バイオ研究データブック

糸 状 菌 (2006)

Aspergillus
Rhizopus
Mucor
Rhizomucor
Mortierella
Absidia
Phycomyces
Penicillium
Acremonium
Trichoderma
Neurospora
Fusarium
Magnaporthe
Cryptococcus
Phanerochaete
Pleurotus
Lentinula
Schizophyllum
Coprinus
Ustilago

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Japan Society for Molecular Biology of Filamentous Fungi

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平成17年度　麹・麹菌の研究業績

酒類

2．麹及び麹菌

中村ら[1]は白麹菌から細胞表層タンパク質の候補CwpAを取得した。本遺伝子のコードするタンパク質のN末端及びC末端には疎水性領域が存在し，GPIアンカー型タンパク質の特徴的な構造を示していた。本タンパク質は細胞分画により膜画分に存在し，PI-PLC処理を行わない場合には界面活性剤層に抽出され，PI-PLC処理を行い，リン脂質部分を切断すると水槽に移行した。またGPI付加シグナルの直前にストップコドンを人工的に導入すると，細胞に保持されず分泌された。以上の結果より，CwpAはGPIアンカータンパク質であることが示された。さらに，GFPを付加したCwpAは細胞表層に蛍光を示したことから，細胞膜にGPIを介してアンカリングされていることが示された。

恩田ら[2]はバクテリオシン産生乳酸菌3株の製麹工程への応用試験を実施した。製麹時に蒸米に麹菌とともに *Lc. lactis* GM 005を添加したところ *Lc. lactis* GM 005は良好に増殖し，バクテリオシンを産生した。麹中の *Lc. lactis* GM 005の生育は，麹菌の生育には影響しなかった。*Lc. lactis* GM 005添加により作成された麹は抗菌活性を有し，*B. subtilis* JCM 1465^{T}の生育を阻害した。バクテリオシン生産菌 *E. faecium* MR 006と *E. durans* MY 411を用いた場合も，同様に汚染菌レベルの少ない製麹が可能であった。

土佐ら[3]は数種類のアミノ酸を製麹時に添加し，黄麹菌のユビキノン生産性（Q-10（H_2）量）について検討を行った。その結果，芳香族アミノ酸，特にPheが影響を与えた。対照区と比較してPheの添加量が多くなるに従って麹あたりの菌体量は減少した。麹あたりのQ-10（H 2）量は対照区と比較して，添加量が5，10，20 mol区でそれぞれ約1.4倍高い値を示した。次にPheとリンゴ酸Naの併用効果について検討した結果Pheの添加量が多くなるにつれて菌体量も減少したが，麹あたりのQ-10（H 2）量は対照区よりも10 mmol Pheと10 mmolリンゴ酸Naを併せて添加した試験区が1.6倍高くなった。また，この麹の酵素力価も測定したところ，AAaseとGAaseは対照区よりも高くなり，リンゴ酸Naの併用は効果があるものと推察された。

麹菌 *Aspergillus oryzae* RIB 40には *Micrococcus luteus* K-3株由来耐塩性グルタミナーゼと一次構造が約40%の相同性を持つグルタミナーゼ（*Micrococcus* 型グルタミナーゼ，AoGls）をコードする遺伝子AoGlsAが存在する。増尾ら[4]はAoGlsのC末端側にヒスチジンタグとV 5-エピトープタグを融合させ（タグ融合型AoGls）精製した。精製したタグ融合型AoGlsの酵素特性への影響をタグの付いていないAoGlsと比較することにより検討した。

北本ら[5]はキシラン分解酵素遺伝子群の誘導発現因子（XlnR）をコードするAoxlnR遺伝子の一部分を麹菌形質転換ベクターpYRG 100に組み込んだ *AoxlnR* 遺伝子破壊用ベクターpDisXR 100を構築し，REMI（Restriction Enzyme Mediated Integration）法を用いて *AoxlnR* 遺伝子が破壊された麹菌を取得した。*AoxlnR* 遺伝子破壊麹菌では，キシロシダーゼ活性およびキシラナーゼ活性が著しく低下していた。

北本ら[6]はデンプン分解酵素遺伝子群誘導発現因子（AmyR）をコードする *amyR* 遺伝子の内部に *pyrG* 遺伝子を組み込んだ *amyR* 遺伝子破壊用ベクターpDisAmR 100で麹菌を形質転換し，*amyR* 遺伝子破壊麹菌を取得した。*amyR* 遺伝子破壊麹菌ではタカアミラーゼ，グルコアミラーゼおよびα-グルコシーダーゼの各遺伝子の発現量が著しく低下し，デンプンを炭素源とする液体培養ではα-アミラーゼ活性およびグルコアミラーゼ活性は検出されなかった。

麹菌 *A. oryzae* RIB 40 ゲノムには 3 M 食塩存在下でも約 50%の残存活性を示す耐塩性グルタミナーゼ (AoGls) をコードする *AoglsA* が存在する。吉宗ら[7]は *AoglsA* を酵母 *Saccharomyces cerevisiae* で大量発現させたところ，細胞壁と細胞膜を含む画分に結合した遺伝子産物が得られた。一方，大腸菌 *Escherichia coli* を宿主とした場合，Aogls は菌体内可溶性画分に大量発現したことから，タンパク質発現の宿主による違いを比較した。

Feng ら[8]は *A. oryzae* の膜面液体培養で特に発現している 22 個の遺伝子を同定した。これらの遺伝子の中にはセリン／スレオニン kinase (*aopk1*) やフォスファターゼ (*aoppt*) などが含まれていた。また，*aopk1* は GenBank に登録されている配列から予測されるアミノ酸配列より N 末端が 150 アミノ酸長いタンパク質をコードしていることが明らかとなった。

Suzuki ら[9]は寒天培地上に生育させたコロニーから FTA カードを用いてゲノム DNA を抽出し，これを鋳型として PCR によるハイスループットな遺伝子型判定方法を開発した。本法は他の菌類でも寒天のスラントや食材からの検出に応用できた。これにより有機溶媒や特別な設備を使用しなくとも糸状菌の遺伝子型判定が可能である。

Uchida ら[10]はフィターゼ遺伝子と pNAN 8142 からなるプラスミドを *A. oryzae* RIB 40 の niaD-株に組み込んだ。デンプンを唯一の炭素源とし，ポリビニルピロリドンを含んだ培地で形質転換体を培養したところ，培地中にフィターゼ活性が測定され，pH 5.5, 37°C で最大活性値 2.0 units/ml を示した。

Kirimura ら[11]は *Aspergillus niger* のクエン酸生産性の指標とするためにオルタナティブオキシダーゼ遺伝子 (*aox1*) に蛍光タンパク質 EGFP の遺伝子を融合し，この融合タンパク質 AOX-EGFP の発現および局在の解析を行った。EGFP の蛍光はミトコンドリア染色試薬の蛍光と一致し，AOX-EGFP がミトコンドリアに局在することが確認された。アンチマイシンを添加した際に AOX の酵素活性が上昇し，かつ EGFP の蛍光強度も上昇していた。また，EGFP の蛍光強度は培地中のグルコース濃度によって変動しなかった。

Oshima ら[12]は Aspergillus sojae 由来の endo-1,4-β-D-xylanase を利用して，アラビノキシランと D-プシコースから糖転移反応によりキシロシルプシコース (XP) を合成することに成功した。糖の組成と分子量を決定するために HPLC で構造を解析し，結合様式を決定するために GC-MS によるメチル化解析を行い，さらに NMR 解析によりグリコシド結合のアノマー構造を解析した。

Ku 遺伝子群は非相同 DNA 組換え経路で重要な役割を果たしている。Takahashi ら[13]は *A. sojae* と *A. oryzae* から *Ku70* 遺伝子及び *Ku80* 遺伝子のホモログを単離し，これらの単独破壊株及び二重破壊株作成した。*Ku70*, *Ku80* とも DNA に損傷を与えるような薬剤に対して感受性にはならなかった。さらに，増殖遅延や分生子形成が悪くなるなどの望ましくない形質は観察されなかった。

1,2-α-マンノシダーゼはタンパク質に結合した N 型糖鎖の 1,2-α-マンノースを特異的に切断する。Akao ら[14]は *A. oryzae* の固体培養より調整した cDNA ライブラリから 1,2-α-マンノシダーゼにホモロジーのある新規な DNA 配列を取得した。*fmanIB* と名付けたこの遺伝子の cDNA は 510 アミノ酸からなるタンパク質をコードしており，class I α マンノシダーゼの共通モチーフが全て保存されていた。麹菌を宿主として全長 c DNA を発現させたところ，細胞内及び細胞外でマンノシダーゼ活性が上昇した。発現した FmanIBp タンパク質は 1,2-マンノビオースを特異的に加水分解し，至適 pH 5.5, 至適温度 45°C であった。また，$Man_9GlcNAc_2$ を基質として反応させた場合，$Man_5GlcNAc$ が最終産物として生成した。本タンパク質の細胞内局在を調べるために，GFP との融合タンパク質を作成して顕微鏡観察した結果，菌糸内の小胞に蛍光が観察された。これらの酵素学的観察及び顕微鏡観察の結果から，FmanIBp がほ乳類のゴルジ型マンノシダーゼアイソザイムであると結論づけた。

Aspergillus 属の CCAAT 結合タンパク質は Hap 複合体として知られており，少なくとも 3 つのサブユニット (HapB, HapC, HapE) から構成されている。Hap のサブユニットには進化的に保存されたコアドメインが存在している。Tanoue ら[15]は *A. oryzae* の HapE サブユニットの遺伝子を一部欠損させることで，遺伝子発現の活性化に必要な領域を探索した。この解析により，他の Hap との保存性の低い領域や *S. cerevisiae* の Hap 4 p recruiting domain と相同性の高い領域は転写活性化に必要でないことが判明した。

麹菌のグルコアミラーゼをコードする *glaB* 遺伝子のプロモーターは固体培養において組み換えタンパク質の分泌に有用である。Isida ら[16] は *glaB* プロモーターのシスエレメントを含む 97 bp の断片を，固体培養でほとんど発現しない *glaA* 遺伝子のプロモーターに導入したところ，本キメラプロモーターは固体培養において 24 倍の活性を示した。またこの 97 bp の断片をタンデムに 8 コピーつなげたプロモーターでは固体培養においてさらに 4.6 倍の活性を示した。この改変したプロモーターで *glaB* を高発現したところ，2 日間で 1524 mg/kg 培地のグルコアミラーゼが得られた。以上のことから改変した *glaB* プロモーターは固体培養でのタンパク質生産に有用であるといえる。

Mabashi ら[17] はマルチサイトゲートウエイシステムを用いた迅速で効率的に発現ベクターを作成できる多用途ベクターを開発した。このシステムは 3 つの断片を同時に，しかも規定したとおりの方向と順番でベクター中に導入できる。これによりプロモーター，融合タグ，選択マーカー等の組み合わせで 1 つのタンパク質につき 80 種類の発現プラスミドを作成することが可能である。このシステムを利用して，クエン酸合成酵素 (AoCit 1) のミトコンドリアへのシグナルペプチドを融合させた EGFP を発現するプラスミドを作成した。このプラスミドを発現させた株では，EGFP によりミトコンドリアの管状構造が可視化された。本システムを利用することで煩雑な DNA 操作なしで多くの発現プラスミドを作成することができる。

Koseki ら[18] は *Aspergillus* 属のタイプ A フェルロイルエステラーゼのアミノ酸配列中にユニークな N-糖鎖結合モチーフ (Asn 79-Tyr-Thr) を見出した。*Pichia pastoris* で発現させた *Aspergillus awamori* のフェルロイルエステラーゼ (AwFAEA) の生化学的特性に関する，フラップの機能，すなわち，そのフラップ領域に存在する N-結合オリゴ糖の役割を，サイトダイレクト変異法により N-糖鎖結合認識部位を除去することによって解析した。アスパラギン (N) 79 をアラニン (A) 又はグルタミン (Q) に置換した。N-糖鎖がない N 79 A 及び N 79 Q 変異酵素は，α-ナフチルブチレート (C 4)，α-ナフチルカプリレート (C 8) 及びフェノリック酸メチルエステルに対する活性が，糖鎖が結合している野生型のリコンビナント AwFAEA 酵素と比較して，活性が低下した。変異酵素の速度論的解析から，触媒効率の低下は，N 79 A の Km 値の上昇及び k_{cat} の低下，及び N 79 Q の k_{cat} の著しい低下によっていた。N 79 A 及び N 79 Q 変異酵素は，また野生タイプの酵素と比較して熱安定性が著しく低下した。

UDP-N-アセチルグルコサミン：α-3-D マンノシド β-1,2-N-アセチルグルコサミニルトランスフェラーゼ I (GnT-I) は高マンノースタイプのオリゴ糖をハイブリッド又は複合型に変換するために必須である。Kasajima ら[19] はラットの GnT-I の全長 cDNA を *A. oryzae* に導入し発現させた。本組み換え体のミクロソーム画分から GnT-I の活性として，N-アセチルグルコサミンから $Man_5GlcNAc$ に転移する反応が検出された。またこの組み換え体の α アミラーゼの N 結合型糖鎖は HPLC 解析において，従来とは異なる保持時間を示したが，この保持時間は *in vitro* で GnT-I 反応を行った場合と同じであった。このピークは β-N-アセチルグルコサミニダーゼ処理により消失した。さらに質量解析を行った結果，本組み換え体の α アミラーゼの糖鎖として $GlcNAcMan_5GlcNAc_2$ が存在することが支持された。GnT-I と GFP とのキメラタンパク質を *A. oryzae* 内で発現させたところドット状のパターンの蛍光が観察されゴルジ小胞に局在していることが示唆された。これらのことから著者らは GnT-I は *A. oryzae* の細胞中で発現させても機能を保持していることが判明した。

Aspergillus nidulans は 16 のデンプン分解酵素遺伝子を有している。Nakamura ら[20] はこれらの遺伝子発現を調べた結果，8 つの遺伝子がイソマルトースにより誘導されることを明らかにした。この誘導には転写制御因子 AmyR が必要であり，上記 8 遺伝子のプロモーターには AmyR 結合配列が存在していた。さらに，マルトースではこれらの遺伝子発現は誘導されなかった。16 遺伝子中 13 遺伝子の mRNA は炭素源飢餓により誘導された。これは CreA 依存的な発現抑制からの脱抑制が主たる原因であったが，5 遺伝子は *creA* 変異株でも発現が上昇していた。以上のことから，炭素源飢餓においては CreA 依存的及び非依存的な機構がデンプン分解酵素遺伝子の発現上昇に関わっている。

解説・総説には秋田の[21]「麹菌ゲノム解析プロジェクトの概要とポストゲノム研究」，山本ら[22] の「麹菌に

も有性世代がある？―ゲノム解析から明らかになったこと―」，阿部[23]の「温故知新―麹菌ゲノム科学が開く，醸造産業の未来」，山下ら[24]の「清酒麹菌の分子育種に関する研究」，北本ら[25]の「ポストゲノム時代を迎えた麹菌」，正路ら[26]の「麹菌を用いた糸状菌細胞生物学の進展」，織田ら[27]の「プロテオミクスで麹菌を照らす」，水谷ら[28]の「糸状菌の形態と細胞壁合成酵素」，北本ら[29]の「醤油の色を麹菌酵素が変える？」，尾関ら[30]の「麹菌は宝の山です！」，小路ら[31]の「麹菌の液体培養による酵素高生産と焼酎製造」などがある。

関連する特許としては，杉山ら[32]の「抗腫瘍物質，これを用いた飲食品及び抗腫瘍物質の製造方法」，佐野[33]の「肝機能強化性健康食品」，廣瀬ら[34]の「日焼け止め用化粧品」，廣瀬ら[35]の「美白作用を有する組成物およびそれを含有する化粧料」，田邊ら[36]の「ACE阻害剤」，石田ら[37]の「アスペルギルス・オリザの新規変異株及び選択マーカー」，廣瀬ら[38]の「老化防止組成物およびそれを含有する化粧料ならびに飲食品」，石田ら[39]の「麹菌のフェリクローム又はそのデフェリ体生合成に関与するオルニチンN5-オキシゲナーゼ遺伝子」，平野[40]の「麹用の包装材及び麹の包装体」，石田ら[41]の「フコース特異的レクチン」，高橋[42]の「相同組換え頻度が上昇した形質転換菌」，杉本ら[43]の「液体麹と固体麹を併用した酒類の製造方法」，小笠原ら[44]の「玄米麹の製造方法，及び該玄米麹を用いる食酢の製造方法」，葛西ら[45]の「抗真菌物質の製造方法」，古林ら[46]の「鉄吸着性高分子物質及び鉄含有高分子物質ならびにこれらの製造方法」，山田ら[47]の「特定の麹菌の同定方法」，山中ら[48]の「新規麹菌及びその用途」，坂本ら[49]の「生存性の低い胞子を作る糸状菌の作成方法」，佐野ら[50]の「肝機能強化性健康食品」，五味ら[51]の「新規乳酸脱水素酵素遺伝子及び乳酸の製造法」，丸山ら[52]「の「プロスタサイクリン生成促進剤」，佐藤ら[53]の「抗マラリア薬有効成分としてのグリオトキシンの新規用途，及びグリオトキシンを有効成分とする抗マラリア薬。」，吉田ら[54]の「血中乳酸値上昇抑制組成物及びそれを含有する飲食品」，鈴木ら[55]の「ポリペプチドの結晶及び阻害薬の設計方法」，楠ら[56]の「α-グルコシダーゼ阻害剤」，山下ら[57]の「ドラム型棚式製麹機」，中川ら[58]の「胞子飛散防止麹搬送装置」，南條ら[59]の「糖化タンパク質の分析装置および方法」，渡部ら[60]の「酸化褐変や加熱増色が低減された色の淡い醤油の製造法」，田中ら[61]の「醤油の製造法」，松田ら[62]の「中性トレハラーゼ遺伝子破壊麹菌」，安田ら[63]の「紅麹を用いた薬剤及びその製造方法」，渡辺ら[64]の「発酵食品用種麹及び該種麹を用いる発酵食品の製造法」，石田ら[65]の「アスペルギルス・オリザの新規変異株及び選択マーカー」，中田ら[66]の「F-91636化合物及びその製造方法」などが公開された。

2．麹及び麹菌

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味噌

(b) 麹　菌

産業技術総合研究所[21]は，わが国において，清酒や味噌，醤油等各種発酵食品に広く使用されている麹菌遺伝子のヌクレオチド配列解析の結果，37 Mb（ミリオン塩基対），遺伝子数12,000が予測されるとした。また，麹菌ゲノム（麹菌が持っている全遺伝情報一式）解析の方法，麹菌ゲノムヌクレオチド配列及び構造の特徴について背景を含めて概説し，今後のゲノム情報の利用についても言及した。柏木[22,23]は，①発酵食品と麹菌，②ゲノム情報とは何か，③麹菌ゲノム解析に何が期待されるか，④麹菌が実際に発現している遺伝子をcDNA（相補的DNA）として捉え，そのESTを決定する，麹菌のEST（末端塩基配列）解析，⑤ゲノムサイズ，遺伝子数確認のための麹菌ゲノムの解読，⑥麹菌ゲノムの特徴，⑦ポストゲノムシーケンス研究などについて解説した。

関連する特許として，味噌等の発酵食品の製造において，抗変異原性等を示す生理活性物質として知られる遊離脂肪酸や脂肪酸エチルエステルの含有量が高いものを製造するに適した麹菌 *Aspergillus oryzae* を選抜し，該麹菌（AOK 138株または139株）を用いた発酵食品の製造法を提供することを特徴とする渡辺ら[24,25]の「発酵食品用種麹及び該種麹を用いる発酵食品の製造法」が公開された。

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醤油

(2) 製　麹

インドネシアの醤油様調味料ケチャップの製造は，蒸大豆に自然に生育してくる *Aspergillus* 属菌などにより大豆麹を製造することから始まる。*Aspergillus* 属糸状菌はカビ毒アフラトキシンを生産する。SULISTYOら[23]は，安全性確保のため，アフラトキシン非生産麹菌 *Aspergillus* K-1から紫外線照射により，誘発した突然変異K-1A系統を用いて種菌培養を行った。種菌培養により，製造過程が通常法（種菌なし）よりも3日間短縮した。培養中に接種系統K-1Aは白い分生子ができるので汚染 *Aspergillus* 属菌と区別できる。ケチャップ麹における汚染された *Aspergillus* 属菌の数は，種菌培養により1/10まで減少した。

総説，解説として，田中[24]の「培養装置の開発とその実用的利用」，中台[25~27]の「醤油麹菌の培養条件による酵素生産（その1～3）」，飯野[28]の「麹；お呪いから育種へ」がある。

関連する特許として，鶴見[29]の「円形発酵槽の撹拌装置」，藤原ら[30]の「回転円盤固体培養装置における培養基質の培養方法」，玉井ら[31]「たまり醤油醸造用麹の製造方法」，山下ら[32]の「ドラム型棚式製麹機」，中川[33]の「胞子飛散防止麹搬送装置」が公開され，斎藤ら[34,35]の「円盤式自動製麹装置の側板洗浄方法および装置」と「サニタリ性に優れた回転式自動製麹装置」，京ら[36~38]の「中心円筒構造および洗浄装置を備える円盤式自動製麹装置」と「出麹装置および昇降装置を備える回転式自動製麹装置」と「自動製麹装置の連続運転装置」，大川ら[39]の「サニタリ性に優れた製麹装置」，荒木ら[40]の「自動製麹装置の通風方法および装置」，藤原ら[41]の「回転円盤固体培養装置における培養基質の培養方法」，田中ら[42]の「固体培養装置における培養原料の盛込装置」，関口[43]の「米糠麹の製造方法」が公告された。

(3) 微 生 物

(a) 麹　菌

SUZUKIら[44]は，寒天プレート上に生育したかびコロニーから複製連鎖反応（PCR）のためのゲノムDNA鋳型の抽出にFTAカードを使用することにより，*A. oryzae* の高効率ジェノタイピングを開発した。この方法を寒天スラントと食品物質からその他のかびの種を検出することに応用し，この方法が有機溶剤や特殊な器具を使用しないで，糸状かびのジェノタイピングのために便利な道具を提供するものとした。

解説として，水谷[45]の「糸状菌の形態と細胞壁合成酵素」，増田ら[46]の「醤油麹菌 *A. sojae* におけるアフラトキシン合成経路の機能不全」，中台[47]の「醤油麹菌の分類とアフラトキシン非生産と安全性」，北本[48,49]の「麹菌ゲノム解析からわかったこと」と「ポストゲノム時代を迎えた麹菌」，正路ら[50]の「麹菌を用いた糸状菌細胞生物学の進展」，北本[51]の「醤油の色を麹菌酵素が変える？」，柏木[52,53]「麹菌ゲノム情報の解読とそれに期待されること」と「発酵食品と麹菌のゲノム解析」，産業技術総合研[54]の「麹菌のゲノム科学と産業利用　日本の伝統的発酵産業の発展に寄与」，BENTLEY[55]の「味噌，酒および醤油から化粧品へ：コウジ酸科学時代」がある。

関連する特許として，渡部ら[56]の「酸化褐変や加熱増色が低減された色の淡い醤油の製造法」，田中ら[57]の「醤油の製造法」が公開された。

（4） 酵素・遺伝子

増尾ら[71]は，*Micrococcus* 型グルタミナーゼ（AoGls）のC末端側にヒスチジンタグとV5-エピトープタグを融合させ（タグ融合型AoGls），均一に精製した。麹菌 *A. oryzae* RIB 40 には *Micrococcus luteus* K-3株由来耐塩性グルタミナーゼ（*Micrococcus* グルタミナーゼ）と一次構造が約40%の相同性をもつグルタミナーゼAoGlsをコードする遺伝子 Ao*glsA* が存在する。精製したタグ融合型AoGlsはAoGlsのそれと異なる性質を示した。ここではタグ融合によるAoGlsの酵素特性への影響をタグの付いていないAoGlsと比較することにより検討した。ヒスチジンタグとV5-エピトープタグの融合により，AoGlsの温度および食塩に対する安定性が低下した。酵素にタグを融合する際にはその効果を検討する必要があるとした。

YANOら[72]は，*M. luteus* K-3からの耐塩性グルタミナーゼ（*Micrococcus* グルタミナーゼ）の構造に基づく変異分析により，3つのアミノ酸残基，S 64，K 67，E 160が触媒反応に必須であることを明らかにした。この結果は，*Micrococcus* グルタミナーゼが *Pseudomonas* 7Aからのグルタミナーゼ-アスパラギナーゼより，むしろクラスAの β-ラクタマーゼと同様と考えられる触媒機構をもつことを示唆した。

吉宗ら[73]は，*A. oryzae* RIB 40由来耐塩性グルタミナーゼの大量生産について検討した。麹菌 *A. oryzae* RIB 40のゲノムには3M食塩存在下でも約50%の残存活性を示す耐塩性グルタミナーゼ（AoGls）をコードする遺伝子 Ao*glsA* が存在する。Ao*glsA* を酵母 *Saccharomyces cerevisiae* で大量発現させたところ，細胞壁画分に結合した遺伝子産物が得られた。一方，大腸菌 *Escherichia coli* を宿主とした場合，AoGlsは菌体内可溶性画分に大量発現することを認めるなど，Ao*glsA* の発現に関するそれら宿主の違いを比較した。

YOSHIMUNEら[74]は，食品たん白質製造工程でのL-グルタミン酸の生成を増加させる新しい方法を開発した。日本の温泉で分離した好酸性，好熱性細菌である *Alicyclobacillus* F-62を耐酸性で耐熱性のグルタミナーゼ生産菌として選択した。L-グルタミンからのL-2-ピロリジン-5-カルボン酸（L-ピログルタミン酸）の非酵素的生成を最小にするpHと温度条件を調べ，L-グルタミン酸生成を最大にするために必要なpH，温度およびグルタミナーゼの最適条件を検討した。醤油の発酵工程においてL-ピログルタミン酸への非酵素的変換を防ぐことにより，グルタミナーゼによるL-グルタミンからL-グルタミン酸の生成量を最大にする方法を確立した。*Alicyclobacillus* F-62のグルタミナーゼを用いてL-グルタミン酸を増加させる最適条件は，pH 5.0，温度30°Cであった。この条件下で1mMのL-グルタミンから，0.96mMのL-グルタミン酸と0.03mMのL-ピログルタミン酸が得られたという。

麹菌のグルタミナーゼは耐塩性でなく，醤油醸造時の18%食塩存在下では，その活性はごくわずかであるため遊離されたグルタミンのすべてがグルタミン酸とはならず，化学的に無味のピログルタミン酸になり，旨味に十分寄与できないとされている。鈴木ら[75]は，γ-グルタミルトランスペプチダーゼ（GGT）がグルタミナーゼ活性をもち，枯草菌のGGTが極めて耐塩性であることを見出したことから，枯草菌のGGTをグルタミナーゼとして醤油醸造時に添加することを検討した。GGT無添加の場合，醸造開始98日目にグルタミン酸の蓄積が65mMであったものが，4.1U/g諸味の野生型GGT添加系では115mMになった。醸造開始98日後に醤油を搾り，呈味性試験をしたと

ころ，10人のパネルメンバーのうち9人までがGGTを添加した方が旨味が強く，おいしいと答えた。このことから醤油醸造時の諸味に枯草菌由来のGGTを添加することは醤油中のグルタミン酸濃度を上昇させ，呈味性改善に効果があることが明らかとなった。

OSHIMAら[76]は，キシロシルプシコース（XPs）を*A. sojae*からのエンド-1,4-β-D-キシラナーゼ（EC 3.2.1.8）を使用して，アラビノキシラン（AX）とD-プシコース（D-psi）の糖転移反応により合成した。XPsの構造は，糖組成と分子量を決定するためにHPLC，架橋を決定するためにGC-MSを使用してメチル化分析，糖架橋のアノマー立体配置を得るために^{1}H-と^{13}C-NMR分光分析により分析した。XPsの構造は，化学分析により，Xyl*p* β1-4 Psi*p*，Xyl*p* β1-5 Psi*p*，Xyl*p* β1-1 Psi*f*，Xyl*p* β1-6 Psi*f*であることが見出された。

VARZAKASら[77]は，カルボキシフルオレセインの蛍光強度の測定により，大豆によるエンドグルカナーゼとエンドキシラナーゼの取込みを研究した。これらの酵素溶液からの酵素の消失速度は，異なるモデルからの予測と比較した。フリードリッヒのモデルは著者らのデータに最もよく適合した。溶液中の酵素濃度は11日後に顕著には変化しなかったので平衡に達したと考えた。平均質量転移係数はエンドグルカナーゼとエンドキシラナーゼの両者への（C_{lav}-C_l*）に対する取込み速度のプロットから計算し，4×10^{-5} ms^{-1}が得られた。K値は薄片と考えた2つの酵素の効率的な拡散の計算のために使用し，Kは長い滞留時間と漸近的で，4.8×10^{-10} m^2 s^{-1}の値が得られることを見出した。

木村ら[78]は，*A. sojae*の液体培養液からエキソ-1,5-α-L-アラビナナーゼを電気泳動的に均一な標品として精製した。精製酵素標品の分子質量は，ドデシル硫酸ナトリウム-ポリアクリルアミドゲル電気泳動（SDS-PAGE）によって41 kDa，ゲルろ過クロマトグラフィーによって43 kDaと決定された。この酵素の等電点は3.7であった。このエキソ-アラビナナーゼ活性はカルボキシメチル（CM）-直鎖アラビナンに対し50°C，pH 5.0で作用させることで最大となった。活性はpH 6.0から8.0，45°Cまで安定であった。精製酵素標品の活性はAg^{+}（1 mM）およびCr^{2+}（1 mM）によって強く阻害され，SDS（5 mM）の添加により促進された。1,5-アラビナンに対するK_m値は5.8 mg/m*l*となった。精製酵素標品の*N*末端アミノ酸25残基は，*Penicillium chrysogenum*のそれと69％という高い相同性を示した。この標品をビート由来の1,5-アラビナンに作用させた場合の主な生成物は，アラビノビオースであり，反応液中にアラビノースの遊離は認められなかった。

MABASHIら[79]は，*A. oryzae*の発現プラスミドの迅速で効率的な調製のために応用できるMultiSiteGateway™に基づく何にでも向くプラスミド構築システムの開発を報告した。このシステムは限定された順番と配向中の目的ベクターと一緒に，侵入クローン中に挿入された3つのDNA断片の同時に結合することを許す。著者らは多様な侵入クローンとプロモーター，担体たん白質と融合タグをコードする遺伝子，選択マーカーを含む目的ベクターを調製し，これが各々の目的タンパク質の80の発現プラスミドを生ずるシステムを作った。このシステムを使用して，クエン酸合成酵素（AoCit 1）のミトコンドリア-目的シグナルと融合したEGFPの発現のためのプラスミドが作られた。ミトコンドリアの微小管構造はAoCit 1-EGFP融合たん白質を発現する形質転換体中に可視化された。このプラスミド構築システムは骨の折れるDNA操作なしに多数の発現プラスミドを調製することでき，*A. oryzae*における分子生物学研究を促進するだろうとした。

TANOUEら[80]は，*A. oryzae*のHapEサブユニットをコードする先端を切った遺伝子のシリーズを，かびの遺伝子の転写促進のために必須の領域を探すために構築した。Hap複合物と知られている*Aspergillus*属かび中のCCAAT結合複合物がHapB，HapC，HapEの少なくとも3つのサブユニットから成り立ち，それぞれのHapサブユニットは進化的に保存された核ドメインを含むこと，*Saccharomyces cerevisiae*のHap 4 p補充ドメインと同一の非保存領域と保存領域はHap複合体が媒介する転写促進のために必要でないことを明らかにした。

ISHIDAら[81]は，*A. oryzae*の固体培養発酵（SSF）中で発現されるグルコアミラーゼをコードする遺伝子（*glaB*）プロモーターの改良を行った。*glaB*プロモーターは*A. oryzae*のSSFにおける形質転換たん白質の生産に非常に有効であるが*glaB*プロモーターの

シス要素を含む97 bpの断片を*glaA*プロモーターに挿入するとSSF中ではわずかしか発現されないことを認めた。このキメラプロモーターはSSFにおいてプロモーター活性で約24倍の増加を示した。97 bpの断片の8コピーが*glaB*プロモーターとタンデムに融合されたが，この改良されたプロモーターはSSFにおけるプロモーター活性の約4.6倍の増加を示した。*glaB*遺伝子はSSFにおいてこの改良された*glaB*プロモーターの調節下で過剰発現され，形質転換グルコアミラーゼの生産は2日で約1524 mg/kgブロスに達した。この改良された*glaB*プロモーターは*A. oryzae*のSSFにおいて形質転換の過剰生産に非常に有効であるとした。

TAKAHASHIら[82]は，麹菌*A. sojae*と*A. oryzae*中の*Ku* 70と*Ku* 80相同物を同定し，*Ku* 70，*Ku* 80，*Ku* 70-80の破壊変異株における表現型の変化の性質を明らかにするために，これらの変異株を構築した。*Ku*遺伝子はDNA 2重鎖の破壊修復において非相同的な末端結合（エンドジョイニング）経路において鍵となる役割を演じており，*Ku* 70と*Ku* 80変異株ともにメチルメタンスルフォン酸（MMS）とフェレオマイシンのDNA損傷試薬に対して著しい感受性を示さなかった。さらに，生育の悪さや分生子形成の阻害のような好ましくない表現型は，*Ku*を破壊した*A. sojae*と*A. oryzae*には観察されなかったことを明らかにした。

北本ら[83]は，キシラン分解酵素遺伝子群の誘導発現因子（XlnR）をコードする*Aoxln*遺伝子に由来する1.6 kbの*Nru* I断片を麹菌形質転換ベクターpYRG 100の*Sma* I部位に挿入して，*AoxlnR*遺伝子破壊用ベクターpDisXR 100を構築した。ターゲッティング効率の高いREMI（Restriction Mediated Integration）法を用いてXlnRをコードする*AoxlnR*遺伝子が破壊された麹菌を取得した。*AoxlnR*遺伝子破壊麹菌ではキシロシダーゼ活性およびキシラナーゼ活性が対照株と比較して約0.2%および検出限界以下へとそれぞれ低下したという。さらに，北本ら[84]は，デンプン分解酵素遺伝子群誘導発現因子（AmyR）をコードする*amyR*遺伝子の*Stu* I - *Xho* I部位に*pyrG*遺伝子を組込んで，*amyR*遺伝子破壊用ベクターpDisAmR 100を構築した。*amyR*遺伝子破壊用ベクターpDisAmR 100で*A. oryzae* KBN 616-P株を形質転換することによって，*amyR*遺伝子が遺伝子置換破壊された麹菌を取得した。*amyR*遺伝子破壊麹菌ではタカアミラーゼ，グルコアミラーゼおよびα-グルコシダーゼの各遺伝子の発現量が著しく低下し，デンプンを炭素源とする液体培養では，α-アミラーゼ活性およびグルコアミラーゼ活性は検出されなかったという。また，北本ら[85]は，醤油の着色を抑制することを目的として取得した*AoxlnR*遺伝子破壊麹菌（Δ*AoxlnR*株）および*amyR*遺伝子破壊麹菌（Δ*amyR*株）の評価を無菌製麹試験および小仕込み試験により実施した。Δ*AoxlnR*株は液体培養ほどではないが，固体培養においてもキシラナーゼ活性およびキシロシダーゼ活性の低下が認められ，セルロース液化酵素活性およびセルロース糖化酵素活性の低下も同時に観察された。Δ*amyR*株は液体培養と同様に固体培養においてもα—アミラーゼ活性，グルコアミラーゼ活性およびα—グルコシダーゼ活性が顕著に低下していた。たん白質分解酵素活性，セルロース分解酵素活性およびキシラン分解酵素活性は増加していた。試醸醤油の成分分析の結果，Δ*AoxlnR*株を使用すれば色の淡い醤油の醸造が可能となり，Δ*amyR*株を使用すれば甘味および旨味が増強され，火入れ近が少ない色の淡い醤油の醸造が可能となると考えられた。濾布を用いた自然垂れ試験を行った結果，植物細胞壁分解酵素の活性が上昇しているΔ*amyR*株の液汁量は対照株およびΔ*AoxlnR*株の約1.2倍となり，Δ*amyR*株は品質向上ばかりでなく圧搾工程の改善にも有用と考えられるとした。

AKAOら[86]は，正式な1,2-α-マンノシダーゼに相同な新規なDNA配列を*A. oryzae*の固体培養から調製したcDNAライブラリーからクローンした。1,2-α-マンノシダーゼはたん白質に架橋した*N*-グリカンからの1,2-マンノースの特異的な分解を触媒する。この*fmanIB*のcDNAは1530ヌクレオチドからなり，510アミノ酸のたん白質をコードし，この中にクラスIのα-マンノシダーゼの全ての共通モチーフは保存されていた。*Aspergillus*を宿主とした1,2-α-マンノシダーゼcDNA全長の発現は，他の糸状菌では稀であるが，*fmanIB*により成功裡にされ，そして，菌体内と菌体外のマンノシダーゼ活性両者の増加を生じた。この発現されたタンパク質（FmanIBp）は最適pH 5.5，最適温度45°Cで，1,2-マンノビオースを特異的

に加水分解した。$Man_9GlcNAc_2$ を基質として，中間の分岐の 1,2-α-マンノース残基の加水分解の間に蓄積された $Man_5GlcNAc_2$ は律速であった。この酵素の菌体内の局在化を試験するため，緑色蛍光たん白質と FmanIBp のキメラたん白質が構築された。これは *Aspergillus* の菌糸内に点々のある蛍光パターンを示し，そしてこれは細胞内の小胞に局在を示した。これらの酵素的と顕微鏡的結果に基づき，FmanIBp は哺乳類のゴルジ体の 1,2-α-マンノシダーゼのアイソザイム IB に対するかびの代用であると推測された。これと *A. oryzae* 中の他の ER タイプのマンノシダーゼの存在に関する前報（吉田ら，2000）は，糸状菌が哺乳類細胞のそれらと同じ N-架橋糖鎖の修飾のステップをもつことの見解を支持した。

KASAJIMA ら[87]は，ラットの UDP-N-アセチルグルコサミン：α-3-D-マンノサイド β-1,2-N-アセチルグルコサミニルトランスフェラーゼ I（GnT-1）の全長を糸状菌 *A. oryzae* で発現させた。GnT-1 は高マンノース型オリゴ糖の混成や複合型への転換に必須の酵素である。形質転換かび（NG 株）からのミクロソーム調製物は，受容体のヘパタオースの $Man_5GlcNAc_2$ へ N-アセチルグルコサミン残基を転移する GnT-1 活性を示した。この株により分泌された α-アミラーゼの N-架橋の糖鎖は，高速液体クロマトグラフィー（HPLC）において *in vitro* の GnT-1 分析の反応生成物と同じ新規な保持時間のピークを示した。このオリゴ糖のピークは β-N-アセチルグルコサミン処理後，HPLC において消失した。質量分析は NG 株からの α-アミラーゼの糖鎖として $GlcNAc_5GlcNAc_2$ の存在を支持した。緑色蛍光たん白質（GFP）と GnT-1 のキメラは，菌糸中で点々のある型を示し，これはゴルジ体に局在することを示唆した。GnT-1 は *A. oryzae* 細胞中に機能的に発現され，そして N-アセチルグルコサミン残基は *in vivo* で α-アミラーゼの N-グリカンへ転移されると結論し，*A. oryzae* は遺伝子改変糖鎖による糖たん白質の生産のための可能性のある宿主として期待されるとした。

UCHIDA ら[88]は，*A. oryzae* フィターゼ遺伝子と pNAN 8142 ベクターにより構築したプラスミドを使用して，*A. oryzae* RIB 40 *niaD*$^-$を形質転換した。炭素源としてでん粉とポリビニルピロリドンを含む培地に生育した形質転換体の培養ブロスは，37℃，pH 5.5 で最大 2.0 単位/m*l* のフィターゼ活性を示したという。

FENG ら[89]は，膜表面液体培養（MSLC）において優先的に発現される *A. oryzae* からの 22 遺伝子を同定し，その内の，Ser/Thr プロテインキナーゼ（*aopk1*）とフォスファターゼ（*aoppt*）遺伝子をクローンした。*aopk1* は GenBank 中に登録された配列から予測されたそれより，N 末端配列が 150 アミノ酸残基長いたん白質をコードすることを明らかにした。

総説，解説として，吉宗ら[90]の「高濃度食塩存在下で働く酵素たん白質の環境適応戦略　旨味増強酵素グルタミナーゼ表面のアミノ酸残基組成の違いに鍵」，織田[91]の「プロテオミクスで麹菌を照らす」，田上[92]の「遺伝子組換え食品に関しての確認事項」がある。

関連する特許として，高石ら[93]の「L-アラビノースの製造方法」，中村ら[94]の「新規なアミダーゼ遺伝子」，仲原ら[95]の「不精臭が低減した飲食品」，山田ら[96]の「特定の麹菌の同定方法」が公開され，バーカーら[97]の「アスペルギルス・オリゼ（*A. oryzae*）における β-ガラクトシダーゼの生産増加」，小畑ら[98]の「α-L-アラビノフラノシダーゼ遺伝子」，アールら[99]の「糸状菌の形態変異体」，柏木ら[100]の「麹菌発現遺伝子プロモーターならびに該プロモーターを利用した外来遺伝子の発現方法」，秦ら[101]の「新規遺伝子，ベクター，それを用いた形質転換体およびその利用」，川戸ら[102]の「アスペルギルス属由来の新規なプロモーター」が公告された。

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日本放線菌学会・糸状菌遺伝子研究会ジョイントシンポジウム
（第 44 回日本放線菌学会講演会・第 27 回糸状菌遺伝子研究会例会）

有用微生物としての放線菌と糸状菌
～基礎から応用まで～

2007 年 2 月 23 日（金）

（北里大学薬学部コンベンションホール）

共催：　日本放線菌学会・糸状菌遺伝子研究会

協賛：　北里大学 21 世紀 COE プログラム

–プログラム–

13:00　　はじめに

武蔵野大学薬学研究所（日本放線菌学会）市瀬浩志

13:05ー13:50　「放線菌からの抗結核活性物質の探索と開発」

五十嵐　雅之　（微生物化学研究センター）

13:50ー14:35　「糸状菌ポリケタイド生合成遺伝子の機能解析」

藤井　勲　（東京大学大学院薬学系研究科）

14:35ー15:20　「鞭毛菌類の収集と保存」

稲葉　重樹

（製品評価技術基盤機構　バイオテクノロジー本部）

休憩（15:20ー15:40）

15:40ー16:25　「ロドコッカス属細菌における発現ベクターの開発と、

同属菌における遺伝子発現制御に向けた技術開発」

中島　信孝　（産業技術総合研究所）

16:25ー17:10　「麹菌のタンパク質大量生産システム」

峰時　俊貴　（大関株式会社　総合研究所）

17:10　　おわりに

酒類総合研究所（糸状菌遺伝子研究会）　山田　修

17:30〜19:30　懇親会　（北里本館　食堂）

放線菌からの抗結核薬の探索

五十嵐　雅之
微生物化学研究センター

WHOの推計によると、世界人口の約32%(20億人)が結核に感染しており、毎年800万人が新たに発病し、200万人が結核により死亡している。これは単独の病原体による死亡としては第一位である[1)]。また、HIV感染者の増加は結核のまん延を加速させ、世界的に深刻な問題となっている。我が国において結核は、1950年まで日本人の死亡原因のトップを占めたが、医療・医学の発展により現在では、死亡原因で20位以下となり、過去の病と考えられるようになっている。しかし結核は「再興感染症」として再び注目すべき疾患となってきている。近年、WHOは、多くの抗結核薬が効かない多剤耐性菌の出現に強い警戒を呼びかけている。「多剤耐性結核菌 (MDR-TB)」とは、リファンピシンやイソニアジドなど２種類の第一選択薬に耐性がある結核菌を指し、さらにカナマイシンやパラアミノサリチル酸など第二選択薬の複数に対し耐性があるものを「超多剤耐性結核菌 (XDR-TB)」と定義している[2)]。

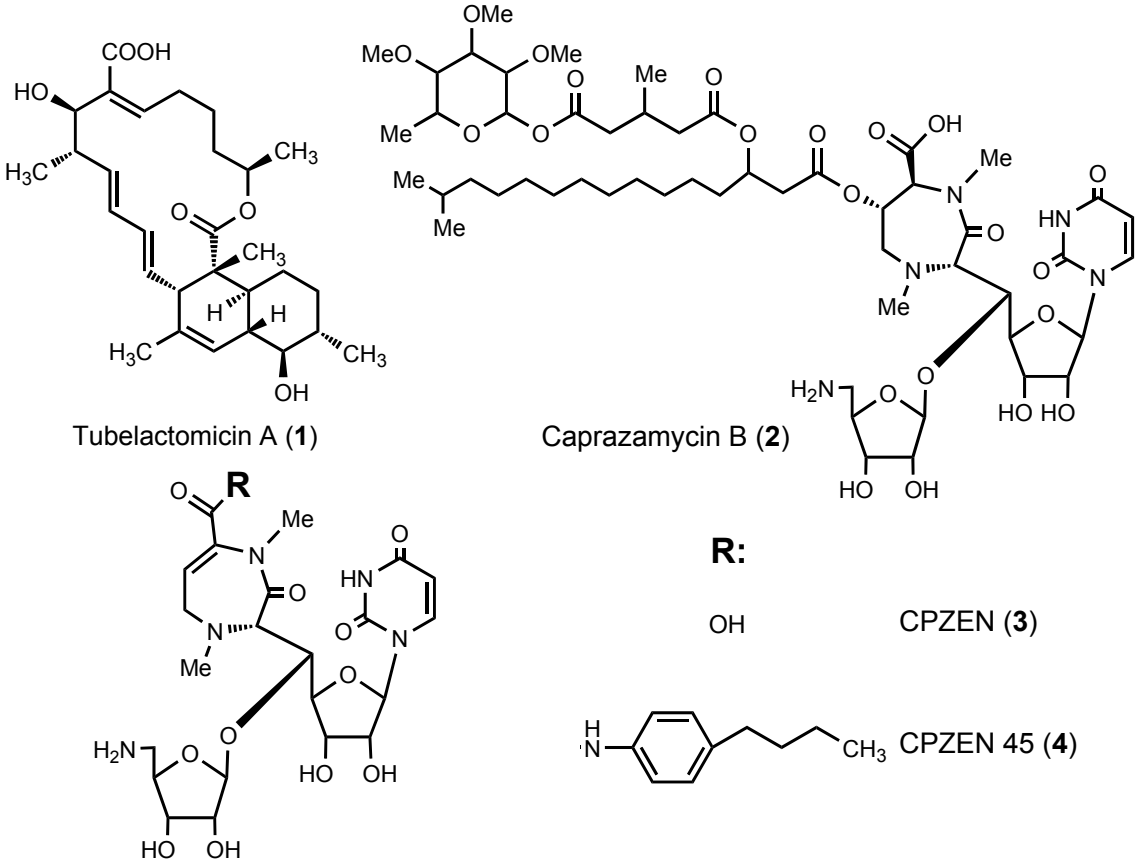
私たちは、現在問題となっているMDR-TB/XDR-TBを含む結核菌に対し特異的に活性を示す有用な化合物を微生物の代謝物から発見すべく探索研究を開始した。その結果、tubelactomicin 類(TLMs)およびcaprazamycin類(CPZs)の系統が異なる２種の有望な化合物を得る事が出来た。これらTLMsおよびCPZsは、いずれも土壌より分離した放線菌（*Nocardia vinacea* MK703-102F1および*Streptomyces* sp. MK730-62F2）の代謝物であった。

TLMsの主成分TLM-A（**1**）は、*Mycobacterium sumegmatis* ATCC607に対し強く且つ選択的な抗菌活性を示した。TLM-Aは、種々の抗結核薬に耐性な抗酸菌に対しても強い抗菌力を示したことより、既存の抗結核薬とは異なる機序を有するものと考えられた。本物質の作用機序は、*Mycobacterium*の脂質合成を特異的に阻害する。さらに、本物質は、イソニアジドと相乗的な効果を示した。

CPZs の主成分 caprazamycin B（CPZ-B, **2**）は、*M. tuberculosis* H37Rv に対し強い抗菌活性を示した。また、本物質は結核菌肺感染モデルマウスに対し優れた治療効果を示し、毒性等もみられなかった。CPZ-B は、CPZs の一成分であり精製に HPLC を必要としたため、共通母核を用いた誘導体の作製を行った。即ち、CPZs をアルカリ加水分解によりアシル側鎖を分離し、共通骨格である CPZEN（**3**）を得た。CPZEN を基に誘導体展開を行

った結果、CPZEN 45（**4**）を発見する事ができた。CPZEN 45 は、CPZ-B よりも優れた抗結核菌活性を有し、結核菌肺感染モデルマウスに対しても有効であった。CPZEN 45 は、現時点で抗結核薬候補として最も有望な化合物と考えている。

本誌ポジウムでは、放線菌から得られた、TLM-A, CPZ-B, および CPZEN45 について生産菌ならびに *in vitro*, *in vivo* 活性と構造活性相関について述べる。



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糸状菌ポリケタイド生合成遺伝子の機能解析

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糸状菌のポリケタイド合成酵素 polyketide synthase(PKS)は、そのほとんどが哺乳類脂肪酸合成酵素 FAS と類似の繰返し型 iterative type I 型であり、ゲノムプロジェクトの進展とも相まってデータベースに登録される糸状菌 PKS 遺伝子数は著しく増大している。しかしながら、その機能、つまり PKS 産物が同定されたものは数少ない。我々は、糸状菌 PKS を異種糸状菌、特に麹菌 *Aspergillus oryzae* を宿主として発現させることにより、芳香族ペンタケタイド合成酵素である *Colletotrichum lagenarium* の PKS1、同ヘプタケタイド合成酵素である *Aspergillus nidulans* の WA、*Aspergillus fumigatus* の Alb1p や、*Phoma* sp.のノナケタイド合成酵素 PNK2 などの発現、機能同定に成功している。WA PKS では、C-末のチオエステラーゼ TE とされていた領域が、単に PKS から生成物を加水分解的に遊離させる TE ではなく、芳香環のクライゼン型閉環に関わるクライゼンサイクラーゼドメイン CYC であることを明らかにした。また、還元型 PKS についても、ソラナピロン生産菌であるバレイショ夏疫病菌 *Alternaria solani* より遺伝子クローニングを試み、*pksN*、*pksF* などの PKS 遺伝子を取得した。*pksN* の *A. oryzae* での発現では、新規化合物アルタナピロンの生産が確認された。アルタナピロンは、デカケタイド鎖にメチオニン由来の8個の側メチルが導入され生成する化合物であり、PKSN 酵素タンパクの中央に存在するメチルトランスフェラーゼ MeT ドメインの働きにより、9回の縮合反応のうち、8回の各縮合反応後にアシルキャリヤープロテイン ACP 上のβ-ケトアシル中間体のα位にメチル基を導入するものと考えられる。また、*pksF*の発現では、PKSF が新規ポリエン化合物アスラニピロン、アスラニオールを主生成物として、11 以上のポリエン化合物を与える興味深い PKS であることを明らかにした。

このように糸状菌発現系は PKS の機能解析系としても、また、化合物生産のための生物合成系としても優れた系であるが、糸状菌の形質転換効率の低さなどが問題点となる。そこで、酵母を宿主とする糸状菌 PKS の発現についても検討を進めてきた。*Aspergillus fumigatus* は、ヒトのアスペルギルス症原因菌であり、その胞子色素生合成遺伝子クラスターに見出された PKS 遺伝子 *alb1* は、ヘプタケタイド YWA1 の合成酵素をコードしている。この Alb1p を *Bacillus subtilis* のホスホパンテテイニル化酵素 Sfp とともに酵母で発現させたところ、YWA1 の生産が確認され、酵母においても糸状菌多環性芳香族 PKS が機能的に発現することが明らかになった。また、Alb1p の CYC ドメイン欠失変異体を発現させると、YWA1 ではなくクライゼン型の閉環を受けず酵素から遊離されたデヒドロシトレオイソクマリン(DHCI)を与えるが、CYC欠失体とその欠失した CYC とを共発現させることにより、YWA1 の生産が回復することを確認した。これは、CYC ドメインが独立の機能タンパクとしてACP 上のヘプタケタイド中間体に作用するCYC 酵素として機能し得ることを示している。

また、*A. fumigatus* 胞子色素生合成遺伝子クラスター中の各遺伝子を順次、酵母に導入することにより胞子色素生合成系を酵母で再構成する試みも進めており、*alb1* と *ayg1* の共発現により、テトラヒドロキシナフタレンが、更に *arp2* を導入することによりシタロンが生産されることを確認している。糸状菌においても、二次代謝産物の生合成遺伝子がゲノム上にクラスターとして存在していることから、その機能同定の手法として、この酵母共発現系が応用できるものと考えられる。

鞭毛菌類の収集と保存

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鞭毛菌類とは、その生活環の一部で鞭毛をもった運動性の胞子（遊走子）を生じる真核従属栄養生物である。遊走子に生える鞭毛の数、位置、種類によって、ツボカビ類、サカゲツボカビ類、卵菌類の 3 分類群に区別される。鞭毛菌類の形態は非常に多様であり、最も単純なものでは宿主組織中に形成された多核の単細胞である。より発達した種類では、生殖器官と仮根からなるものや、分枝した菌糸からなるものがある。

鞭毛菌類は淡水や海水または土壌中の有機物質上で腐生菌もしくは寄生菌として生活する。そのため、天然環境から採取した有機物質上で多くの種を観察することができる。また、釣菌法も鞭毛菌類の分離によく用いられる。釣菌には新鮮な水試料や土壌試料を用いる。釣菌法の目的は、天然に近い条件下で観察できるよう菌類の個体数を次第に増加させ、かつ純粋培養に適した発達段階（例えば遊走子形成）を得ることにある。釣菌法では釣り餌として幅広い有機物質が用いられ、主なものとしてアサの種子、マツ花粉、セロファン、タマネギの皮、昆虫の翅、ヘビの皮、脱脂した毛髪などが挙げられる。釣り餌の種類によって得られる種がある程度決定される。

従来、凍結乾燥・L-乾燥・凍結保存などの長期保存方法は鞭毛菌類には有効ではないとされてきた。しかし、NITE の生物遺伝資源部門(NBRC)では液体窒素を用いた超低温下での鞭毛菌類の長期保存を行っている。低温での保存のためには、前培養法、凍結保護剤の種類、凍結速度および解凍速度が重要となる。このうち、最も重要なのは凍結速度である。急速な凍結は細胞内に氷の核を発達させ、細胞を傷つける。一方、低速で凍結すると、細胞外で著しく氷が形成され、細胞周囲の凍結保護剤の浸透圧が高まるため、脱水と細胞膜の不可逆的破壊をもたらす。約 1 °C/分で冷却することにより、両過程のバランスが最適となり、細胞の生存可能性が高まる。NBRC では、プログラム設定が可能な液体窒素凍結装置を用いることにより、液体窒素中での超低温保存を可能とした。凍結保護剤としては、5%トレハロースと 10%グリセロールの混合溶液を用いている。また、30 °C に設定したウォーターバスに凍結チューブを浸すことによって、比較的速やかに解凍することも重要である。このような鞭毛菌類の長期保存方法の確立は、鞭毛菌類研究の進歩に貢献することと思われる。

ロドコッカス属細菌における発現ベクターの開発と、同属菌における遺伝子発現制御に向けた技術開発

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組換えタンパク質を大量調製するために、大腸菌が宿主として最も広く用いられている。大腸菌以外では、昆虫細胞（Sf9 細胞）、酵母菌、*Pichia pastoris*、バチルス属細菌なども宿主として用いられており、これらは標的組換えタンパク質の性質の違い等によって使い分けられている。しかし、いずれの宿主を用いても、標的組換えタンパク質の生産が困難な場合がある。そこで我々は、利用可能な宿主を増やす目的で、ロドコッカス属細菌を宿主とした組換えタンパク質発現システムを構築することを目指した。ロドコッカス属細菌は、高 GC 含量のゲノムをもつグラム陽性菌で、有機溶媒耐性をもち、脂肪族、芳香族および複素環式化合物などを変換する生体触媒活性も強い細菌である。特に我々が使用しているロドコッカス・エリスロポリスは、4 から 35 度という広範囲な温度域で増殖可能であるという特徴も併せ持ち、これまで使用されている汎用型の宿主とは大きく異なる表現型を有する。これらのことから、ロドコッカス属細菌は次世代宿主候補として世界的に期待されていたが、同属菌の汎用型発現ベクターが利用できる状況には至っていなかった。我々は、１．大腸菌とロドコッカス属細菌とのシャトルベクターで、２．薬剤で発現を厳密に調節でき、３．マルチクローニング部位並びに 6×ヒスチジンタグをコードする配列を持つロドコッカス属細菌用発現ベクター、pTip ベクターを作成し、4 から 32 度までの温度で、様々な組換えタンパク質を相当量生産することができることを証明した。この発現ベクターを基にした発現システムを用いれば、これまでは発現・精製が困難だった有用タンパク質を大量調製することが可能になると考えられる。本講演では、pTip ベクターを用いたロドコッカス属細菌での組換えタンパク質発現システム開発を中心に、pTip ベクターを基にした、組換えタンパク質の多重発現法、ランダム遺伝子破壊法、有用化合物の微生物変換法、遺伝子発現制御法等の開発を報告する。

麹菌のタンパク質大量生産システムの開発

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遺伝子組換え技術により生産された有用タンパク質(酵素)は、医薬用途、産業用途など現在では幅広い分野で利用されており、現代社会に多くの恩恵をもたらしていることは、周知のとおりである。これらの有用タンパク質をより効率的に生産するために、様々な微生物、動植物を宿主とするタンパク質発現系が開発されており、演者らの開発した麹菌(*Aspergillus* 属)を宿主とする発現系もその中の１つである。麹菌を宿主として利用するメリットは、主に２つある。１つは、産業用酵素の多くは麹菌由来であることからも示唆されるように、麹菌は、本質的に多種多様な酵素を生産し、且つその生産能力が他の宿主より優れていることである。もう１つは、麹菌 *A. oryzae* と *A. niger* は、酒、味噌，醤油等の醸造食品の製造に長年にわたって使用されており、菌体及びその生産物の安全性が極めて高いため、物質生産の宿主としても安全であると評価されていることである。

遺伝子工学により目的のタンパク質を生産する場合、その生産効率は、使用するプロモーターによる転写量、転写後の翻訳効率、発現したタンパク質の糖鎖付加などの翻訳後修飾、遺伝子のコピー数、宿主プロテアーゼによるタンパク質の分解など様々な因子によって規制されるが、まず第一に、目的タンパク質の転写量が多くなければ、転写量以上の発現分泌は望むべくもない。この観点から、我々はアミラーゼ遺伝子プロモーターに存在するシスエレメントを利用した改良プロモーター(PagdA142、P-No8142、PenoA142 等)を開発した。また、大量に転写されたmRNA からのタンパク質への翻訳量を増やす試みも重要なポイントの１つである。最近、我々は麹菌においても、5′非翻訳領域(5′UTR)がタンパク質の翻訳効率に重要な役割を担っていることを証明し、翻訳効率を高める 5′UTR を見出した。本講演では、5′UTR と改良プロモーターを組合わせて利用した麹菌のタンパク質高生産システムについて紹介する。

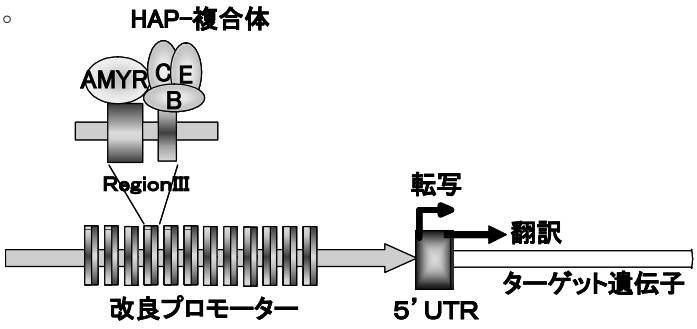

図　麹菌のタンパク質生産システムの発現カセット構成

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第２８回例会　講演要旨

日　時　　平成１７年６月１日（金）

会　場　　北とぴあ　第２研修室　（７Ｆ）

演　題

「麹菌の転写制御因子の網羅的解析」
　　小川真弘（(財）野田産業科学研究所）

「アーバスキュラー菌根共生におけるシグナル物質の同定」
　　秋山康紀（大阪府立大学大学院　生命機能化学分野）

「糸状菌の交配と関連する遺伝子」
　　有江 力（東京農工大学大学院　植物病理学研究室）

「シイタケの遺伝子解析技術の現状と収穫後の子実体で発現が変化する遺伝子」
　　佐藤利次（財）岩手生物工学研究センター微生物利用研究部

麹菌の転写制御因子の網羅的解析

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小川　真弘

麹菌 (*Aspergillus oryzae*) は日本酒、味噌および醤油の醸造など、わが国の伝統的な発酵産業にとって欠かすことのできない微生物であり、産業用酵素の生産にも利用されている。また、本菌は安全性が確認されている生物であるため、今後バイオテクノロジー分野や医薬品分野での活用が期待される微生物である。

麹菌の品種改良は、品質や生産性の向上を目的として以前から行われてきたが、従来の研究手法では莫大な手間と時間を費やす必要があった。その問題を解決するべく麹菌ゲノム解析コンソーシアムが組織され、（独）製品評価技術基盤機構との共同研究により本菌のゲノム解析が行われた。その結果は 2005 年 12 月の *Nature* 誌に掲載された [1)]。また、麹菌のゲノムが解析されていた同時期に、海外では他の *Aspergillus* 属糸状菌のゲノム解析が盛んに行われていた。今日までに *A. nidulans*[2)]をはじめ *A. fumigatus*[3)]、*A. niger*[4)]、*A. flavus* および、*A. terreus* などのゲノム解析が行われている。このような状況から、糸状菌研究はポストゲノム時代の真っ只中にあるといえよう。

我々は現在、ゲノム情報を活用した高機能麹菌の育種を目指している。ゲノム解析の結果によると、麹菌には 12,000 個以上の遺伝子が存在していると予測されているが、その中の多くのものが機能未知である。そのため、これら機能未知遺伝子の解析が急務である。我々はこれらの中でも、一つの遺伝子で複数の遺伝子の発現を制御しており、その解析結果が高機能麹菌の育種につながりやすいと考えられる「転写制御因子」をターゲットとして研究を行っている。本シンポジウムでは、その進捗状況と今後の展開について報告する。

1. 転写制御因子の強制発現による網羅的解析

麹菌のゲノム情報をもとに、転写制御因子に関連した遺伝子を抽出した。次に目的遺伝子の ORF を PCR 法により増幅後、*amy*B プロモーターに連結した強制発現用ベクターを作製し、麹菌の *nia*D 領域に導入した。本株を誘導培地に植え目的遺伝子を強制発現させたところ、生育特性、胞子形成および、酵素生産性に変化が見られる形質転換株が得られた。

2. 転写制御因子の破壊による網羅的解析

2-1. *ku*70 破壊により相同組換え効率を向上させた麹菌の作製

ある生物の機能未知遺伝子の機能を明らかにするためには、その遺伝子を破壊して表現型などの変化を調べる方法が有効である。遺伝子破壊株作製にはジーンターゲティングを効率的に行うために、その生物の相同組換え効率が高いほうが有利である。麹菌は多核であるうえに相同組換え効率が低いため、遺伝子破壊による網羅的解析を行うことは非常に困難であった。そこで高橋らは、相同組換え効率を向上させた麹菌の作製を試みた[5]。麹菌をはじめとする糸状菌には、外来 DNA を染色体上にランダムに組み込む「非相同組換え」の経路があることが知られていた。この非相同組換え経路に関与する遺伝子の一つである *ku*70 を破壊したところ、本菌の相同組換え効率が飛躍的に向上した。この麹菌*Δku* 70 株を宿主とし転写制御因子遺伝子の網羅的破壊を行った。

2-2. 破壊株の網羅的作製と表現型の解析

我々は麹菌の有する全転写制御因子を対象に、網羅的な破壊を行い解析することを試みている。遺伝子破壊の方法は以下の通りである。標的とする遺伝子領域の左右 1.2-1.5 kb の配列を PCR 法により増幅後、マーカー遺伝子である *pyr*G に結合させた遺伝子破壊用ベクターを作製した。これを *A. oryzae* RIB40 *Δku*70 *Δpyr*G 株に導入し最少培地により選択することで、目的遺伝子の破壊株を得た。破壊株作製のハイスループット化のため、遺伝子破壊用ベクターの作製にはライゲーションや大腸菌のトランスフォーメーションを行う必要がない Fusion PCR 法を用いた。また Fusion PCR の各種条件を詳細に検討することで、画一的な反応条件でベクターを作成することに成功し、さらなる効率化を図ることが出来た。これまでに約 200 株の破壊株を作製し、その中には生育特性や胞子形成に変化が見られた株や、色素生産が確認されるようになった株が得られている。また、生育阻害が見られた株では菌糸の多分岐化が観測されたものもある。今後は、破壊株のトランスクリプトーム解析やメタボローム解析を行うことにより、転写制御因子の機能解析を進めていく予定である。

本研究の一部は地域新生コンソーシアム研究開発事業（平成 15 年）および生研センター基礎研究推進事業（平成 17 年）の支援を受け実施したものである。

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アーバスキュラー菌根共生におけるシグナル物質の同定

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「菌根」とは菌類と植物根との共生形態であり，菌根を形成する菌類を「菌根菌」と呼ぶ。アーバスキュラー菌根菌（arbuscular mycorrhizal fungi，AM 菌）は 80%以上もの陸上植物と共生する自然生態系で最も広く見られる菌根菌であり，根の皮層細胞内に樹枝状体（arbuscule）と呼ばれる栄養交換器官を形成することからその名が付けられている。AM 菌は土壌から吸収したリン酸やミネラルを宿主植物に与える有用微生物であり，農業生産や自然生態系において極めて重要な役割を果たしている。

AM 菌は隔壁のない菌糸をもつことから接合菌類に分類されてきたが,最近になってグロメロ菌門 (Glomeromycota)という 4 億年前に類縁系統から分岐した独自の系統であることが明らかになった。実際，4 億 6 千万年前のオルドビス紀の地層から胞子の化石が発見されている。ちょうどこのころ，植物が陸上に進出していることから,無機栄養素が乏しい陸上で植物が生存していくのに AM 菌が重要な役割を果してきたと考えられている。AM 菌は絶対共生菌であり，菌単独ではほとんど生育せず，次世代の胞子も形成できない。AM 菌にとっても植物との共生は，生存し子孫を残すのに絶対的に必要なことなのである。4 億年以上もの進化の歴史の中で，AM 菌と植物はどのようにして互いの存在を発見し，今日まで共生関係を保ち続けてきたのであろうか？

AM 菌と植物はそれぞれが生産するシグナル物質により互いの存在を認識する。植物の根から分泌されるシグナル物質は AM 菌の宿主認識反応である菌糸分岐を誘導することから branching factor（BF）と呼ばれている。AM 菌は菌糸分岐を起こすときに，植物の共生応答を引き起こすシグナル物質を菌糸から分泌することが分かっており，これは Myc factor (MF)と呼ばれている。我々は 10 年にわたり，これらシグナル物質の単離同定を目標に研究を行ってきた。

AM 菌の菌糸は宿主の根の近傍に達すると BF を感受して激しく分岐する。この菌糸分岐は非宿主であるアブラナ科やアカザ科などの植物では見られないことから，AM 菌の宿主認識反応であると見なされている。BF は根から分泌される分子量 500 以下の脂溶性物質であり，その単離が試みられてきた。しかし,根から極微量しか分泌されず，化学的にも不安定であるため，単離に成功した例はなかった。2005 年，我々はマメ科モデル植物であるミヤコグサ（*Lotus japonicus*）の根分泌物から世界で初めて BF の単離に成功し，これを 5-デオキシストリゴールと同定した。本物質はストリゴラクトンと総称される根寄生雑草の種子発芽刺激物質として単離されていた化合物であった。ストライガやオロバンキなどの根寄生雑草は他の植物の根に寄生して養水分を奪う難防除性の強害雑草であり，世界中で農作物に甚大な被害を与えている。寄生を受けてしまうのにもかかわらず，なぜ植物が根からストリゴラクトンを分泌するのか，ながらく謎であった。本成果により，ストリゴラクトンの本来の役割は，AM 菌に対する共生シグナルであり，根寄生雑草はこれを傍受することにより寄主となる植物の位置を突き止めているということが明らかになった。

AM 共生研究者らは，AM 菌が共生シグナルを生産すると推定してきたが， MF が実在

するという実験的証拠はながらく得られなかった。しかし,マメ科モデル植物を用いた研究により, 菌根共生と根粒共生との間で共生初期のシグナル伝達系に共通過程が存在することや, 根粒菌や根粒菌の生産するシグナル物質である Nod ファクターによって誘導される初期ノジュリン遺伝子(*ENOD*)のいくつかが AM 菌の感染初期にも誘導されることが明らかになり,MF 発見の期待が高まってきた。そしてついに 2003 年, AM 菌が根の近傍で菌糸分岐を起こすときに, タルウマゴヤシの *MtENOD11* の発現を誘導する物質を菌糸から分泌することが報告された。根での *MtENOD11* の発現は菌糸分岐を起こしている菌糸の近くのみで見られることから, このシグナル物質の生産・分泌は BF, すなわちストリゴラクトンにより誘導されると考えられている。我々も AM 菌の胞子中にミヤコグサの *ENOD* 遺伝子の一つである *LjCbp1* のプロモーターを活性化させる物質が含まれていることを突き止めている。MF 同定はフランス, スイスなど海外のグループとの国際的な競争の中で行われている。どのグループが一番乗りするかはともかく, 近い将来に MF が解明されるのは間違いない。

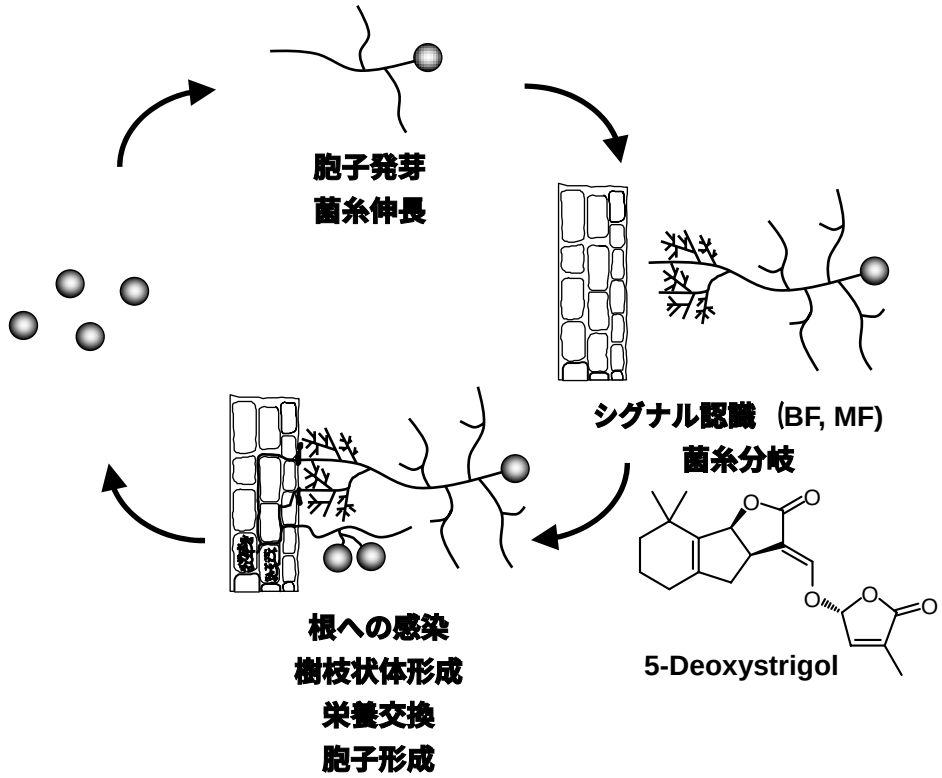

図. AM 菌の生活環と BF として同定された 5-deoxystrigol の構造

Akiyama, K., Matsuzaki, K. & Hayashi, H. *Nature*, **435**, 824-827 (2005).
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「糸状菌の交配と関連する遺伝子」

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はじめに

糸状菌の生活環は無性世代と有性世代から構成される。子嚢菌では、菌糸、菌核、分生子、厚膜胞子、bud cell（酵母状細胞）などが核相 n の無性世代であり、菌糸の伸長、菌核や分生子などの形成によって遺伝的に同一の後代（クローン）を生み出している（無性生殖）。多くの子嚢菌では、無性生殖が繰り返され、生活環の大部分が無性世代によって占められている。

一方、環境条件が整うと、糸状菌は有性世代へとその生活環を切り替える場合がある。子嚢菌では子嚢、担子菌では担子器のような器官を、それぞれ子嚢果（子嚢殻など）、担子器果（いわゆる、きのこ）等の子実体上に形成し、そこで有性生殖を行い、後代を形成する。有性生殖では、核相 n の２つの細胞が融合し、n+n の状態（異核共存体）になり、その後、核融合（2n）および減数分裂によって核相 n の後代をつくる。減数分裂の過程での染色体シャフリング等によって、遺伝的に親と異なる後代が生じる可能性がある。

子嚢菌は基本的に雌雄同体であるが、有性生殖の際に相手を要求するものとしないものが存在する。相手を必要としない性質をホモタリック、相手を必要とする性質をヘテロタリックと呼ぶ。ホモタリックは自家和合性（self-compatible）、ヘテロタリックは自家不和合性（self-incompatible）と理解することが可能である。子嚢菌の基本的な有性生殖様式はヘテロタリックであり、ホモタリックな菌はヘテロタリックな菌から生じたと考えられている（後述）。

交配型と交配型遺伝子

ヘテロタリックな子嚢菌では、１種のうちに２つの交配型（MAT1-1 と MAT1-2）の菌株が存在する。MAT1-1 の菌株と MAT1-2 の菌株が適した環境条件の下で出会うと、細胞融合が起こり、有性生殖（上述）の一連の過程が進み、後代を形成する。これを、交配（outcross または単に cross）と呼ぶ。MAT1-1 の菌株同士、あるいは MAT1-2 の菌株同士では交配は起きない。菌株の交配型を決定しているのは、交配型遺伝子領域（*MAT1*）に存在する１組の対立遺伝子（*MAT1-1-1* および *MAT1-2-1*）であり、各菌株はこの対立遺伝子のどちらかを保持している。両遺伝子産物とも、DNA 結合モチーフを有しており、交配の初期調節因子であることが示唆されている（Shiu & Glass 2000）。

既述のように、子嚢菌にはホモタリックな菌が存在するが、これらの多くは染色体上に、*MAT1-1-1* および *MAT1-2-1* の両方を持つ、あるいは、*MAT1-1-1* と *MAT1-2-1* が融合したような遺伝子を持つことが知られており、ヘテロタリックな菌の *MAT1* 領域の乗換え等によってこのような構造が生じたことも示唆されている（Yun et al. 1999）。

一方、子嚢菌には、有性生殖をしない、あるいは有性生殖が発見されていない種が存在する。いわゆる不完全菌（asexual ascomycetes）である。これまでの解析で、交配能を有する菌と同様に、不完全菌もゲノム上に *MAT1* 領域を有すること、*MAT1* 領域に存在する遺伝子（*MAT1-1-1* あるいは *MAT1-2-1*）が発現しており、しかもその産物が機能を保持していること、種の中に両交配型の株が存在していることが示されている

（Arie et al. 2000; Yun et al. 2000）。しかし、不完全菌が交配しない理由についてはまだ明らかになっていない。

近年、複数の子嚢菌において *MAT1* 領域周辺の解析が進み、比較の結果、DNA リアーゼや膜貫通タンパク質などをコードする遺伝子が普遍的に存在することが判明してきている（Debuchy & Turgeon 2006）。*MAT1* 領域に存在する遺伝子と併せ、それらの機能の解明に興味が持たれる。

交配に関わるシグナル伝達系と遺伝子

既述のように、交配型遺伝子産物は交配の初期調節因子として機能する。子嚢酵母の研究成果を参考にすると、交配型遺伝子産物が、フェロモンやフェロモンレセプターの発現を調節していること、膜上に存在するフェロモンレセプターがフェロモンを受容するとその信号が三量体Gタンパク質および MAPK カスケードを経て、核移行調節因子である STE12 のリン酸化を制御、STE12 が核内に移行して、有性生殖の遂行に関与する多数の遺伝子の発現が調節されていることが想定される（Elion 2000）。近年、複数の子嚢糸状菌において、シグナル伝達系関連遺伝子、フェロモン受容体遺伝子などが同定され、それらの破壊実験などが行われている。その結果、複数の子嚢糸状菌において、MAPK カスケードの代わりに、cAMP を介したシグナル伝達系が有性生殖の調節に重要な役割を担っている可能性が示唆されている（Lengeler & Heitman 2000; D'Souza & Heitman 2001）。

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シイタケの遺伝子解析技術の現状と収穫後の子実体で発現が変化する遺伝子

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シイタケ（*Lentinula edodes*）は、日本で最も重要な栽培キノコの一つであるが、未だゲノム解析は行われておらず、解析されている遺伝子も限られているのが現状である。本講演では、我々がこれまで検討してきたシイタケの遺伝子解析技術の現状と、収穫後の子実体で発現が変化する遺伝子についての解析結果を紹介する。後者に関しては、収穫後の子実体保存中に問題となる現象として、子実体ヒダの褐変化と、抗癌剤として臨床で使用されているシイタケ子実体由来のレンチナンの減少に注目した。

1）シイタケ遺伝子解析技術の現状

シイタケの形質転換は、遺伝子の機能解析手段として重要であるが、我々がシイタケの研究を開始した当時（1993 年頃）には確立されていなかった。そこで我々は、宍戸らが開発したシイタケ由来の遺伝子プロモータを利用したベクターpLC1-hph を用いてシイタケ形質転換方法について検討した。その結果、restriction enzyme-mediated integration（REMI）法により、従来法に比べて約 20 倍高い効率でシイタケを形質転換することができた（Sato *et al.* 1998）。

また、より高発現するシイタケベクターの開発を目的に、構成的に発現している解糖系の酵素、glyceraldehyde-3-phosphate dehydrogenase 遺伝子やキチン合成酵素遺伝子のプロモータ を利用したベクター pChG など複数のベクターを開発した。さらに、シイタケ由来のマーカー遺伝子として、カルボキシン耐性遺伝子を作製した。現在、これらのベクターを利用して、アンチセンス法や RNAi 法によるシイタケの遺伝子発現抑制について検討しており、いずれの手法でも遺伝子発現抑制が可能であることが示唆されている。

2）収穫後の子実体で発現が変化する遺伝子

・シイタケ収穫後の子実体ヒダの褐変に関与する遺伝子

シイタケやマッシュルームなどの食用キノコでは、収穫後の子実体のメラニン生成による褐変化は、その商品価値を減少させる。そこで、褐変しにくい保存性の高いシイタケの育種を目的に、褐変化の生化学的メカニズムの解明を試みた。褐変物質であるメラニンの生合成に関与する酵素として、チロシナーゼとラッカーゼに着目して解析を行った。その結果、両酵素は、子実体の保存に伴って、転写及び翻訳レベルでその発現が上昇することなどから、シイタケ子実体の褐変に関与していることが明らかとなった（Kanda *et al*. 1996、Nagai *et al*. 2003）。一方、ラッカーゼに関しては、環境汚染物質のモデル物質として用いられるレマゾールブリリアントブルーR（RBBR）等の色素類を脱色できること（Nagai *et al*. 2002、2004）なども明らかとなっている。

・シイタケ子実体保存中のレンチナン分解に関与する遺伝子

レンチナンは、シイタケ子実体から抽出される細胞壁の構成成分であるβ

-1,3-1,6-glucan からなる多糖類で、抗癌活性を有し、医薬品として利用されている。レンチナンは、子実体収穫後の保存中にグルカナーゼ活性が上昇してその含量が減少することが明らかとなっており、レンチナンの収量が安定しない原因となっている。そこで、レンチナンが分解されにくいシイタケの育種を目的に、レンチナン分解に関与するグルカナーゼ遺伝子の単離を試みた。これまで報告されているグルカナーゼ遺伝子等に関する情報をもとに、数種類のグルカナーゼ遺伝子を単離した。その結果、*exg2* と *tlg1* の２種類の遺伝子が子実体保存中にその発現が上昇することや、精製した両タンパク質がレンチナン分解活性を有することが明らかとなり（Sakamoto *et al*. 2005、2006）、 *exg2* と *tlg1* がレンチナンの分解に関与することが示唆された。

・シイタケ子実体の保存前後で発現が変化する遺伝子

シイタケ子実体の保存過程における遺伝子発現パターンの変化を網羅的に解析するために、新鮮子実体と保存３日目の子実体のひだから抽出した mRNA を用いて、PCR-select-cDNA subtraction 法（Clontech）による解析を行った。その結果、保存後に発現が上昇する遺伝子としては、褐変関連遺伝子としてチロシナーゼやラッカーゼ遺伝子、レンチナン分解関連遺伝子としてグルカナーゼ遺伝子、また、それ以外にも老化等に係わると考えられる遺伝子としてキチナーゼやプロテアーゼなどがクローニングされた。一方、新鮮な子実体で発現が高かった遺伝子の中には、転写因子様遺伝子などがクローニングされた。単離した両者の遺伝子群を比較すると、保存後に発現が上昇する遺伝子の中には未知遺伝子が多かった。

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例会演題及び演者一覧

第１回　研究会（平成２年６月 20 日）　東京都北区滝野川　醸造試験所
１．糸状菌分子育種の将来の展望：東京大学教授　別府　輝彦
２．黄麹菌（*Aspergillus oryzae*）の分子育種の現状：醸造試験所　五味　勝也
３．くものすかび（*Rhizopus*）の分子育種の現状：東京大学農学部　堀内　裕之

第２回　研究会（平成２年９月 26 日）　東京ガーデンパレス（湯島会館）
１．有用タンパク質の分泌生産－現状と展望－：名古屋大学農学部　鵜高　重三
２．Molecular genetics of nitrate assimilation in *Aspergillus nidulans* : University of St. Andrews Dr. James R. Kinghorn
３．Transformation in *Aspergillus niger* and *Aspergillus oryzae* using the niaD system：University of St. Andrews　Dr. Shiela. Unkles
４．*Penicillium* genes and heterogus expression　The University of Sheffield Dr. G. Turner

第３回　研究会（平成３年６月４日）　東京都北区　北とぴあ
１．黄麹菌（*Aspergillus oryzae*）の α–アミラーゼ、グルコアミラーゼ遺伝子のプロモーター領域の解析：醸造試験所　北本　勝ひこ
２．*Trichoderma* のセルラーゼについて：Resarch Laboratories, Alko Ltd.　Sirpa Aho
３．白麹菌のグルコアミラーゼと生澱粉分解機作について：九州大学農学部　林田　晋策

第４回　研究会（平成３年 10 月 22 日）　東京都北区　北とぴあ
１．セファロスポリン修飾酵素遺伝子のクローニングと糸状菌 *Acremonium chrysogenum* における発現：藤沢薬品（株）検索研究所　磯貝　隆夫
４．麹菌 *Aspergillus oryzae* の２種類のプロテアーゼの構造と酵母における発現：キッコーマン（株）研究本部　辰巳　宏樹
５．*Rhizopus* の宿主－ベクター系と遺伝子の解析と利用：東京大学農学部　高木　正道

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１．世界の糸状菌関連酵素の業界について：新日本化学工業（株）　山本　綽
２．*Aspergillus kawachii* のキシラナーゼ遺伝子の解析：醸造試験所　伊藤　清
３．*Aspergillus niger* の α–グルコシダーゼ遺伝子の解析：東京大学農学部　魚住　武司

第６回　研究会（平成４年 10 月 28 日）　東京都北区　北とぴあ
１．*Penicillium camembertii* のモノおよびジアシルグリセロールリパーゼ遺伝子の解析：天野製薬（株）筑波研究所　山口　庄太郎
２．セリンカルボキシペプチダーゼ遺伝子の構造解析：東京農工大学農学部　竹内　道雄

３．タカアミラーゼＡ遺伝子の発現制御：名古屋大学農学部　塚越　規弘

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２．*Aspergillus oryzae* のデンプン分解酵素系遺伝子の解析：醸造試験所　五味　勝也
３．*Aspergillus* の酵素の新規機能の探索：東北大学農学部　一島　英治

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１．黄麹菌による異種タンパクの分泌生産：協和発酵工業（株）食品酒類研究所　土屋　幸三
２．高脂血症治療薬プラバスタチンと糸状菌 *Penicillium citrinum* の分子育種系の開発：三共（株）第二生物研究所　奈良　太
３．カビによる脱窒とチトクロームＰ-４５０nor：筑波大学応用生物化学系　祥雲　弘文

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２．*Mucor* rennin（凝乳酵素）と糖鎖：東京大学農学部　堀之内　末治
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２．新しいモデル生物としてのイネいもち病菌：植物病原性に関わる分子遺伝学研究：理化学研究所微生物制御研究室　鎌倉　高志
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３．*Mortierella* 属糸状菌による有用脂肪酸の生産：京都大学農学部　清水　昌

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１．*Aspergillus niger* 由来の高発現プロモーターの取得と解析：大関（株）総合研究所　尾関　健二

２．菌類系統解析の障壁を打ち砕いた分子系統：理化学研究所　西田　洋巳

３．第１回　Fungal Genome Workshop の報告：キッコーマン（株）研究本部　阿部　敬悦

４．糸状菌酵素の機能検索：東北大学農学部　一島　英治

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１．*Penicillium camembertii* のリパーゼ遺伝子(*mdlA*)を利用した異種タンパク質の発現システム：天野製薬株式会社　山口　庄太郎

２．麹菌 *Aspergillus oryzae* の遺伝子解析－ゲノム、cDNA などのパイロット的解析：通産省生命工学工業技術研究所　町田　雅之

３．麹菌 *Aspergillus oryzae* の菌体内ヌクレアーゼと特異的阻害蛋白の遺伝生化学的研究：東京大学農学生命科学研究科　魚住　武司

第 16 回研究会（平成 9 年 11 月 11 日）　東京都北区滝野川会館

１．麹菌 *Aspergillus oryzae* の新規グルコアミラーゼ遺伝子の単離と解析：月桂冠株式会社　秦　洋二

２．糸状菌のポリケタイド化合物生合成遺伝子：東京大学薬学部　藤井　勲

３．*Aspergillus niger* のイソプルラナーゼの構造と機能に関する研究－イソプルラナーゼはデキストラナーゼか？－：東京農工大学農学部　坂野　好幸

第 17 回研究会（平成 10 年６月５日）　東京都北区北とぴあ

１．麹菌のアミラーゼ遺伝子のマルトース誘導に関与する転写因子遺伝子：東北大学農学研究科　五味　勝也

２．担子菌トレハロースホスホリラーゼの利用とその性質及び遺伝子：呉羽化学工業株式会社　高橋　栄作

３．微生物ゲノム研究の現状：奈良先端科学技術大学院大学　小笠原　直毅

第 18 回研究会（平成 10 年 11 月 6 日）　東京都北区滝野川会館

1．保存配列を利用した改良プロモーターによる麹菌の高発現システムの開発：大関株式会社　峰時　俊貴

2．真菌の二次代謝産物生産阻害物質アフラスタチンの構造と作用：東京大学農学生命科学研究科　作田　庄平

3．アカパンカビにおける形態的突然変異遺伝子：関東学院大学工学部　村山　肇子

4．Protein secretion and processing by *Aspergillus niger*：Institute of Food Reserch, UK　Dr. D.B.Archer

第 19 回研究会（平成 11 年 6 月 4 日）　東京都北区北とぴあ

1．*Aspergillus saitoi* alpha-1,2-mannosidase を用いた酵母糖鎖の改変：新エネルギー・産業技術開発機構　千葉　靖典

2．糸状菌 *Mortierella alpina* による高度不飽和脂肪酸の生産：サントリー株式会社　藤川　茂昭　　京都大学　清水　昌

3．糸状菌の液胞形成関連遺伝子の解析とその機能：東京大学農学生命科学研究科　北本勝ひこ

第 20 回研究会（平成 11 年 11 月 4 日）　東京都北区北とぴあ

1．*Aspergillus niger* 由来のオリゴ糖生産用酵素遺伝子とその改変：明治製菓（株）薬品技術研究所　矢内　耕二

2．アルカリ環境で生育する糸状菌の生産するセルラーゼ：大阪府立大学農学部応用生物化学科　　川口　剛司

3．菌類の多様性と生態系とのからみあい：筑波大学名誉教授　　椿　啓介

第 21 回研究会（平成 12 年 6 月 2 日）　東京都北区北とぴあ

1．麹菌由来キシランに作用する枝切り酵素：国税庁醸造研究所　小関　卓也

2．麹菌 *Aspergillus oryzae* 由来 phospholipaseA1－遺伝子、蛋白、及びその食品への応用－：(株) 三共バイオメディカル研究所、探索研究所、化学研究所　渡辺　一郎

3．イネいもち病菌の感染初期過程：モデル植物病原糸状菌イネいもち病菌遺伝子研究の世界的動向を踏まえて：理化学研究所　鎌倉　高志

第 22 回研究会（平成 13 年 6 月 7 日）　東京都北区北とぴあ

1．白麹菌の分泌酵素と菌体外可溶性多糖（Extracellular soluble polysaccharide:ESP）：独立行政法人酒類総合研究所　岩下　和裕

2．清酒のムレ香生成に関与する麹菌の新規アルコールオキシダーゼ遺伝子：白鶴酒造株式会社　山下　伸雄

3．クエン酸生産糸状菌におけるシアン非感受性呼吸の機能：早稲田大学理工学部応用化学

科　桐村　光太郎

第 23 回研究会（平成 14 年 6 月 6 日）　東京都北区北とぴあ

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2．醤油麹菌 *Aspergillus sojae* におけるアフラトキシン合成遺伝子の発現抑制：キッコーマン研究本部　松島　健一朗
3．麹菌（*A. oryzae*）のフェリクローム生合成遺伝子クラスターの解析：月桂冠（株）総合研究所　石田　博樹
4．糸状菌アミラーゼ遺伝子の転写誘導機構：名古屋大学大学院生命農学研究科　小林　哲夫

第 24 回研究会（平成 15 年 6 月）

1．麹菌を用いたコク味の創出　－天然系調味料「コウジ・アジ」の開発－：味の素㈱ 食品研究所 発酵醸造素材開発室　岡村　英喜
2．糖鎖を削る酵素・糸状菌のα-マンノシダーゼ：弘前大学 農学生命科学部 細胞工学講座 吉田　　孝
3．アカパンカビ遺伝解析の現状：埼玉大学 理学部 生体制御学科 遺伝学研究室　　井上弘一

第 25 回研究会（平成 16 年 6 月）

1．イネいもち病菌における形態形成と感染力：（独）農業生物資源研究所　遺伝資源研究グループ　西村　麻里江
2．紙パルプ産業での利用を目指した白色腐朽菌の育種：王子製紙株式会社　研究開発本部 新技術研究所　　杉浦　純
3．*Aspergillus aculeatus* のセルラーゼ：中部大学応用生物学部　環境生物科学科　　荒井基夫

第 26 回研究会（平成 17 年 6 月）

1．麹菌における Woronin body の機能と動態解析：東京大学大学院農学生命科学研究科　丸山潤一
2．アスプジンシン亜鉛結合モチーフ・プロテアーゼの構造的特性と特異性に関する研究：独立行政法人 科学技術振興機構　土井ゆうこ
3．マイクロアレイを利用した麹菌の遺伝子発現制御機構の解析：独立行政法人　酒類総合研究所　坂本和俊
4．ゲノム情報を利用した麹菌有用遺伝子の単離と活用：月桂冠（株）総合研究所　東田克也、（秦　洋二）

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１．国菌・麹菌：糸状菌遺伝子研究会会長　一島　英治

２．麹菌 EST 解析の成果と意義：（独）酒類総合研究所　赤尾　健

３．麹菌ゲノムの特徴：（独）産業技術総合研究所　町田　雅之

４．種麹と麹菌の今昔：（株）ビオック　和久　豊

５．麹菌ゲノムはアトキンソンの再来か？：（独）酒類総合研究所　岩下　和裕）

６．ゲノム解析情報を利用した醤油用麹菌育種の可能性について：（株）キッコーマン　畑本　修

７．固体培養からみた麹菌の産業利用 ― 今までとこれから：(株) フジワラテクノアート　狩山　昌弘

８．麹菌の酵素をもっと活用する！酵素生産メカニズムの解析と有用酵素の探索：（株）月桂冠　秦　洋二

９．ゲノム情報から麹菌の細胞システムを理解する：東京大学大学院農学生命科学研究科　北本　勝ひこ

１０．麹菌プロテオームの解析：東京農工大学応用生物科学科　竹内　道雄

１１．麹菌のマイクロアレイを用いた遺伝子の発現プロファイル解析：東北大学大学院農学研究科　五味　勝也

１２．麹菌ゲノム科学からの新産業の展望：東北大学大学院農学研究科　阿部　敬悦

１３．高峰譲吉博士の麹学から：日本醸造学会会長　秋山　裕一

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１．放線菌からの抗結核活性物質の探索と開発：微生物化学研究センター　五十嵐　雅之２．糸状菌ポリケタイド生合成遺伝子の機能解析：東京大学大学院薬学系研究科　藤井　勲

３．鞭毛菌類の収集と保存：製品評価技術基盤機構 バイオテクノロジー本部　稲葉　重樹

４．ロドコッカス属細菌における発現ベクターの開発と同属菌における遺伝子発現制御に向けた技術開発：産業技術総合研究所　中島　信孝

５．麹菌のタンパク質大量生産システム：大関株式会社　総合研究所　峰時　俊貴

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１．麹菌の転写制御因子の網羅的解析：（財）野田産業科学研究所　小川真弘

２．アーバスキュラー菌根共生におけるシグナル物質の同定：大阪府立大学大学院　生命機能化学分野　　秋山康紀

３．糸状菌の交配と関連する遺伝子：東京農工大学大学院　植物病理学研究室　有江 力

４．シイタケの遺伝子解析技術の現状と収穫後の子実体で発現が変化する遺伝子：（財）岩手生物工学研究センター微生物利用研究部　　佐藤利次

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