIC	FROG	ZEBR	mHUM	mRAT	c1CO	DROS	c2CO	HEV2	HEV1	BRAS	ARAB	ORIZ	PINE	POMB	CAEN	SPEP	BORR	MEKA	*AERO*	*MYCO*
cRAT	-																						
cHUM	95	-																					
CHIC	86	86	-																				
FROG	81	82	85	-																			
ZEBR	79	80	82	81	-																		
mHUM	66	67	67	68	68	-																	
mRAT	65	65	65	67	66	91	-																
c1CO	64	64	64	65	66	63	63	-															
DROS	63	63	64	64	63	60	60	67	-														
c2CO	60	59	60	60	61	55	54	60	67	-													
HEV2	49	50	49	50	50	50	50	51	49	50	-												
HEV1	50	50	49	50	49	50	50	52	49	50	94	-											
BRAS	48	49	48	50	49	48	49	50	47	48	84	84	-										
ARAB	48	49	49	50	49	47	48	49	48	47	83	81	94	-									
ORIZ	52	52	51	51	53	50	49	51	49	50	78	76	76	74	-								
PINE	50	51	51	50	51	50	50	50	50	50	73	73	72	72	73	-							
POMB	47	47	48	46	46	47	50	48	46	46	44	44	43	43	44	44	-						
CAEN	43	42	43	41	41	44	44	44	40	42	39	39	40	40	39	39	33	-					
SPEP	28	29	27	28	28	30	31	27	28	29	27	27	28	28	26	28	28	28	-				
BORR	27	27	27	27	26	28	27	25	25	25	25	27	27	27	26	25	25	27	29	-			
MEKA	24	25	24	25	25	24	23	24	24	26	27	27	29	28	29	26	28	23	27	24	-		
AERO	24	24	23	22	22	20	21	21	21	20	24	24	23	24	24	24	25	19	24	19	55	-	
MYCO	20	19	21	18	18	19	19	20	21	20	20	20	20	18	18	19	23	19	18	23	23	23	-

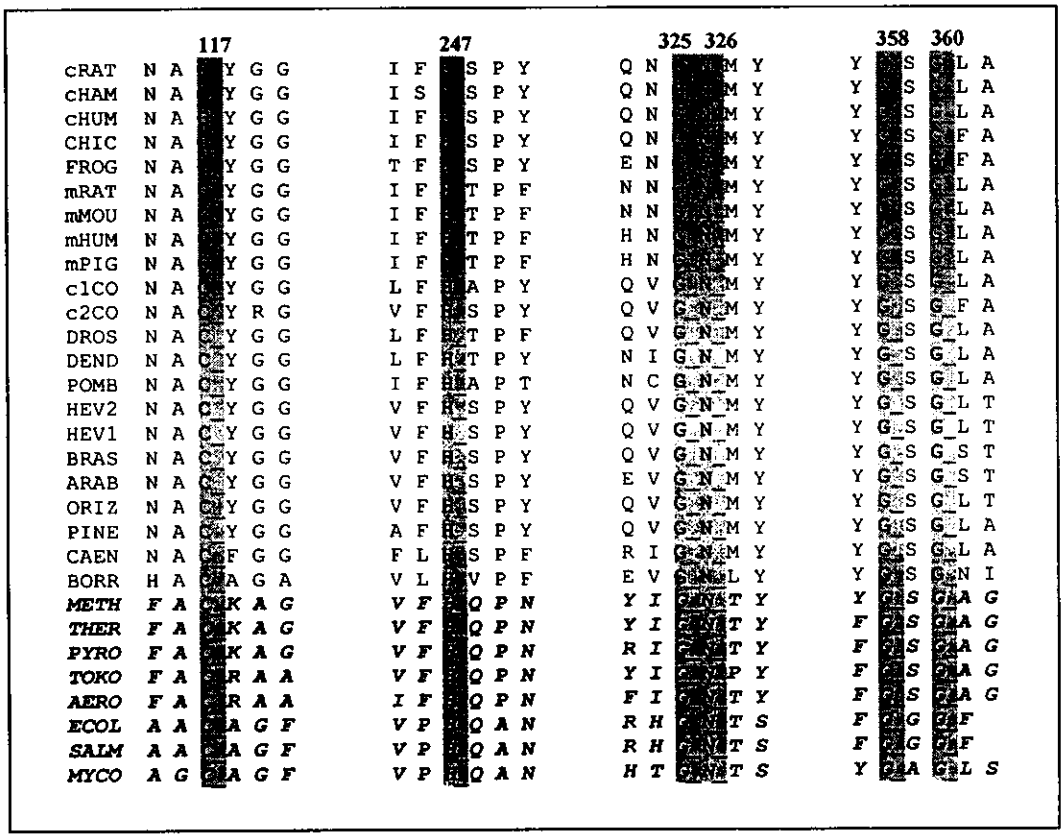

Figure 24. Alignment of deduced amino sequences for HMG-CoA synthase and ACP synthase III (Boldface type) to indicate the conserved residues.

Letters in gray highlight invariant amino acids among both proteins. The number on top refers to *H.brasiliensis* HMG-CoA synthase. See Figure 23 for abbreviation of HMG-CoA synthase sequences. ACP synthase III abbreviations are: METH: *M.jannaschii*, THER: *T. volcanium*, PYRO: *P. furiosus*, TOKO: *S. tokodaii*, AERO: *A. pernix*, ECOL: *E. coli*, SALM: *S. typhimurium*, MYCO: *M. tuberculosis.*

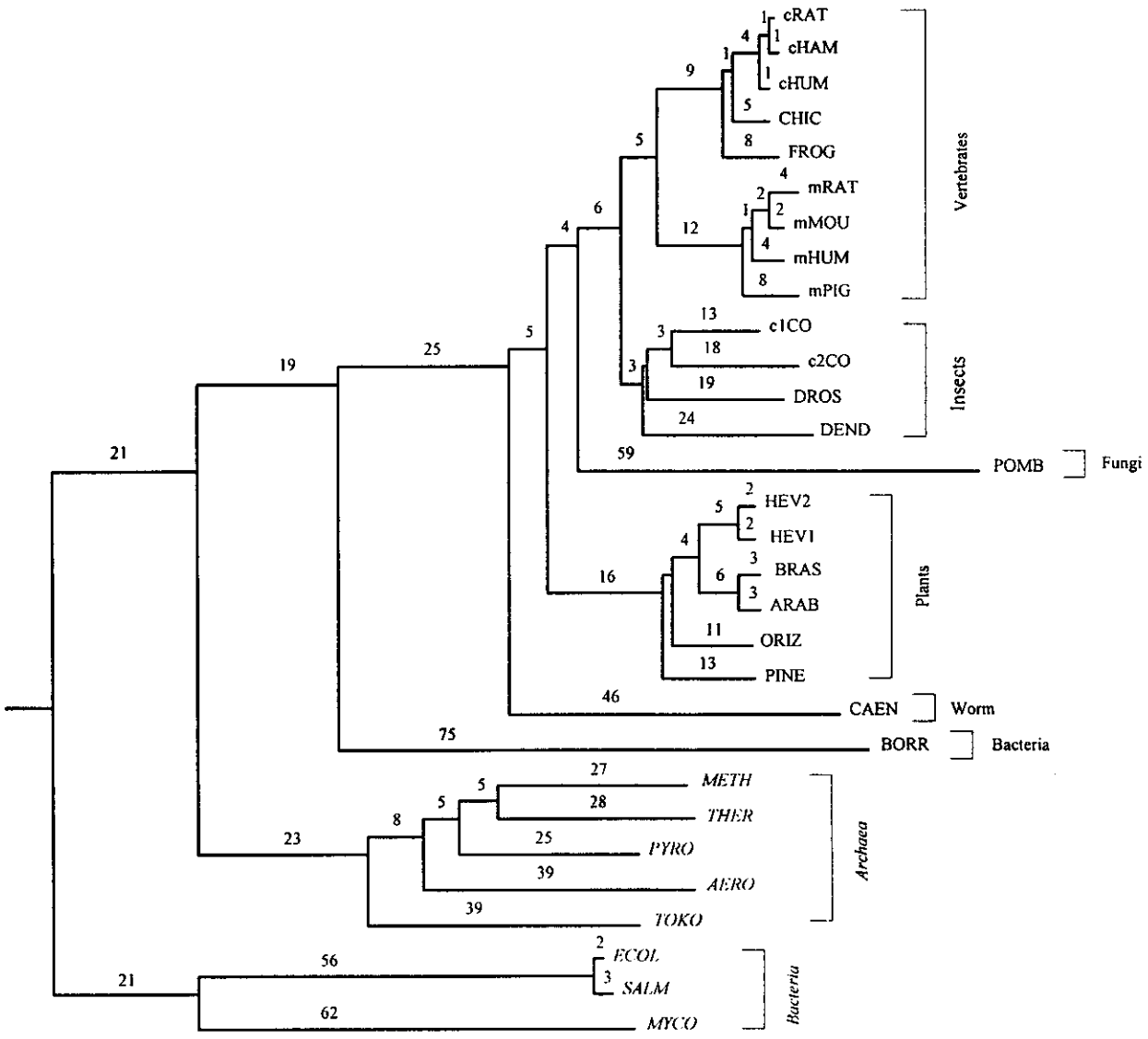
cRAT
cHAM
cHUM
CHIC
FROG
mRAT
mMOU
mHUM
mPIG
Vertebrates
c1CO
c2CO
DROS
DEND
Insects
POMB
Fungi
HEV2
HEV1
BRAS
ARAB
ORIZ
PINE
Plants
CAEN
Worm
BORR
Bacteria
METH
THER
PYRO
AERO
TOKO
Archaea
ECOL
SALM
MYCO
Bacteria

Figure 25. Phylogenetic relationship of HMG-CoA synthase and ACP synthase.

ACP synthase III sequences from bacteria in italics and HMG-CoA synthase sequences from plants, mammals, chicken, insects, frog, worm, and bacteria. The tree was constructed based on growing methodologies and corresponds to the progressive alignment to determine the branching order of the sequences. Numbers indicate branch lengths. See Figure 23 and 24 for abbreviations. GenBank accession numbers: cRAT (NP_058964), cHAM (P13704), cHUM (NP_002121), CHIC (P23228), FROG (AAH42929), mRAT (NP_775117), mMOU (NP_032282), mHUM (NP005509), mPIG (U90884), c1CO (P54961), c2CO (P54870), DROS (NM_079972), DEND (AF166002), POMB (NP_593859), HEV2 (AY534617), HEV1 (AF396829), BRAS (AAG32924), ARAB (NM_117251), ORIZ (NP_912446), PINE (X96386). CAEN (NP_504496), BORR (NP_212817), METH (NP_248554), THER (BAB59274), PYRO (NP_578701), TOKO (NP_377303), AERO (NP_148228), ECOL (BAB34892), SALM (NP_460163), MYCO (CAB08984).

4. The expression of recombinant *H. brasiliensis hmgs1* in *E. coli* and comparison of the enzymes activity

The multiple alignment of HMG-CoA synthase sequences and those of ACP synthase III, shows three totally conserved residues; Cys^{117}, His^{247}, and Asn^{326}. The effects of Asn^{326} on catalysis of which in HMG-CoA synthase have not been investigated. In this study, the specific activity of the mutant HMG-CoA synthase 1 was compared to recombinant wild type enzyme. The mutant ORFs of *hmgs1* at Cys^{117}, Asn^{326}, and the double mutant at Cys^{117} Asn^{326} were performed by PCR amplification for site-directed mutagenesis, as described in the method. The 350 bp 5' 'megaprimer' and 115 bp 3' 'megaprimer' were used in the amplification of an ORF of C117A and N326A *hmgs1* gene, respectively. The double mutation, C117/N326A *hmgs1* was performed by using the C117A *hmgs1* gene as template, mutating Asn^{326}, and amplifying with the 3' 'megaprimer'. The expression plasmids encoding alanine substitutions for Cys^{117}: (C117A), Asn^{326}: (N326A), and $Cys^{117}Asn^{326}$: (C117/N326A) were transformed into *E. coli* M15 (pREP4) and used for heterologous protein expression. The whole pathway from acetyl-CoA to mevalonate is absent in this bacterium and in most of the gram-negative bacterial species (Rohmer 1999 and Wilding et al. 2000). In order to express wild type and mutant *hmgs1* in *E. coli*, an open reading frame of *hmgs1* cDNA was amplified with 5' flanking and 3' flanking as forward and reverse primer, respectively. The expected size of 1.4 kb amplification products were observed in agarose gel electrophoresis (Figure 26). The pQE-31 plasmid not containing ORF *hmgs1* was amplified and also subjected to gel electrophoresis as a control to compare with recombinant pQE-31 plasmid.

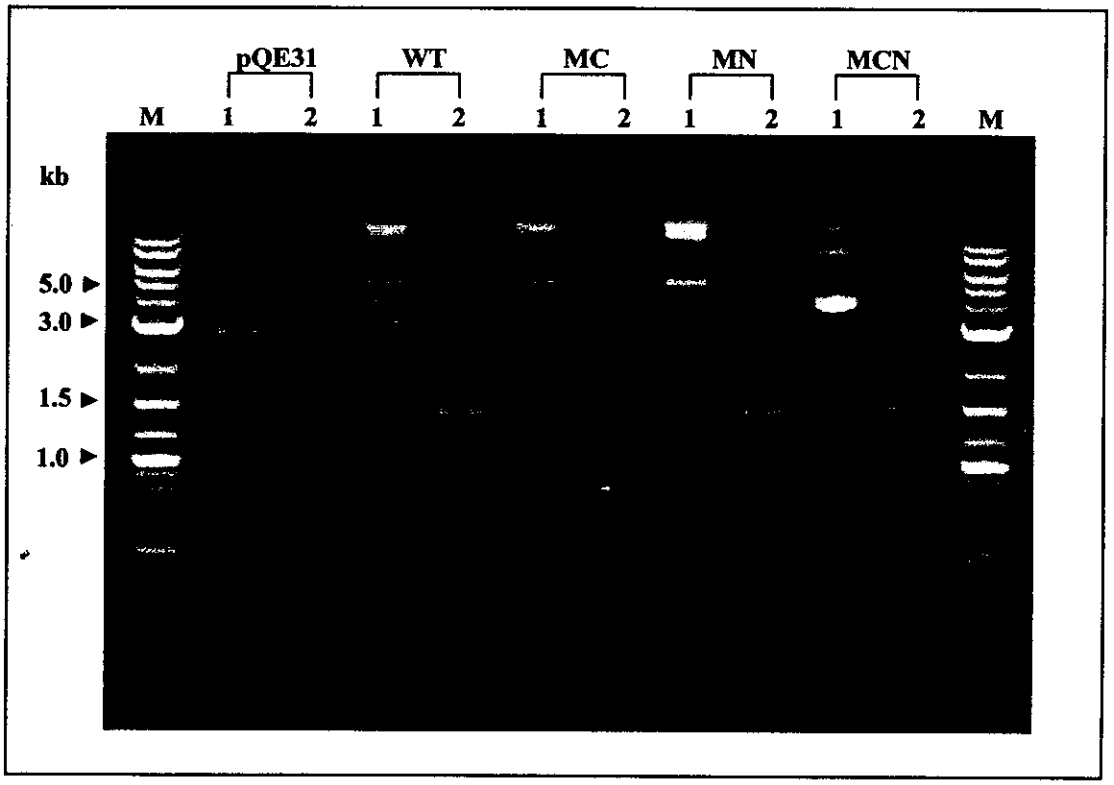

Figure 26. PCR amplification products of open reading frame *hmgs1* in expression vector transformed to *E. coli* M15 (pREP4).

1% agarose gel electrophoresis with ethidium bromide strain, pQE31 is no *hmgs1* in pQE31. The WT, MC, MN, and MCN are plasmid encoding wild type, C117A, N326A, and C117/N326A HMG-CoA synthase, respectively.

Lane M: 2 log DNA marker

Lane 1: Plasmid template

Lane 2: PCR products (1.4 kb)

The optimum condition for expression of wild type HMG-CoA synthase 1 induced by IPTG under T5 promoter was determined using various IPTG final concentrations; it was found that the optimum concentration of IPTG for the expression is 0.5 mM as shown in Figure 27. The time-course for induction with IPTG of 1-5 h showed that the amount of expressed protein increased as the induction time increased and reached maximum within 4-5 h. (Figure 28). Therefore, the optimum condition for expression of HMG-CoA synthase 1 in *E. coli* using this system is induced with 0.5 mM IPTG (final concentration) for 4-5 h at 37°C.

The $(His)_6$-HMG-CoA synthase 1 fusion proteins with mutation were produced at levels comparable to the wild type proteins (Figure 29). These expressed proteins exhibit subunit molecular masses of those observed for a *H. brasiliensis* HMG-CoA synthase in the C-serum of rubber latex. The HMG-CoA synthase activity from crude extract of the expressed enzyme at various concentrations of protein was determined for wild type and mutant enzymes (Figure 30). An enzyme activity in wild type is comparatively higher than the activity of mutant HMG-CoA synthase at Cys^{117}, Asn^{326}, and Cys^{117} Asn^{326} including pQE31 vector at all concentrations of the protein used in the assay. The specific activities of the mutant enzymes are lower than that of wild type as shown in Table 11. The kinetic parameters for the recombinant HMG-CoA synthase 1 could not be determined since the enzyme activities in mutated enzymes, expressed in *E. coli* are lower than the activity of pQE31 vector (Figure 30).

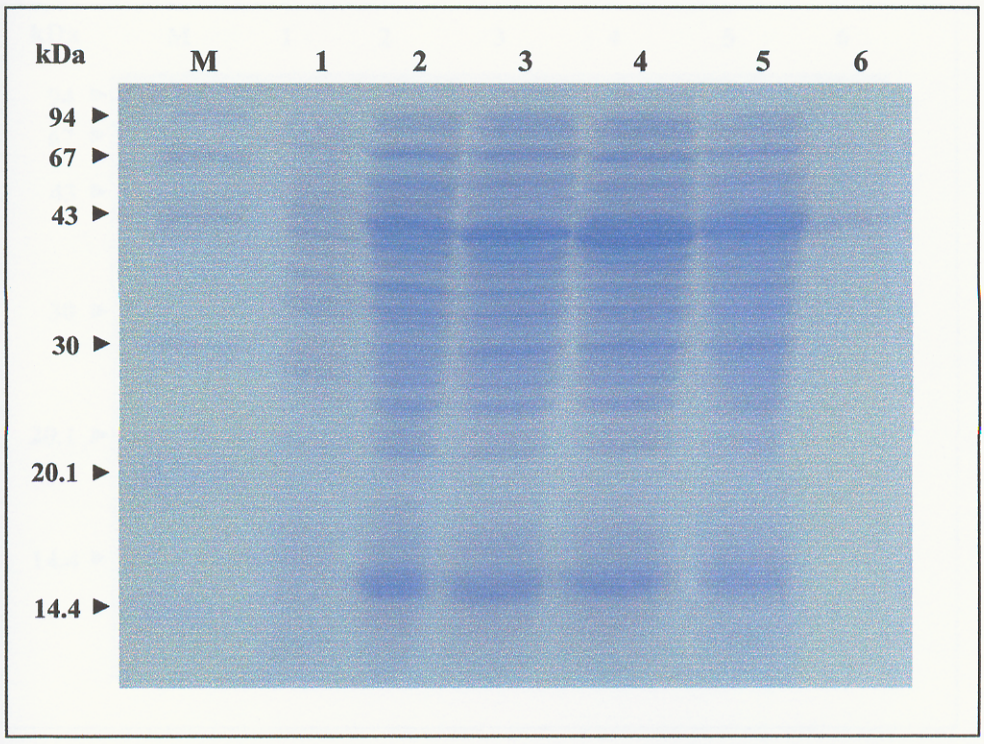

Figure 27. SDS-polyacrylamide gel electrophoresis of *E. coli* lysate from wild type HMG- CoA synthase1 at different IPTG concentrations

The expressed proteins were induced with various concentrations of IPTG in the culture grown for 4 h post-induction.

Lane M: Low molecular weight standard

Lane 1: Non-induced *E. coli*

Lane 2-5: Total lysate of *E. coli* induced with 0.05, 0.1, 0.5, and 1 mM final concentration of IPTG, respectively.

Lane 6: Purified HMG-CoA synthase from C-serum of rubber latex.

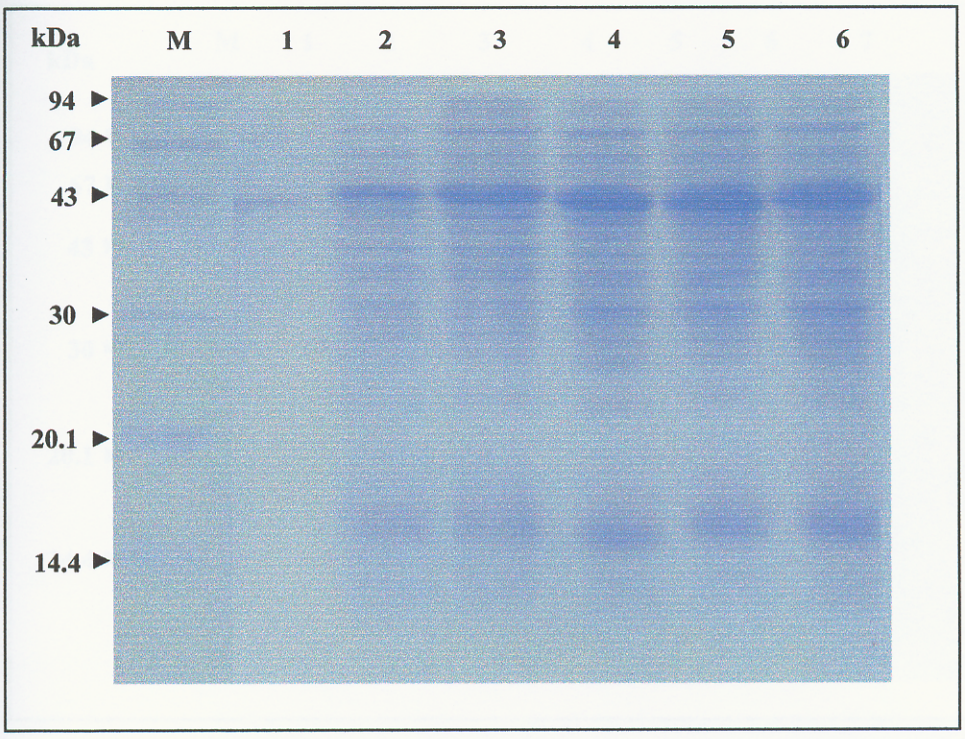

Figure 28. SDS-polyacrylamide gel electrophoresis of *E. coli* lysate from wild type HMG-CoA synthase1 at different incubation times.

Lane M: Low molecular weight standard

Lane 1: Purified HMG-CoA synthase from C-serum of rubber latex

Lane 2-6: Expressed proteins after induction with 0.5 mM IPTG final concentration for 1,2,3,4, and 5 h, respectively.

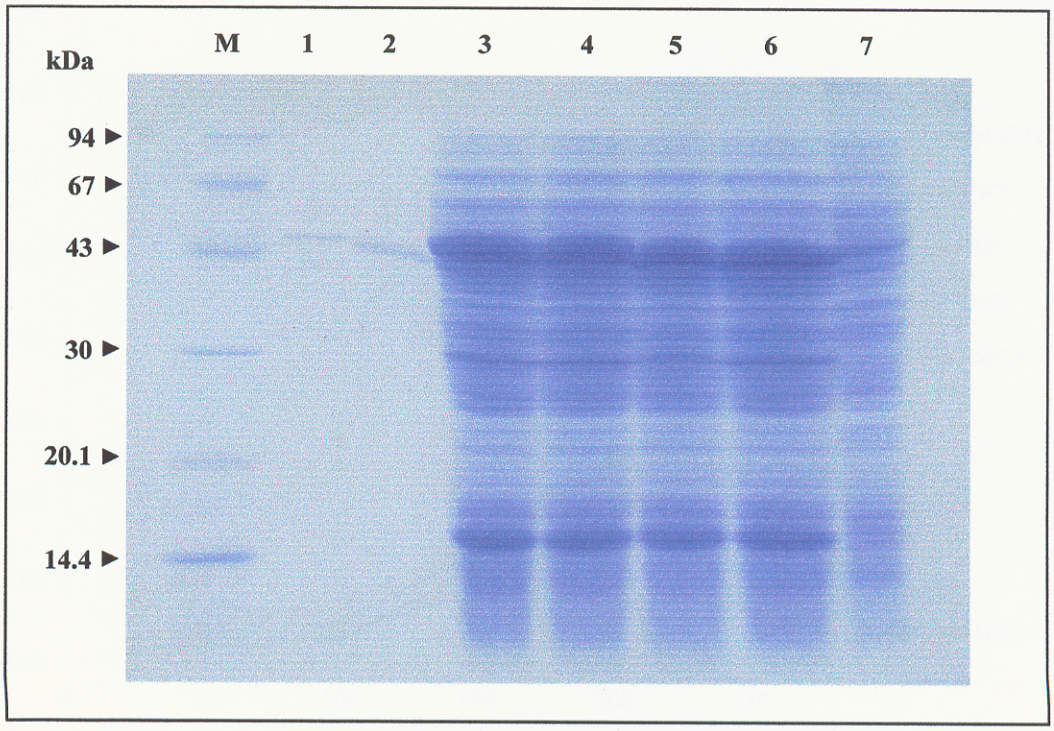

Figure 29. SDS-polyacylamide gel electrophoresis of *E. coli* lysate from recombinant wild type and mutant HMG-CoA synthase1

Lane M: Low molecular weight standard.

Lane 1: Purified expressed HMG-CoA synthase

Lane 2: HMG-CoA synthase purified from C-serum of latex.

Lane 3-7: Total cell lysate of *E. coli* bearing pQE31 encoding C117A/N326A, N326A, C117A, wild type HMG-CoA synthase1, respectively.

Lane 7: Total cell lysate of *E. coli* bearing vector pQE-31.

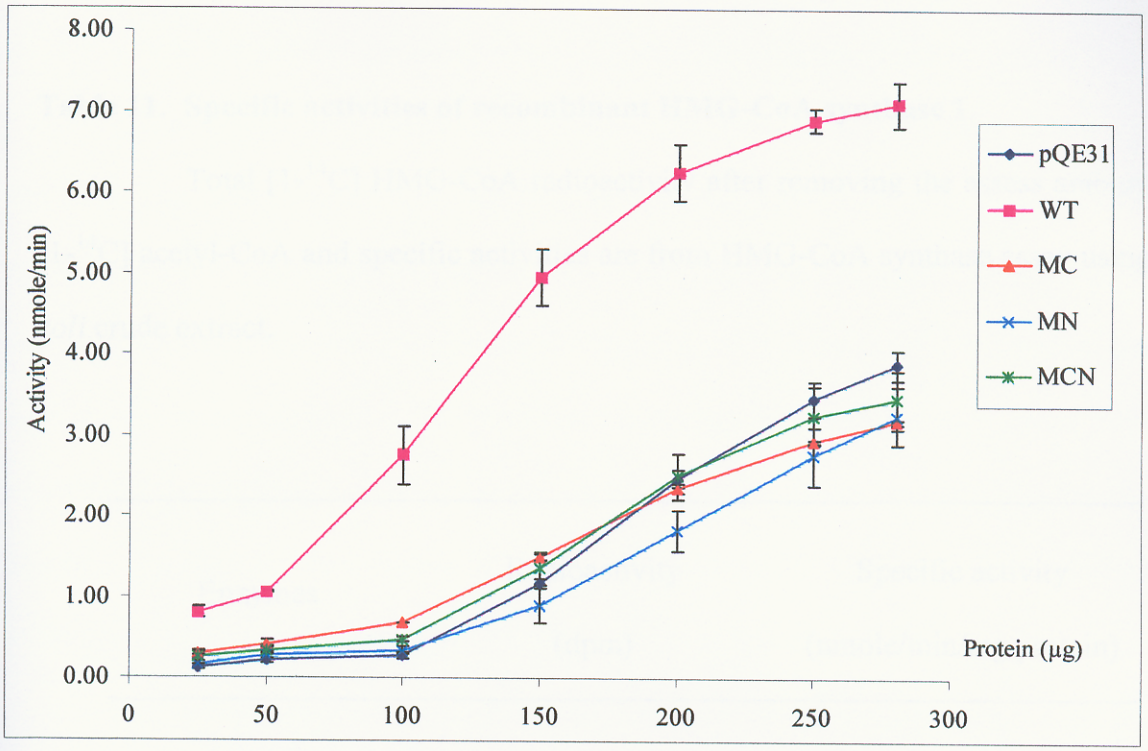

Figure 30. The activity of recombinant HMG-CoA synthase 1 in crude extract at various protein concentrations.

The pQE31, WT, MC, MN, and MCN are enzyme activity from crude extract of *E. coli* bearing only vector pQE-31, pQE-31 encoding wild type, $Cys^{117}Ala$, $Asn^{326}Ala$, and $Cys^{117}/Asn^{326}Ala$ HMG-CoA synthase 1, respectively .

Table 11. Specific activities of recombinant HMG-CoA synthase 1.

Total [1-^{14}C] HMG-CoA radioactivity after removing the assess amount of [1-^{14}C] acetyl-CoA and specific activities are from HMG-CoA synthase assay using *E. coli* crude extract.

Enzymes	Radioactivity (dpm)	Specific activity (nmole/min/mg protein)
Vector pQE31	188	9.64 ± 0.52
Wild type	780	41.25 ± 2.9
C117A	240	12.50 ± 0.6
N326A	168	8.13 ± 0.88
C117/N326A	219	11.25 ± 1.77

5. The predicted secondary structure of *H. brasiliensis* HMG-CoA synthase

Three dimensional structure of HMG-CoA synthase has not been studied in any organism, and the search in GenBank with BLAST found many non-HMG-CoA synthase relatives. As it happens, there are a number of related sequences belonging to the acyl carrier protein (ACP) synthase family, and there is a structure for 3-oxoacyl-acyl carrier protein synthase III from *Mycobacterium tuberculosis* with about 20% identity to the *H. brasiliensis* HMG-CoA synthase gene. Therefore, this protein was selected as a recognizable relative of HMG-CoA synthase for predicting the secondary structure of *Hevea brasiliensis* HMG-CoA synthase in this study.

The tertiary structure was obtained from the X-ray crystallography of *M. tuberculosis* ACP synthase III obtained from the Protein Data Bank (PDB), 1HZP (Figure 31). The information of amino acid residues in the secondary structure of ACP synthase III in the PDB file were used to compare with amino acid residues of HMG-CoA synthase by structural alignment (Figure 32). Then, the overall possible secondary structure of *Hevea brasiliensis* HMG-CoA synthase was predicted as shown in Figure 33. The possible secondary structure arrangement of *Hevea brasiliensis* HMG-CoA synthase is mainly composed of two β-sheets of five β-strands each and three sets of two α-helices. Each β-sheet is located between two α-helices and in the centre are two α-helices which are sandwiched with two β-sheets. Amino acid residues; Cys^{117}, His^{247}, and Asn^{326} play catalytic role in the active site.

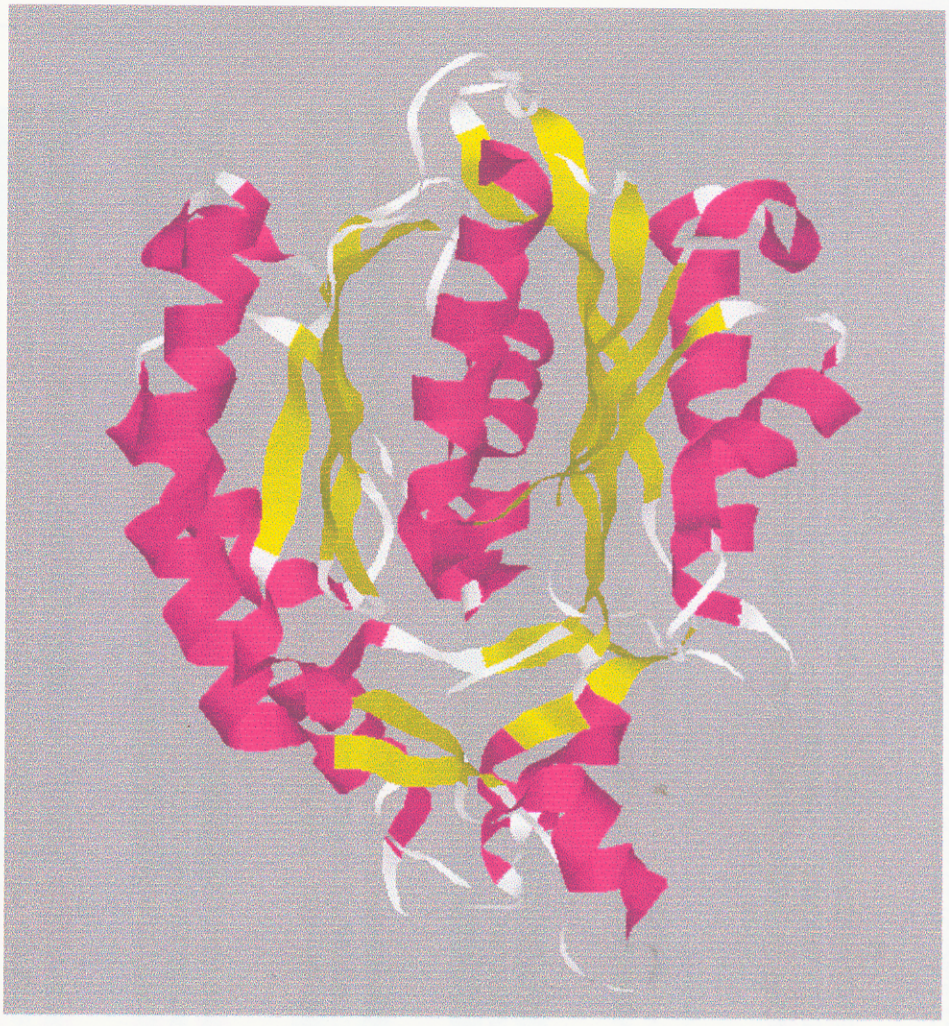

Figure 31. Ribbon diagram of *M. tuberculosis* ACP synthase III

Pink, yellow, and white strands represent α-helices, β-strands, and turns, respectively (Scarsdal et al. 20001).

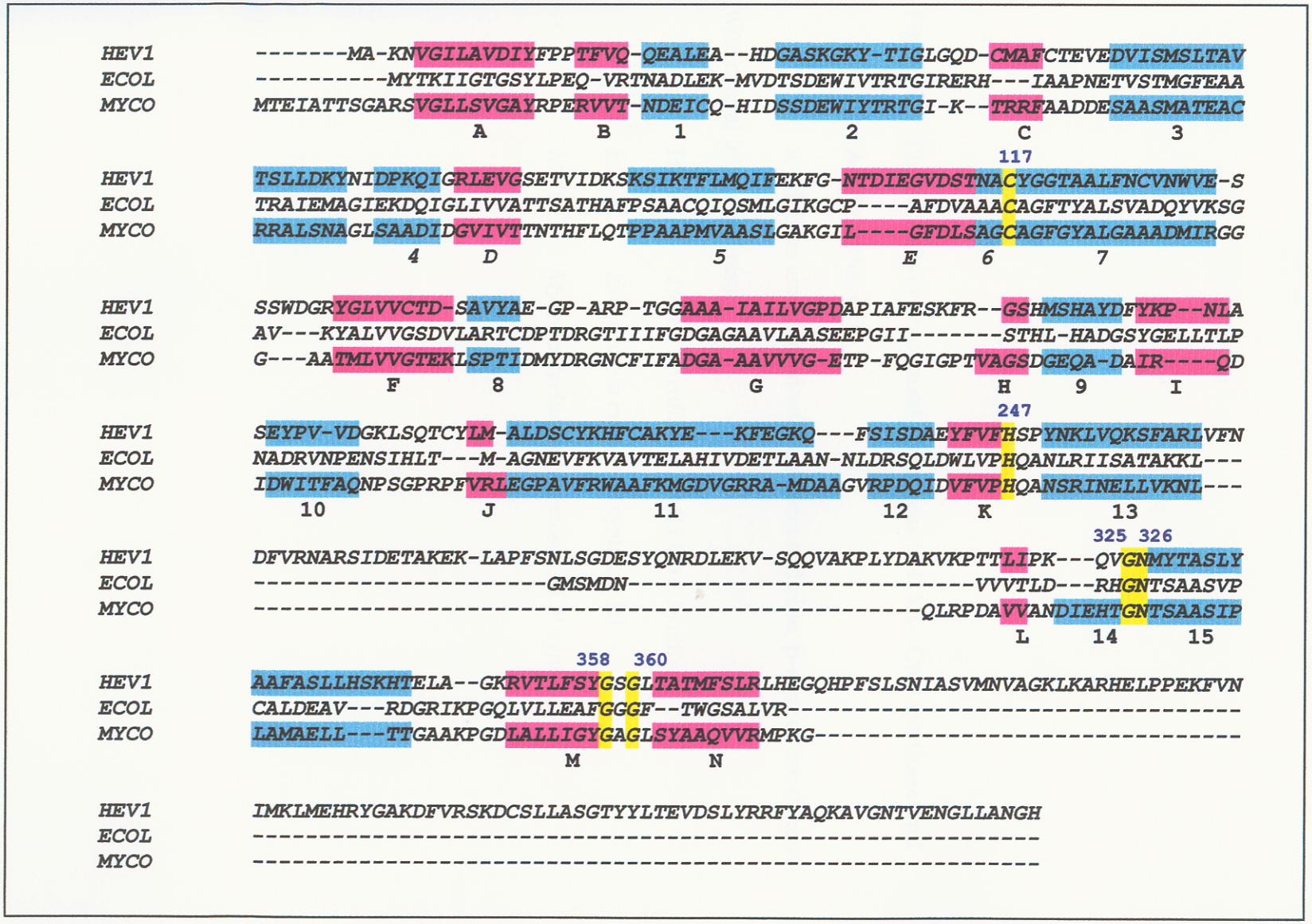
HEV1 -------MA-KNVGILAVDIYFPPTFVQ-QEALEA--HDGASKGKY-TIGLGQD-CMAFCTEVEDVISMSLTAV
ECOL ----------MYTKIIGTGSYLPEQ-VRTNADLEK-MVDTSDEWIVTRTGIRERH---IAAPNETVSTMGFEAA
MYCO MTEIATTSGARSVGLLSVGAYRPERVVT-NDEICQ-HIDSSDEWIYTRTGI-K--TRRFAADDESAASMATEAC
A B 1 2 C 3
117
HEV1 TSLLDKYNIDPKQIGRLEVGSETVIDKSKSIKTFLMQIFEKFG-NTDIEGVDSTNACYGGTAALFNCVNWVE-S
ECOL TRAIEMAGIEKDQIGLIVVATTSATHAFPSAACQIQSMLGIKGCP----AFDVAAACAGFTYALSVADQYVKSG
MYCO RRALSNAGLSAADIDGVIVTTNTHFLQTPPAAPMVAASLGAKGIL----GFDLSAGCAGFGYALGAAADMIRGG
4 D 5 E 6 7
HEV1 SSWDGRYGLVVCTD-SAVYAE-GP-ARP-TGGAAA-IAILVGPDAPIAFESKFR--GSHMSHAYDFYKP--NLA
ECOL AV---KYALVVGSDVLARTCDPTDRGTIIIFGDGAGAAVLAASEEPGII-------STHL-HADGSYGELLTLP
MYCO G---AATMLVVGTEKLSPTIDMYDRGNCFIFADGA-AAVVVG-ETP-FQGIGPTVAGSDGEQA-DAIR----QD
F 8 G H 9 I
247
HEV1 SEYPV-VDGKLSQTCYLM-ALDSCYKHFCAKYE---KFEGKQ---FSISDAEYFVFHSPYNKLVQKSFARLVFN
ECOL NADRVNPENSIHLT---M-AGNEVFKVAVTELAHIVDETLAAN-NLDRSQLDWLVPHQANLRIISATAKKL---
MYCO IDWITFAQNPSGPRPFVRLEGPAVFRWAAFKMGDVGRRA-MDAAGVRPDQIDVFVPHQANSRINELLVKNL---
10 J 11 12 K 13
325 326
HEV1 DFVRNARSIDETAKEK-LAPFSNLSGDESYQNRDLEKV-SQQVAKPLYDAKVKPTTLIPK---QVGNMYTASLY
ECOL ----------GMSMDN----------VVVTLD---RHGNTSAASVP
MYCO ----------QLRPDAVVANDIEHTGNTSAASIP
L 14 15
358 360
HEV1 AAFASLLHSKHTELA--GKRVTLFSYGSGLTATMFSLRLHEGQHPFSLSNIASVMNVAGKLKARHELPPEKFVN
ECOL CALDEAV---RDGRIKPGQLVLLEAFGGGF--TWGSALVR----------
MYCO LAMAELL---TTGAAKPGDLALLIGYGAGLSYAAQVVRMPKG----------
M N
HEV1 IMKLMEHRYGAKDFVRSKDCSLLASGTYYLTEVDSLYRRFYAQKAVGNTVENGLLANGH
ECOL ----------
MYCO ----------

Figure 32. The secondary structural alignment of HMG-CoA synthase and ACP synthase III

α- Helices are denoted with numbers and blue; β-strands are denoted with capital letters and pink.

HEV1: *Hevea brasiliensis* HMG-CoA synthase

ECOL: *Escherichia coli* ACP synthase III

MYCO: *Mycobacterium tuberculosis* ACP synthase III

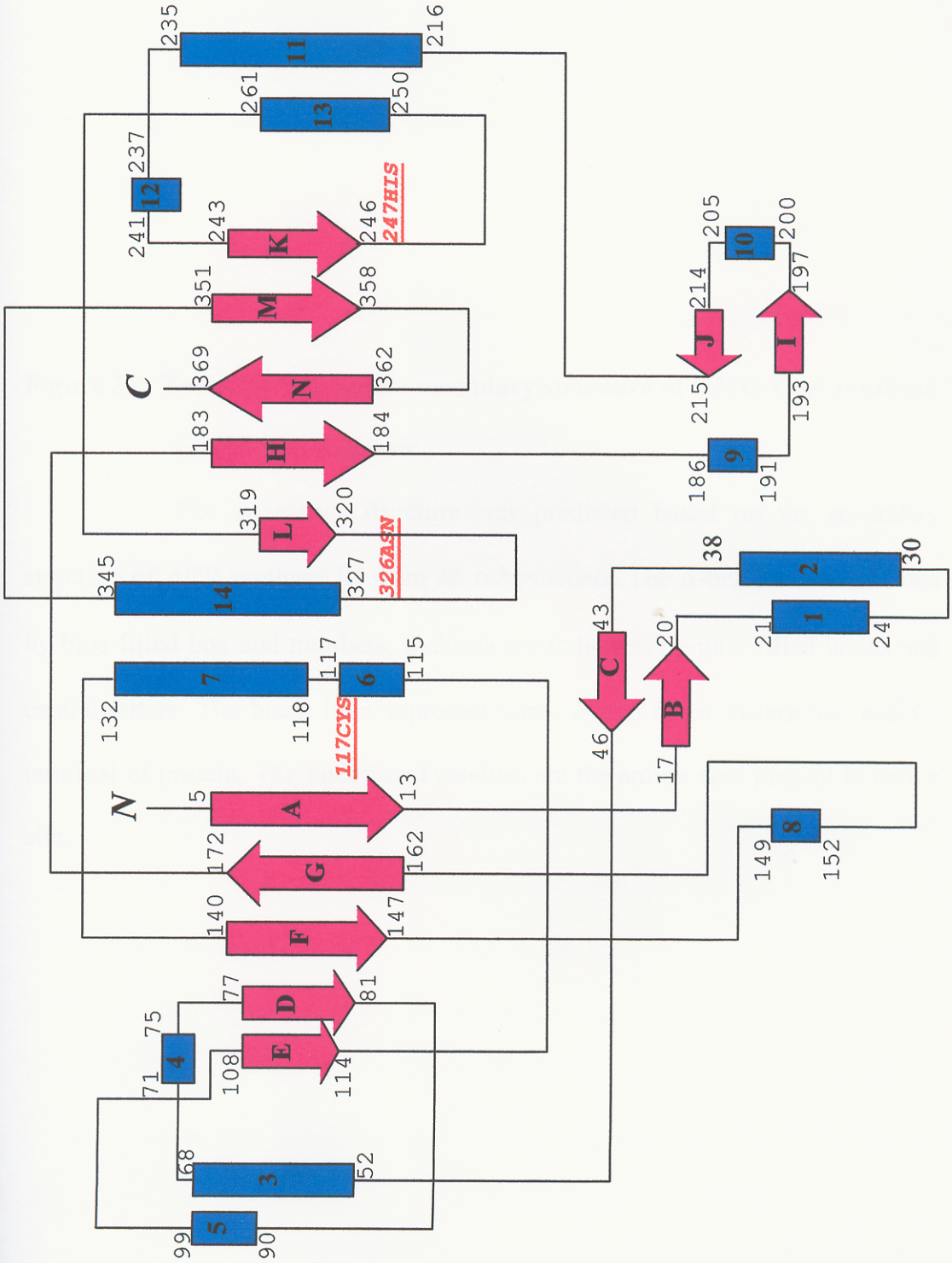
N
C
A
B
C
D
E
F
G
H
I
J
K
L
M
N
1
2
3
4
5
6
7
8
9
10
11
12
13
14
117CYS
326ASN
247HIS

Figure 33. Topology of possible secondary structure of HMG-CoA synthase in *Hevea brasiliensis*.

The secondary structure was predicted based on the secondary structure of ACP synthase III from *M. tuberculosis*. The α-helices are indicated by blue filled box and numbers, β-sheets are indicated by pink filled boxes and capital letters. The black lines represent turns. *N* and *C* are N-terminal and C-terminal of protein. The underlined residues are the amino acid present in active site.