バイオ研究データブック

糸 状 菌 (2008)

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

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Japan Society for Molecular Biology of Filamentous Fungi

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糸状菌遺伝子研究会

Aspergillus nidulans 遺伝子

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平成 20 年度　麹･麹菌の研究業績

（許可を得て醸造協会誌より転載）

酒類

2.　麹及び麹菌

高橋ら[1]は麹の現在のプロテアーゼ活性測定方法が，実際のアミノ酸遊離量を十分に反映していない可能性があるとして，プロテアーゼ活性の新たな評価方法について検討した。酒造米の主要蛋白質であるグルテリンを基質に用いたニンヒドリン反応を利用する方法をペプチダーゼ総合活性（TPase 活性）とし，既存の，カルボベンゾキシーグルタミルーチロシンを基質とした酸性カルボキシペプチダーゼ活性（ACPase 活性）との比較を行った。麹抽出液の TPase 活性及び ACPase 活性の測定値と，実際に蒸米を消化した際のアミノ酸生成量との相関を調べたところ，秋田酒こまちの麹 33 点を用いた実験では，TPase 活性と ACPase 活性は，ともにアミノ酸生成量と相関があったが，相関係数は TPase 活性の方が高かった。また，異なる 5 品種の麹 15 点を用いた検討では，TPase 活性とは高い相関があるが，ACPase 活性とは相関がなかった。したがって，

TPase活性による評価方法は，既存の方法よりも，清酒製造工程での米のタンパク質分解をより正確に評価していると考えられる。

同じく，高橋ら[2]は麹米5品種，種麹菌株25菌株を用いて125通りの製麹を行い，製麹において麹米と麹菌株がタンパク質分解酵素の生産に有意に影響することを示した。また，窒素源としてアミノ酸を使用した寒天培地での検討から，タンパク質分解酵素生産とアミノ酸資化量との間には高い正の相関関係があることを示した。さらに，麹米がタンパク質分解酵素生産に与える影響は，麹米のタンパク質組成に起因し，グルテリン含有量と負の相関が，グロブリン，プロラミン含有量とは正の相関があることを示した。

Hisada[3]らは，麹菌 *Aspergillus oryzae* のカタラーゼ遺伝子 *catB* のプロモーターについて，β-グルクロニダーゼ（GUS）をレポーターとしたプロモーター欠失解析を行った。その結果，−1,000から−800の200 bpの欠失でGUS活性が著しく低下することが分かった。さらに，ゲルシフト解析を行うことで，熱ショックエレメント配列およびCCAAT配列を含む−1,000から−975の領域が，過酸化水素ストレスへの応答に重要であることを明らかにした。

Kimuraら[4]は麹菌 *A. oryzae* によるヒトリゾチーム生産量を改善するために，生産されたリゾチームの分解に関与するプロテアーゼの探索を行った。液体培養において，ヒトリゾチーム生産性が培養後期に低下することから，ゲノム解析より見出された132個のプロテアーゼ遺伝子のうち，同時期に発現量が増加するプロテアーゼを，DNAマイクロアレイを用いて探索したところ，すでに分解への関与が指摘されている酸性プロテアーゼの遺伝子 *pepA* などのほかに，中性プロテアーゼをコードする *nptB* が培養後期に発現が増加する遺伝子として見出された。そこで遺伝子破壊株を作製したところ，ヒトリゾチーム生産量が最大で20%改善することが明らかとなった。

Nishinoakiら[5]は *A. saitoi* 由来消化剤であるモルシンで豆腐を処理すると，イソフラボンであるDaidzeinやGenisteinが増加することを見出した。モルシンに多く含まれているアスペルギロペプシンIの阻害剤であるペプスタチンを加えても，イソフラボンの増加が見られたことから，βグルコシダーゼによる配糖体からの遊離が生じた結果であると考えた。また，モルシン処理により，テクスチャが変化することから，本方法が豆腐の機能性及び物性を変化させる方法として有効であることを示した。

Miyakeら[6]は以前に米麹中の抗酸化物質として，ピラノニグリン-Aを見出しており，本報告では，米麹中の新たな抗酸化物質の探索を行った。その結果，*A. saitoi* の米麹より，ピラノニグリン-Aの誘導体であるピラノニグリン-Sを見出した。ピラノニグリン-SのDPPHラジカル消去能は，ピラノニグリン-Aより高かった。また，ピラノニグリン-Sは，*A. oryzae*，*A. sojae* の麹からは検出されなかったが，*A. saitoi* のほか，*A. awamori*，*A. kawachii* の麹からは検出された。さらに筆者らは，ピラノニグリン-Aがヒト臍帯静脈血管内皮細胞におけるTNF-α による血管細胞接着分子の発現を抑制することを見出した。

Yeら[7]は小胞体におけるタンパク質の品質管理機構のひとつであるUPR(Unfolded Protein Response)に関与する，*hacA*，*bipA*，*pdi*，*ppi*，について，*A. oryzae* における発現を調べた。液体培地と寒天培地でのこれら遺伝子の発現量を比較したところ，寒天培地での方が強く発現することが分かった。一方，最小液体培地より，*amyB* の発現量が2.7倍高くなるDPY液体培地でも，UPRの誘導にかかわる，スプライシングされた *hacA* mRNAの量に差はなかったことから，液体培養においては，*amyB* の発現量の増加はUPRを引き起こしていないことを示した。さらに，液体培地，寒天培地において，熱ショックを与えた場合の，UPR関連遺伝子の発現量変化を調べたところ，両培養条件において，熱ショックにより，UPR関連遺伝子の発現量は増加した。従って，培養条件の変化がUPRの誘導には重要であると結論した。

Shakeriら[8]は人工染料を含む排水の処理について，脱色酵素として作用する *Geotricum candidum* 由来ペルオキシダーゼの麹菌 *A. oryzae* による生産を報告している。筆者らは，麹菌を宿主とした生産において，0.1%硝酸ナトリウムと1%マルトースを用いた，繰り返し回分培養システムが，高い脱色効率を維持でき，効率的な脱色方法であることを示した。

Sakaiら[9]は麹菌 *A. oryzae* による異種二次代謝産物の生産について，*Monascus purpureus* 由来のシトリニンをモデルに検討した。4つの生合成遺伝子を含む，約20 kbのシトリニン生合成遺伝子クラスターをカバ

ーするDNA断片をコスミドベクターに導入し，さらに *sC* を形質転換マーカーとして麹菌に導入した。その結果，微量ながらも麹菌によるシトリニン生産に成功した。そこで，この形質転換体に，さらにシトリニン生合成遺伝子クラスターの転写因子をコードする *ctnA* を *A. nidulans* の *trpC* プロモーター下流に連結して導入したところ，生合成遺伝子の発現量が増加し，それに伴い，シトリニン生産量が増加することを報告した。

Watanabeら[10]は麹菌 *A. oryzae* 実用菌株3株における遺伝子破壊の効率を調べるために，ピリチアミン耐性遺伝子をマーカー遺伝子として，フェリクリシン合成遺伝子，*dffA* の遺伝子破壊株の作製を行った。得られた438ピリチアミン耐性形質転換体について *dffA* 破壊の有無をPCR解析により調べた結果，遺伝子破壊効率は42.9%～64.1%であり，それまで報告されている *A. oryzae* RIB 40株における結果よりも高いことが分かった。また，*dffA* を破壊した実用菌株を用いて米麹を作製したところ，フェリクリシンは生産されず，アミラーゼ等の酵素力価は変化しなかったことから，本方法が効率的な実用株の育種に利用できることを示した。

解説・総説には北本ら[11]の「醤油醸造における麹菌多糖類分解酵素の機能解析」，堀尾[12]の「麹菌（糸状菌）の菌糸先端成長のしくみ」，松村[13]の「清酒麹菌のタンパク質でガンを診る」，中島ら[14]の「麹菌による味覚修飾タンパク質ネオクリンの発現生産」，山田[15]の「麹菌 *Aspergillus oryzae* のアフラトキシン生合成遺伝子ホモログクラスタの解析-麹菌がアフラトキシンを作らない理由」，窪寺[16]の「麹菌から見つかったリボスイッチ」，田中ら[17]の「麹菌における異種遺伝子ORF内への異常poly（A）付加によるnonstop mRNA生成」，山内ら[18]の「麹菌をタンパク質工場にする化学　いつでも好きなだけタンパク質をつくれるスイッチRNA」，佐野ら[19]の「麹菌株の違いによる製麹中の微生物の動向」，杉山[20]の「麹菌と *Aspergillus* 属についての一考察」，丸山[21]の「隔てられても心はひとつ？-麹菌の隔壁孔を介した細胞間コミュニケーション」，正路ら[22]の「糸状菌オルガネラの形態とその極性依存的な配置　麹菌の小胞輸送経路の解析からみえてきたもの」，中島[23]の「麹菌の安全性について」がある。

特許には，平澤[24]の「清酒の製造方法」，工藤ら[25]の「植物活力剤」，田中ら[26]の「麹菌由来のグルタミン酸アミノペプチダーゼ」，山田ら[27]の「特定の麹菌の同定方法」，井口ら[28]の「機能性食品とその製造方法」，辻ら[29]の「アスペルギルス属菌由来新規グルコースデヒドロゲナーゼ」，川俣ら[30]の「円型通風製麹装置」，仁平ら[31]の「紅麹菌由来生理活性物質生産誘導因子の同定およびその応用」，仁平ら[32]の「RNAi法を用いた紅麹菌におけるカビ毒シトリニン生産抑制方法」，徳井ら[33]の「チアミン高生産変異微生物」，坂本ら[34]の「生存性の低い胞子を作る糸状菌の作成方法」，福田ら[35]の「デフェリフェリクリシン高生産変異株，シデロフォア生産用の液体培地，シデロフォアの製造方法」，外山[36]の「焼酎用麹菌株の選択法及び選択キット」，稲福ら[37]の「爆砕発酵処理食物繊維含有組成物」，徳田ら[38]の「蜂蜜酒の製造方法」，瀧井ら[39]の「α－グルコシダーゼB，α－グルコシダーゼB遺伝子，当該α－グルコシダーゼB遺伝子を含むベクター，当該ベクターを組み込んだ形質転換体，α－グルコシダーゼBの製造方法」，阿部ら[40]の「新規グルカン及びその製造方法」，石川ら[41]の「麺類の食感改良剤」，稲福ら[42]の「爆砕発酵バガス含有ACE阻害剤」，岩井ら[43]の「焼酎蒸留廃液を用いた醸造酢の製造方法及び加工食品」，柏田ら[44]の「梅スピリッツの製造方法」，矢部ら[45]の「染色体広範囲遺伝子領域を欠失した生物の作出法」，桜井ら[46]の「液体麹及び速醸型味噌様食材」，山元[47]の「生酸性麹菌を利用したアルコール飲料」福岡ら[48]の「混合原料米紅麹およびその製造方法」，松本ら[49]の「糸状菌由来グルコース脱水素酵素の製造方法」北林ら[50]の「糸状菌由来グルコースデヒドロゲナーゼを組換え体で高発現するための方法」，久野[51]の「PMS改善剤」，松島ら[52]の「麹菌分生子形成を増大させる遺伝子，タンパク質，組換えベクター」，辻ら[53]のアスペルギルス・オリゼ由来グルコースデヒドロゲナーゼの製造方法」，山元[54]の「生酸性麹菌を利用した食品廃棄物の処理方法」，原山[55]の「乳酸菌およびこれを用いる製麹方法」，潘[56]の，「抗アレルギー素材およびその製造方法」，内山ら[57]の「甘藷を用いた焼酎の製造方法」，辻ら[58]の「アスペルギルス・オリゼ由来グルコースデヒドロゲナーゼ」，川南ら[59]の「改変型フラビンアデニンジヌクレオチド依存性グルコースデヒドロゲナーゼ」，渡邊ら[60]の「アンジオテンシン変換酵素阻害剤およびその製造方法」，渡邊ら[61]の「抗アレルギー剤」，

鈴木ら[62]の「米麹を用いた降圧組成物」，小出ら[63]の「小豆発酵食品」，高橋ら[64]の「染色体大領域の欠失方法」，井上ら[65]の「飲食品中のジアセチル前駆体の定量法」，郷司ら[66]の「イモ焼酎及びその製造方法」，大岩[67]の「泡盛麹，紅麹および黄麹による紅芋発酵食品とその製造方法」，入江ら[68]の「アンギオテンシン変換酵素阻害ペプチド混合物及びその製造方法」などがある。

（徳岡昌文）

3．酵　　素

菅原ら[1]は，麹菌は製麹の条件により生成 α-アミラーゼが異なると推測されている複数の α-アミラーゼを分析する場合に電気泳動法を用いると視覚的に α-アミラーゼ活性を確認することが可能であることを示した。本報告では 1）電気泳動法を使用した分析における α-アミラーゼの反応条件の検討，2）米麹菌と *Aspergillus kawachii* の異種麹菌における α-アミラーゼの発現差異，3）米麹と液体培地上での α-アミラーゼの発現差異，および 4）味噌熟成過程における α-アミラーゼ活性の推移について概説した。

高橋ら[2]は，清酒醸造における清酒の風味は製麹において生産された各種蛋白質分解酵素が酒造米蛋白質を分解し，さらに酵母による吸収，排出を経て種々のアミノ酸等に変換されて清酒の風味を形成することを示した。さらに，酒造米の主要蛋白質であるグルテリンを主体とした米蛋白質を基質に用いたペプチダーゼ総合活性（TPase）の測定法について検討した。

MUSSATTO ら[3]は，醸造粕（セルロース含有量 16.8%，ヘミセルロース含有量 28.4%）を酵素製剤 Cellclast（Tichoderma reesi 代謝産物）で加水分解する条件として，撹拌速度，酵素添加量，醸造粕濃度を各 3 水準とする実験計画法に従って実験し，グルコース収率及びセルロース分解率を調べた。グルコース収率には，酵素量が高度に有意（多いほど高い）であり，濃度は有意ではないが低い方がやや高かった。セルロース分解率には，酵素量が高度に有意（多いほど高い）であり，濃度も有意（低い方が高い）であった。最適条件においてセルロースからグルコースへの転化率は 93.1%，グルコース及びセロビオースへの転化率は 99.4%に達した。

谷本ら[4]は，バナナ様の香りの酢酸イソアミルおよびリンゴ様の香りのカプロン酸エチルは，香りを特徴とする吟醸酒の品質において最も重要な香気成分であることから，協会 601 号および 901 号から，3-カルボキシ-4-オクチル-2-メチレンブチロラクトン（C 75）に耐性を示す変異株としてそれぞれ 28 および 24 株を取得した。これらを発酵試験した結果，酢酸イソアミルを高生産する株を各親株からそれぞれ 3 および 1 株の高頻度で分離できた。選抜株 K-901-11 は親株に比して，耐性付与によりカプロン酸エチルを高生産することで知られるセルレニンに対する耐性はやや弱かったが，酢酸イソアミルを 1.4 倍生産し，発酵力は同等で，生酸性は低かった。また酒質は官能的に良好であった。アルコールアセチルトランスフェラーゼ活性が高まったことが酢酸イソアミル高生成の 1 要因と示唆された。

KOTAKA ら[5]は，3 つの β-グルコシダーゼをコードする遺伝子および 2 つのエンドグルカナーゼをコードする遺伝子を *Aspergillus oryzae* からクローニングし，それらの遺伝子産物を清酒酵母 *Saccharomyces cerevisiae* GRI-117-UK の細胞表面に提示させた。β-グルコシダーゼ（AO 090009000356）を提示する GRI-117-UK/pUDB 7 は，多くの基質に対して最も高い活性を示し，セロビオースから効率的にエタノールを生産した。一方，エンドグルカナーゼ（AO 090010000314）を提示する GRI-117-UK/pUDCB は，オオムギ β-グルカンをグルコースおよびより小さいセロオリゴ糖に分解した。β-グルコシダーゼおよびエンドグルカナーゼの両方を共提示する GRI-117-UK/pUDB 7 CB を作出した。この共提示菌株で，モデル基質の 20 g/L オオムギ β-グルカンからの直接エタノール発酵を行うと，エタノール濃度は 24 時間の発酵で 7.94 g/L に達した。β-グルカンからのエタノール転換率は，20 g/L オオムギ β-グルカンから生産される理論的エタノール濃度の 69.6%であった。*A. oryzae* セルロース分解酵素を表示する清酒酵母は，セルロース系材料からのエタノール生産に利用できることが分かり，本報告で作出した株は，これまでに報告されたものに比べてより高いエタノール生産能を有していた。

KOTAKA ら[6]は，*glaA* および *glaB* によってコードされる *A. oryzae* グルコアミラーゼと *Rhizopus oryzae* グルコアミラーゼが，酒酵母 *S. cerevisiae* GRI-117-UK および実験室酵母 *S. cerevisiae* MT 8-1 の細胞表面で発現させた。構築された変異体の中で，*R. oryzae* グルコアミラーゼを発現する MT 8-1/pUD-

GAR はその細胞表面で最高のグルコアミラーゼ活性を持っていたが，*glaA* グルコアミラーゼを発現する GRI-117-UK/pUDGAA は液化澱粉からエタノールを最も多く生産したことを報告した。

KAYA ら[7] は，日本の食品工業で長期間安全であると認識されている清酒酵母と *A. oryzae* とを組み合わせて機能性食品イソフラボンアグリコンを生産するためのプラットホームを構築した。最初に，*A. oryzae* ゲノムから新規 β-グルコシダーゼ遺伝子を 3 種類スクリーニングし，それぞれの β-グルコシダーゼを清酒酵母細胞表面上に提示した。これら組換酵母を全細胞生物触媒として用い，イソフラボングリコシドの加水分解によるイソフラボンアグリコン生産を試みた。三 β-グルコシダーゼの内，BGL 1 が最高活性とイソフラボングリコシドに対する広範囲な基質特異性を示した。遊離するグルコースによって β-グルコシダーゼ活性が阻害されたが，残存イソフラボングリコシド比率は 6.2%に減少できた。

総説・解説記事としては，岡戸[8] の「開発進む酵素製品 プロテアーゼの開発-多様性を求めて」，松村[9] の「清酒麹菌のタンパク質でガンを診る」，川端[10] の「酵素反応と微生物資材開発」，谷本[11] の「酵素剤仕込みによる生酒の貯蔵中におけるムレ香の生成抑制」，蓮尾[12] の「食品加工における微生物・酵素の利用（第 14 回）清酒の機能性」，神戸[13] の「アルコール発酵と亜鉛」，真岸[14] の「麹菌の固体培養　温故知新」などがある。

特許関係では，平澤ら[15] の「清酒の製造方法」，坂東ら[16] の「セルロース分解酵素を表層提示する酵母及びその利用」，福田ら[17] の「鉄により生産が抑制される生物生産物の製造方法」，瀧井ら[18] の「α—グルコシダーゼ B，α—グルコシダーゼ B 遺伝子，該 α—グルコシダーゼ B 遺伝子を含むベクター，該ベクターを組み込んだ形質転換体，α—グルコシダーゼ B の製造方法」，菊川ら[19] の「α—D—グルコピラノシルグリセロールの製造方法」，徳井ら[20] の「チアミン高生産変異微生物」石原ら[21] の「マトリックスメタロプロテアーゼ—1 産生阻害剤」などがある。

（水谷　治）

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54) 山元：特開 2008-178787
55) 原山：特開 2008-187955
56) 潘：特開 2008-189590
57) 内山ら：特開 2008-228620
58) 辻ら：特開 2008-228740
59) 川南ら：特開 2008-237210
60) 渡邊ら：特開 2008-239521
61) 渡邊ら：特開 2008-239522
62) 鈴木ら：特開 2008-247888
63) 小出ら：特開 2008-263904

64) 高橋ら：特開 2008-263927
65) 井上ら：特開 2008-263977
66) 郷司ら：特開 2008-27195
67) 大岩：特開 2008-278865
68) 入江ら：特開 2008-308445

3. 酵 素

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2) 高橋仁，伊藤俊彦，中沢伸重，岩野君夫，高橋仁：米タンパク質を基質とした清酒麹のペプチダーゼ総合活性の測定法，日本醸造協会誌，**103(8)**, 638-645, (2008)
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5) KOTAKA Atsushi, BANDO Hiroki, KAYA Masahiko, SAHARA Hiroshi, HATA Yoji, KATO-MURAI Michiko, KURODA Kouichi, UEDA Mitsuyoshi, KONDO Akihiko：Direct Ethanol Production from Barley β-Glucan by Sake Yeast Displaying *Aspergillus oryzae* β-Glucosidase and Endoglucanase, J. Biosci. Bioeng., **105(6)**, 622-627, (2008)
6) KOTAKA Atsushi, SAHARA Hiroshi, HATA Yoji, ABE Yasuhisa, KONDO Akihiko, KATO-MURAI Michiko, KURODA Kouichi, UEDA Mitsuyoshi：Efficient and Direct Fermentation of Starch to Ethanol by Sake Yeast Strains Displaying Fungal Glucoamylases, Biosci. Biotechnol. Biochem., **72(5)**, 1376-1379, (2008)
7) KAYA Masahiko, KOTAKA Atsushi, MATSUMURA Kengo, BANDO Hiroki, SAHARA Hiroshi, HATA Yoji, ITO Junji, OGINO Chiaki, KONDO Akihiko, SHIBASAKI Seiji, KURODA Kouichi, UEDA Mitsuyoshi：Isoflavone aglycones production from isoflavone glycosides by display of β-glucosidase from *Aspergillus oryzae* on yeast cell surface, Appl. Microbiol. Biotechnol., **79(1)**, 51-60, (2008)
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11) 谷本昌太：酵素剤仕込みによる生酒の貯蔵中におけるムレ香の生成抑制，日本醸造協会誌，**103(2)**, 74-79, (2008)
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13) 神戸大朋：アルコール発酵と亜鉛，生物工学，**86(5)**, 243, (2008)
14) 真岸範浩：麹菌の固体培養　温故知新，生物工学，**86(6)**, 293, (2008)
15) 平澤ら：特開 2008-161144
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18) 瀧井ら：特開 2008-072956
19) 菊川ら：特開 2008-005735
20) 徳井ら：特開 2008-054513
21) 石原ら：特開 2008-024638

(2) 製　麹

総説，解説として，真岸[54]の「麹菌の固体培養—温故知新」がある。

関連する特許として，原山[55]の「乳酸菌およびこれを用いる製麹方法」，原田ら[56,57]の「回転円盤固体培養装置」，川俣ら[58]の「円型通風製麹装置」，大浦ら[59,60]の「通風式製麹装置の調湿空気供給装置」と「回転円板式製麹装置の軸受構造」，工藤ら[61]の「植物活力剤」が公開され，田中ら[62,63]の「固体培養装置」と「回転円盤固体培養装置の洗浄装置」，岡本ら[64]の「回転円盤固体培養装置における培養床と培養室壁面との摺接構造」，原田ら[65]の「回転円盤固体培養装置」，臼井[66]の「回転円盤固体培養装置における培養基質の盛込み方法」，平田ら[67]の「回転円盤固体培養装置における培養基質の盛込み装置」，脇本ら[68]の「培養原料排出装置およびこれを用いた培養原料排出方法」，荒木ら[69,70]の「回転式製麹装置の側板周辺構造」と「円盤外周部残存麹の除去装置」，大川ら[71]の「連続通風による製麹方法および装置」，杉本ら[72~76]の「黄麹菌を用いた液体麹の製造方法」，「液体麹の製造方法」，「麹菌の培養方法」，「液体麹を用いた穀類または芋類の液化方法」，「植物繊維溶解酵素が増強された液体麹の製造方法，該方法により得られた液体麹およびその用途」，市川ら[77]の「酒粕麹の製造方法および酒粕醤油の製造方法」が公告された。

(3) 微　生　物

(a) 麹　菌

佐野ら[78]は，麹菌株の違いによる製麹中の微生物の動向を調べた。調査した *Aspergillus oryzae* の約3割の菌株が抗菌物質生産能を有していた。抗菌物質生産能を示す麹菌を使用することにより，微生物汚染を受けやすい製麹工程において有害微生物を低減することができた。麹菌の抗菌物質生産能を麹菌の選択指標として用いることで醤油醸造における製麹時に有害微生物の抑制が容易となり，高品質な醤油醸造へ繋がる可能性を考えた。主な抗菌性物質を単離・精製し，ピラジン環を主骨格する化合物を2種同定した。ピラジン環化合物が抗菌性を示すにはピラジン環のNに結合するOH基の構造が必要であった。

豊島ら[79]は，麹菌へのイオンビーム照射の影響と，遺伝子の変異スペクトル解析を行い，またイオンビームがDNAの二重鎖切断を引き起こすことを利用し，遺伝子組換え体からのマーカー遺伝子除去が可能かを検討し，さらにイオンビーム照射の醤油醸造用麹菌育種への応用研究について紹介した。麹菌はゲノム情報の公開された *A. oryzae* RIB 40株を親株とし，亜硝酸要求性の付与されたniaD 300株を使用した。イオンビーム照射はAVFサイクロンを用いて，$^{12}C^{5+}$ を加速し，229 MeVのエネルギーで300～700 Gy照射した。これらの結果，イオンビーム照射は従来の変異処理方法では得られなかった，麹菌遺伝子に対して大きな構造変化を引き起こし，マーカー遺伝子の除去が可能であることを明らかにした。このため，今まで得ることができなかった，麹菌を育種することが可能になるとした。

麹菌として用いられる *A. oryzae* は酵素生産性が高く，酵素工業的に応用されている。近年組換えDNA技術により，各種高生産株が作出されている。*A. oryzae* TB 1株は，醤油麹菌宿主株KBN 616-39株から

作出され，宿主の500倍のセルラーゼを高生産する菌株である（KITAMOTO N. *et al.*, *Appl.Microbiol. Biotechnol.*, **46**, 538 (1996)）。楠本ら[80]は，TB1株，KBN 616-39株，および野生株PTR-1株の分生子の土壌中の生残性を比較するため，プラスチックチューブに入れた黒土にこれらの分生子を接種し，25℃で保温した。経時的に，希釈平板法により形成コロニー数を計測し，*A. oryzae* 生菌数を決定した。生残試験を反復した結果，TB1株の生残率減少速度の平均および分散はKBN 616-39株およびPTR-1株と同程度であるとした。これらの菌株は，供試土壌環境中で25℃において7週間以上生残するとした。生菌数が増加する菌株は見られなかったことから，供試した土壌中では，供試菌株の分生子は発芽生育しないと考えた。

斉藤ら[81]は，アフラトキシン産生菌 *A. flavus* および *A. parasiticus* の茨城県および千葉県内の畑土壌における分布を調査した結果，茨城県では調査土壌の22.4%，千葉県では27.0%から *A. flavus* が分離され，*A. parasiticus* が茨城県内の土壌1試料から分離した。分離菌株のアフラトキシン産生について調べた結果，*A. flavus* 9菌株および *A. parasiticus* 1菌株がアフラトキシンを産生するとした。

解説として，山田[82]の「麹菌 *A. oryzae* のアフラトキシン生合成遺伝子ホモログクラスタの解析—麹菌がアフラトキシンを作らない理由—」，中島[83]の「麹菌の安全性について」，杉山[84]の「麹菌と *Aspergillus* 属についての一考察」，小西ら[85]の「カビ毒のリスク評価と国際的な動向」，山本ら[86]の「UJNR有毒微生物専門部会第42回日米合同部会」，菊地[87]の「醤油黄麹菌の育種—古くて新しい古典育種—」，堀尾[88]の「麹菌（糸状菌）の菌糸先端成長のしくみ」，丸山[89]の「隔てられても心はひとつ？—麹菌の隔壁孔を介した細胞間コミュニケーション—」，畠山ら[90]の「麹菌も光に応答する」がある。

関連する特許として，山田ら[91]の「特定の麹菌の同定方法」，阿部ら[92]の「微生物に対する突然変異誘発方法」が公開された。

54) 真岸範浩：生物工学，**86**, 293
55) 原山文徳：特開2008-187955
56) 原田年康，田中，大松，藤原，藤原：特開2008-29357
57) 原田年康，田中，大松，藤原，藤原：特開2008-67713
58) 川俣　聡，大浦：特開2008-43305
59) 大浦雅己：国際公開WO 2006/035485
60) 大浦雅己，渡辺，植竹：国際公開WO 2006/035487
61) 工藤りか，石田，垣渕：特開2008-7474
62) 田中　栄，藤原，藤原：特許第4047851号
63) 田中　栄，藤原，藤原：特許第4130245号
64) 岡本久司，岡野，藤原，藤原：特許第4130241号
65) 原田年康，田中，大松，藤原，藤原：特許第4056579号
66) 臼井康朗：特許第4155748号
67) 平田利雄，臼井：特許第4209643号
68) 脇本勝正，藤原，藤原：特許第4176851号
69) 荒木和鬼夫，荒木：特許第4078702号
70) 荒木和鬼夫，荒木：特許第4085401号
71) 大川勝利，荒木：特許第4114108号
72) 杉本利和，小路：特許第4068649号
73) 杉本利和，小路：特許第4083194号
74) 杉本利和，洞口，長谷川：特許第408724号
75) 杉本利和，小路，洞口：特許第4096026号
76) 杉本利和，小路：特許第4113252号
77) 市川英治，本多，菊島，西岡，大方：特許第4069165号
78) 佐野芳仁，村松：醤研，**34**, 121
79) 豊島快幸，田中，赤川，山崎，佐藤，長谷，鳴海：放射線と産業，No. 119, 22
80) 楠本憲一，古川，北本，木村，松下，鈴木，柏木：食総研報，No. 72, 53
81) 斉藤道彦，岡崎，田中，久城：食総研報，No. 72, 77
82) 山田　修：醸協，**103**(9), 665
83) 中島　佑：味噌の科学と技術，**56**, 203
84) 杉山圭一：生物工学，**86**, 557
85) 小西良子，杉山：食衛誌，**49**, 1
86) 山本茂貴，小西，鈴木，長嶋，村田，梅原：食品衛生研究，**58**(5), 9
87) 菊地　護：千葉大学真菌医学研究センター報告，**11**, 57
88) 堀尾哲也：醸協，**103**(6), 411
89) 丸山潤一：生物工学，**86**, 292
90) 畠山理広，北本：醸協，**103**(7), 525
91) 山田　修，坂本，リー，秋田：特開2008-11751
92) 阿部知子，市田，福西，龍頭：特開2008-306991

（b） 麹菌など

渡辺ら[6]は，麹菌の発酵能を利用して茶葉の呈味改善に関する検討を行い，茶カテキンによる生育阻害を受けにくい麹菌菌株を用いて茶葉を発酵させ，カテキン類，アミノ酸の含有量，香味に及ぼす影響を検討した。白味噌用，赤味噌用，焼酎用の麹菌の各菌株ともに，ガレート体カテキンを低下し，加水分解体カテキンおよび没食子酸含有量を上昇させる機能を示した。白味噌用麹菌菌株により発酵した煎茶用茶葉の官能検査を行ったところ，成分変化に対応して渋みの呈味が低下する結果を得た。

Machida ら[7]は，麹菌のゲノム情報を活用して，麹菌（*Aspergillus oryzae*），工業用黒麹菌，*A. nidulans*，*A. fumigatus*，*A. flavus* のゲノム情報を比較し，各菌のゲノム領域のシンテニーについて解説した。

澤山[8]は，食品に関連するカビの有用性と有害性について解説するとともに，生育抑制のため，水分・栄養・温度および酸素・pH・浸透圧の環境条件との関連を解説した。

Machida ら[9]は，発酵食品製造に多く用いられている *A. oryzae* は，固体培養において菌体外ヒドロラーゼが大量に発現生産され，原料分解，うまみ生成，フレーバー生成に関与することを解説した。

6) 渡辺祐子，植野洋志，早川潔：日本家政学会誌，59，999-2004
7) M. Machida, M. Sano, GA. Payne, J-J. Yu, TE. Cleaveland, WC. Nierman：Food Addit. Contam. Part A, 25, 1147-1151
8) 澤山茂：製菓製パン，74，132-134
9) M. Machida, O. Yamada, K. Gomi：DNA Res., 15, 173-183

第 30 回例会　講演要旨

日　時　　平成 21 年 6 月 5 日（金）　　13 時 30 分～16 時 45 分

会　場　　北とぴあ　第二研修室　（7Ｆ）
東京都北区王子 1 丁目 11 番地 1 号　　Tel 03-5390-1100

演　題

13:30～14:15 窪寺隆文　先生（白鶴酒造株式会社）

「麹菌 *A. oryzae* のチアミン合成遺伝子のリボスイッチによる発現制御機構」

14:15～15:00　吉田　誠　先生（東京農工大学）

「担子菌に特有のセルロース分解メカニズム-子嚢菌類の機構とは何が違うのか？-」

15:00～15:15　　休憩

15:15～16:00　加藤雅士　先生（名古屋大学）

「*Asperillus* 属糸状菌のシグナル伝達及び転写制御機構の *in vitro* 解析」

16:00～16:45　堀尾哲也　　先生（カンザス大学）

「糸状菌 *Aspergillus nidulans* の先端成長機構」

麹菌 *A. oryzae* のチアミン合成遺伝子のリボスイッチによる発現制御機構

白鶴酒造　品質保証部　窪寺　隆文

麹菌（*Aspergillus oryzae*）は、清酒、醤油および味噌などの日本の伝統的な醸造産業に必要不可欠な微生物であり、さらに物質生産・分泌能力の高さと世界的に認められた安全性から産業用微生物としても注目されている。この有用特性を遺伝子レベルで知ることは麹菌の能力を最大限に活用することにつながることから、麹菌における分子生物学的手法の充実に伴い、今後の研究成果が期待されている。

これまで、麹菌や酵母などの真核微生物おける遺伝子発現制御機構の解析は、主に遺伝子上のプロモーター領域（cis 領域）を中心に、蛋白質で構成される転写因子（trans 因子）との cis-trans 相互作用（転写制御系）について進められてきたが、遺伝子の発現は転写制御ばかりではなく、mRNA の修飾や安定性、翻訳過程などの転写後調節機構による制御も受ける。近年この転写後調節機構におけるノンコーディング RNA の関与（RNAi）が注目されており、これまで遺伝情報伝達の担い手としか見なされなかった RNA の役割が見直されつつある。

1967 年 Carl Woese らは RNA は、高度な二次構造を形成することから蛋白質と同様の機能（触媒、物質の識別と結合）を示す可能性を示唆している。そして 1980 年代に Thomas R. Cech と Sidney Altman は触媒作用を持つ RNA をそれぞれ *Tetrahymena thermophila* およびバクテリア由来の Rnase P 複合体から発見し、これらはリボザイム（ribozyme）と呼ばれている。さらに 2002 年に Ronald R. Breaker らは *E. coli* のチアミン合成遺伝子 *thiM* よりチアミン二リン酸（TPP）を特異的に認識して結合することで下流域にコードされている遺伝情報の翻訳を制御する RNA 制御因子「リボスイッチ」を発見した。*E. coli* の TPP 結合型リボスイッチは、mRNA 転写以後に TPP と結合することで、リボスイッチ近傍配列と蛋白質合成を担うリボゾーム結合領域である Shine-Dalgarno 配列がステムループ構造を形成し、蛋白質への翻訳を阻害する。当時このリボスイッチによる遺伝子発現制御機構は原核生物のみで確認されており、真核生物での報告はまだなかった。

ところで、清酒醸造において、醪中のチアミン不足により木香様臭と呼ばれるオフフレーバーが発生するが、我々は木香様臭の発生防止を目的として、麹菌のチアミンアナログ（Pyrithiamine）耐性変異株からチアミン高生産性株の取得を試みていた。真核微生物におけるチアミンの供給機構については出芽酵母（*Saccharomyses cerevisiae*）や分裂酵母（*Schizosaccharomyses pombe*）をモデルとした研究が進められており、一般的にチアミン合成にかかわる遺伝子は環境中のチアミンにより非常に厳密な発現抑制を受けることが知られている。本研究の過程で、麹菌の Pyrithiamine への耐性化がチアミン合成遺伝子（*thiA*）の発現様式の変異（脱抑制）に起因することと共に、本遺伝子がリボスイッチによる転写後調節機構を有するこ

とを発見し、真核微生物でもリボスイッチが機能していることを報告した。

A. oryzae thiA の 5’上流領域は約 500ｂの長い 5’非翻訳領域(5’-UTR)を有し、この 5’-UTR 上に二つのイントロン（イントロン 1:-414～-359, イントロン 2:-338～-18）が含まれる特徴的な構造であった。イントロン 2 はチアミンによる発現抑制が解除される変異点を含み、変異点の周辺領域には *thiA* 相同遺伝子の 5’上流領域における高度保存領域(regionA：-75～-63, regionB：-98～-76)が存在した。興味深いことにイントロン 2 のスプライシング様式は、環境中のチアミンによる影響を受け､チアミン存在下ではスプライシングを受けない又は 5’側のスプライシング位置が下流側へ 85ｂ移動した。このスプライシングの制御には regionA, B が必須であり、これらの領域を欠失することで環境中のチアミンによるスプライシング様式の変化および発現抑制は失われた｡またイントロン2の欠失でも脱抑制することから、*thiA* は転写後レベルで制御されており、制御にはイントロン 2 のスプライシング様式の変化が関与することが確認された。

region A, B は *E. coli thiM* の制御領域である *thi* box と相同性が高いことが既に確認されていたが、その後 *thi* box を含む周辺領域が TPP 結合型リボスイッチであることが Breaker らにより報告された。実際に *thiA* のリボスイッチは TPP と結合し、結合には二価のマグネシウムイオンが重要な役割を果たしていた。この TPP 結合型リボスイッチ様配列は *A. oryzae* 以外の糸状菌にも存在しており、興味深いことにいずれも *thiA* 同様に推定チアミン合成関連遺伝子の 5’-UTR 上のイントロンに含まれていた。その後 Breaker らにより *Neurospora crassa* のチアミン合成遺伝子 *NMT1* においても *A. oryzae thiA* と同様の制御機構が報告されており、これは TPP 結合型リボスイッチによる制御機構が糸状菌において広汎に存在することを示唆している。

現在 Breaker の研究グループを中心にリボスイッチの小分子認識機構および発現制御機構の解析が進められている。我々は *thiA* のリボスイッチ周辺領域を仮説に基づき改変することで制御様式が逆転することを既に報告しているが、今後さらにリボスイッチのメカニズムが解明されることで、より理論的に発現制御因子のデザインが可能になるのではと期待している。

担子菌に特有のセルロース分解メカニズム
–子嚢菌類の機構とは何が違うのか？–

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１　はじめに

植物系バイオマスからのバイオ燃料生産に対する期待の高まりを背景に，セルロース分解性糸状菌のゲノム情報が次々と開示されており，その結果，糸状菌類のセルロース分解メカニズムに関する新たな知見が続々と得られてきている。そこでここでは，演者が研究対象としている担子菌のセルロース分解メカニズムについて，子嚢菌の情報を交えながら概説したい。

２　木材腐朽菌と軟腐朽菌とは？

植物細胞壁分解菌の研究においては，担子菌や子嚢菌という分類に換えて，木材腐朽菌や軟腐朽菌といった分類を用いる場合が多い。これらの用語は，木材保存学の分野で古くから用いられてきたものであり，子嚢菌や担子菌といった科学的手法による分類ではなく，あくまで腐朽材の形態的特徴から分類されたものである。木材腐朽菌とは木材腐朽能力を持つ真菌類を指すが，”腐朽”とは ”微生物が木材の化学成分を分解し，自身の栄養源として摂取することにより，比較的短い期間に木材の組織構造を破壊し，その結果木材に形態的損傷と激しい強度劣化を生ずる現象”と定義されることから，木材分解能力の高い真菌類と見なすことができる。一方，軟腐朽菌は，高含水率の木材表面を軟化させる真菌類を指し，木材腐朽菌と比較して，木材分解能力の弱い真菌類と言える（あくまで木材分解能力であり，セルロース分解能力が弱いわけではない）。前述した通り，これらの分類は，腐朽材観察における経験則的な色合いの強い分類法であるが，科学的な分類とほぼ一致する。すなわち，多くの場合，木材腐朽菌は担子菌が属し，軟腐朽菌は子嚢菌が属する。したがって，大まかな捉え方としては，木材腐朽菌とは担子菌を指し，軟腐朽菌は子嚢菌を指すと考えてよい。

３　木材腐朽菌と軟腐朽菌の植物細胞壁分解様式

木材腐朽菌は，その腐朽材の外観によって，白色腐朽菌と褐色腐朽菌という２種類に分類される。前者は，植物細胞壁の主成分であるセルロース，ヘミセルロース，リグニン全てを分解し，腐朽材は白色を呈する。一方，後者は，多糖類は分解可能であるが，リグニンは一部を変質させるものの，その多くが残存することから腐朽材は褐色を呈する。一般に，両者の違いは，リグニン分解能であると理解されているが，セルロース分解機構も大きく異なっており，白色腐朽菌は結晶性セルロースを分解可能なセルラーゼを生産するが，褐色腐朽菌にはそれに相当するセルラーゼが存在しないことが知られており，その代わりに，水酸化ラジカルが関与していることが示唆されている[1)]。また，軟腐朽菌は，褐色腐朽菌同様，リグニン分解能を保持しないものの，そのセルロース分解系は白色腐朽菌と同様に，一連の酵素系で進行すると考えられている。

４　白色腐朽菌と軟腐朽菌のセルロース分解酵素系の違い

上述した通り，褐色腐朽菌のセルロース分解機構は，他の２つの菌類とは大きく異なっていることが示唆されている。一方，白色腐朽菌と軟腐朽菌はどちらも極めて良く似た機構を有すると考えられてきた。そのため，これまでに行われてきた研究例のほとんどは，軟腐朽菌（特に，子嚢菌 *Hypocrea jecorina*：不完全世代 *Trichoderma reesei*）を研究対象として行われており、白色腐朽菌はリグノセルロースを単独で完全分解可能な唯一の生物であるにも関わらず，研究例は少ない。しかし最近になり、担子菌類の全ゲノム解析の結果が公開されるにつれ，白色腐朽菌の植物細胞壁多糖類の分解プロセスが軟腐朽菌とは異なることが明らかとなってきた。

セルラーゼはその酵素活性様式により，セルロース鎖の末端から作用するセロビオヒドロラーゼ（CBH）と，鎖の内部をランダムに加水分解するエンドグルカナーゼ（EG）に分類され

る。CBH は, さらに, セルロース鎖の還元末端に作用する CBHI と非還元末端に作用する CBHII とに分けられる [2]。EG が結晶性セルロースを分解できないのに対して，CBH は分解可能であること，また，CBH が菌体外の細胞壁分解酵素に占める割合は極めて大きいことから，CBH はセルロース分解における最も重要な酵素であると考えられている。

CBH の研究も他のセルラーゼと同様，*T. reesei* が生産するものを中心に研究されてきた。本菌において，CBHI，CBHII は共に，それぞれ 1 つの遺伝子がコードしていることが知られている。これは他の多くの軟腐朽菌においても同様である。一方，白色腐朽菌では，これらの酵素遺伝子はマルチコピーとして存在することが明らかとなってきた。例えば，白色腐朽菌のモデル菌である *Phanerochaete chrysosporium* においては，CBHI 様遺伝子が少なくとも 7 種類存在することが明らかとなっており，これらの遺伝子は，様々な環境条件に応じてそれぞれ異なる発現挙動を示すことが報告されている [3]。また，演者らのグループにおいても，担子菌 *Coprinopsis cinerea* が CBHII 様遺伝子を 5 種類保持することを明らかにした [4]。これらの遺伝子も，*P. chrysosporium* 由来の CBHI 様遺伝子と同様，それぞれ異なる遺伝子発現挙動を示した。そればかりでなく，非常に興味深いことに，5 種の酵素の中には，従来の CBHII とは大きく異なる活性を示す酵素も存在していた [5,6]。これらのことは，白色腐朽菌には軟腐朽菌とは異なるセルロース分解酵素系が存在することを示唆しており，CBH 様の酵素の多様性が白色腐朽菌のセルロース分解系を特徴付ける因子の一つであることが予想される。

5　おわりに

多くの研究分野がそうであるように，糸状菌類のセルロース分解機構に関する研究も，社会的な背景に大きな影響を受ける。セルラーゼは、1900 年代前半には既に見いだされていた酵素であり，1950 年代にはメカニズムに関する仮説が提唱された。しかしながら，その研究が花開いたのは，1970 年代のオイルショックを背景としたバイオマス利用への社会的期待の高まりを受けた頃からであろう。当時からこれまでの研究を紐解くと，糸状菌類のセルロース分解研究の歴史は，*T. reesei* のセルロース分解研究の歴史であると言っても過言ではなく，現在でもセルラーゼ研究の多くは，*T. reesei* に対するものである。一方，担子菌，特に白色腐朽菌においては，そのセルロース分解に見られる現象の多くが特有のメカニズムとは見なされず，*T. reesei* のメカニズムを当てはめることで理解しようとされてきた。しかしながら，ここで概説した通り，褐色腐朽菌だけでなく白色腐朽菌においても，軟腐朽菌とは異なる特有のセルロース分解メカニズムが存在していることが明らかとなってきた。このことは，白色腐朽菌が植物細胞壁を単独で完全分解できる唯一の生物であるということを考えれば，至極当然のことのように思える。現在のリグノセルロース系バイオマス変換の糖化プロセスにおいて酵素糖化が主要な手法の一つとなっていることを考慮すると，高いリグノセルロース分解力を呈する白色腐朽菌のセルロース分解酵素系は大きな魅力である。おそらく，現在までに見いだされている白色腐朽菌に特有のセルロース分解酵素は，氷山の一角であろう。したがって，これらの酵素を特定していくことは，今後のバイオマス変換への応用に大きく寄与するものと思われる。

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Asperillus 属糸状菌のシグナル伝達及び転写制御機構の *in vitro* 解析

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生物は外界の刺激に応答し、ゲノム中に保有する遺伝子を柔軟に使い分けて発現させ、環境に適応し生育する。麹菌 *Aspergillus oryzae* に代表される糸状菌についても例外でないことは言うまでもない。我が国の醗酵工業の基盤となった真核微生物群である糸状菌の環境応答および遺伝子発現機構を解明することは基礎生物学に貢献するとともに糸状菌酵素遺伝子の高度利用に不可欠である。演者らのグループでは転写制御機構については特に麹菌の生産するデンプン分解酵素タカアミラーゼ A 遺伝子の転写発現機構をモデル系として解析を行ってきた。環境応答機構（シグナル伝達機構）についても研究を着手し、興味深い研究結果を得ている。本講演では、シグナル伝達については各種ストレス応答に関与する二成分制御系、転写制御機構については広域転写因子 Hap 複合体に関する最近の知見を交えた研究結果を *in vitro* 解析という切り口で紹介したい。

広域転写因子Hap複合体の解析

糸状菌は酵素の宝庫と呼ばれ、アミラーゼ、キシラナーゼ、セルラーゼ等の糖質関連酵素を始めとした多数の有用酵素を生産している。糸状菌は分解すべき多糖が外界に存在する時に、それに対応する分解酵素を誘導する。タカアミラーゼ A はデンプンやマルトースが存在すると誘導され、グルコースが存在すると酵素合成が抑制される典型的な誘導調節型の酵素である。 *Aspergillus nidulans* を宿主に麹菌タカアミラーゼＡ遺伝子(*taa*)を導入したところ、麹菌の場合と同様な誘導発現が見られ、*A. nidulans* においても麹菌と同様なアミラーゼ遺伝子の発現調節機構が存在していることが示唆された。演者らはこの *A. nidulans* の系をモデル系として *taa* の発現調節機構を解析してきた。解析の過程で、*taa* プロモータには真核生物の代表的なシスエレメントである CCAAT-box が存在しており、この配列が *taa* 遺伝子の転写量を増加させていると結論し、転写促進因子 CCAAT-box 結合因子（Hap 複合体）の解析に着手した。*A. nidulans* からの核蛋白質の精製法を確立し、ゲルシフトアッセイによりプロモータとの相互作用を解析した。また、糸状菌としては初めて核蛋白質による DNaseI フットプリント解析に成功し、Hap 複合体の結合配列の特性の詳細を調べた。その結果、本因子は CCAAT 配列を含む 36 塩基対の領域に特異的に結合していることが明らかとなった。さらに、この Hap 複合体は *taa* 遺伝子のみでなく、アセトアミダーゼ遺伝子をはじめとするいくつかの遺伝子のプロモータにも特異的に結合し得ることが証明され、この因子が広域転写因子であることが判明した。その後、他の多くのグループの研究から *Aspergillus* 属の糸状菌はもとより *Trichoderma* など多くの糸状菌で多様な遺伝子の転写促進および抑制に関与していることが示されている。演者らは Hap 複合体の *in vitro* 再構成系の構築に成功し、サブユニット HapB, HapC, HapE の３量体を形成して CCAAT 配列に結合することを示した。この再構成系を用いて本因子のアセンブリ機構の詳細も明らかにした。HapB/C/E 複合体と相互作用する因子として演者らが見出した転写因子 HapX が鉄のホメオスタシスに関わる重要な因子であることが最近明らかとなった。

最近の知見を交えてHap複合体の新たな研究展開についても紹介したい。

His-Aspリン酸リレー系（二成分制御系）の解析

His-Aspリン酸リレー系は細菌、真菌、高等植物に広く分布する情報伝達機構であり、原核生物では二成分制御系として知られる。この情報伝達系は３つのタイプの因子 i）ヒスチジンキナーゼ活性を有するセンサー（HK） ii）リン酸基受容部位をもつレスポンスレギュレータ(RR) iii）その間をつなぐリン酸基転移因子（HPt）より成る。真核生物のHKはその内部にHKドメインとRRドメインの両方を持つハイブリッド型のHKであり、HK（His→Asp)→HPt（His)→ RR（Asp)と多段階のリン酸基転移を引き起こすと考えられている。最近、糸状菌のHis-Aspリン酸リレー系は抗真菌剤のターゲットとしても注目を集めてきている。演者らはモデル糸状菌 *Aspergillus nidulans* を対象としてHis-Aspリン酸リレーネットワークの網羅的解析を進めてきた。研究に着手した時点で、*A. nidulans* では既に４つのHK（TcsA, TcsB, FphA, NikA）についての報告があったが、ゲノム情報の解析の結果、全体で15のＨＫ，１つのHPt（YpdA)，４つのRRが存在することが明らかとなった。*Saccharomyces cerevisiae* の場合（1つのHK, 1つのHPt, 2つのRR）と比較すると *A. nidulans* ではHKの数がHPt, RRに比べて非常に多い。様々なシグナルを感知したHKが一つのHPtに集中し、限られたRRへとシグナルが伝達されるとするシンプルなモデルではシグナル応答の多様性が説明できない。すなわち、本当に全てのHKのリン酸転移の相手がYpdAであるかを検討する必要があった。このような背景から、演者らは *A. nidulans* におけるHis-Aspリン酸リレーの *in vitro* リン酸転移系の構築を試みた。まず全てのコンポーネントをHis-tagを付けた組換えタンパク質として発現させ、精製を行った。精製できたすべてのHKについて自己リン酸化反応を試みたが自己リン酸化条件を見出すことができなかったため、大腸菌のヒスチジンキナーゼArcBをリン酸基のドナーとしたヘテロな系の構築を試みた。ArcBを大量発現した細胞質膜画分を用い、γ-[^{32}Ｐ]-ATPで自己リン酸化させた後にYpdAを加えると速やかにYpdAにリン酸基転位が起こった。さらにSrrAを加えるとSrrAへの転移も確認された。これは糸状菌においてHis-Aspリン酸リレー系が存在していることを示した最初の生化学的証拠である。同様にもう一つのRRであるSskAについても同様の解析を行ったところ、YpdAからのリン酸基転移を確認できた。さらに興味深いことに、YpdAからフィトクローム型ヒスチジンキナーゼFphAへの逆方向のリン酸基転移も明らかとなった。また最近、当研究グループの金丸らは酸素濃度センサーと思われるHKを見出し、詳細な解析を進めている。解析結果をもとに *A. nidulans* におけるHis-Aspリン酸リレー系の全体像について考える。

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糸状菌 *Aspergillus nidulans* の先端成長機構

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糸状菌は、食品や医薬品等の有用物の生産に利用される一方、生体への感染、アレルゲンや食品の腐敗原因となり、その生育が有害となる場合もある。糸状菌の成長機構の解明は、その生育の制御、促進したい場合と抑制したい場合の双方、について不可欠な知見をもたらすと考えられる。糸状菌の成長機構の最大の特徴は、胞子・分生子からの出芽後、その成長が菌糸の先端のみでおこる極性成長を行うという点と、多くの場合で核分裂と細胞質分裂が同調せず、多核体を形成するという点である。糸状菌の成長端は、生命活動のホットスポットであり、代謝や分泌などの多くの活動が集中して起こる場所でもある。

糸状菌のみならず全ての真核細胞の成長と移動には、細胞骨格線維が主要な役割を果たす。細胞骨格には、アクチンが重合して形成される細い線維であるアクチン線維と、チューブリンの重合体である太い線維の微小管があり、文字通り細胞の骨としてその成長と形態を支えている。どちらの線維がどの様に先端成長に寄与するかは、生物種によって異なっており、それは糸状菌の場合も同様である。本講演では糸状菌 *Aspergillus nidulans* の先端成長機構について、蛍光標識蛋白質を用いた生細胞観察法による知見を中心に、最近の研究成果を紹介する。

細胞骨格のうち、アクチン線維が先端成長を含むあらゆる種類の細胞の拡張に必須である事は一般的に知られているが、糸状菌の先端成長に微小管がどの程度先端成長に関与しているかについては、あまりはっきりとしていなかった。*A. nidulans* でも、微小管阻害剤存在下でも分生子の出芽が見られるという古い実験結果から、微小管は先端成長にはそれほど重要ではないと考えられていた。しかし、早く成長する成熟した菌糸に対して微小管阻害剤を作用させると、菌糸成長が著しく阻害される事が明らかになった。またこの菌においては、分裂期にスピンドルに置き換えられて消失するはずの細胞質微小管が、先端近傍では特異的に分裂期を通して保持されている事が明らかになった。

どうやら微小管は、成長には必須ではないが速く安定した先端成長には不可欠なようである。先端成長における微小管の可能な機能としては、

1. キネシンによる細胞内物質輸送
2. 成長端マーカー分子の先端への埋込み
3. 先端成長マシーナリーの構造的基盤

等が考えられる。

1. キネシンによる細胞内物質輸送

A. nidulans のゲノム中には、10 クラス 11 種のキネシンがコードされている。我々は、これらの全てについて遺伝子破壊、蛍光タンパク質標識と生細

胞観察を行い、その機能を調べた。11 種のキネシンのうち、BIMC が必須である事は以前から知られていたが、残りの 10 種は全て必須ではなかった。驚いた事に、これら 10 種のキネシンのうちの 2 種を欠損させても、致死となる組み合わせはなかった。蛍光標識キネシンを用いた生細胞観察により、少なくとも 5 種のキネシンについて細胞質微小管上を移動する像が観察され、多くのキネシンが細胞内物質輸送に関与している事が示唆された。これらの結果は、*A. nidulans* の細胞内でキネシンは、それぞれ別個の役割を担っているのではなく、それぞれが互いにオーバラップした機能を担っているという事を示唆している。核・スピンドルには５種のキネシンが局在し、それぞれのキネシンの局在の場所と時期には、それぞれの特徴が見られた。また、隔壁形成部位に局在するキネシンがある事も明らかになった。

2.　成長端マーカー分子の先端への埋込み

他研究室による研究により、酵母と同様の成長端マーカー分子が微小管によって先端に輸送され、成長方向制御に重要な役割を果たしている事が分って来た。

3.　先端成長マシーナリーの構造的基盤

速く成長する成熟した菌糸先端では、パッチ状のアクチン線維が成長端やや後方に多数存在し、成長とともに先端からの距離を保ちながら前方に移動する。このアクチンクランプの局在は微小管に依存し、微小管が壊されるとアクチンクランプの先端直下への局在も破壊される。輸送小胞の膜融合を司る SNARE タンパク質は、このアクチンクランプで細胞膜から回収され再利用されており、アクチン機能を阻害すると、SNARE タンパク質の分布が後方の細胞膜まで広がる。

微小管が破壊されても細胞の成長が完全に止まる訳ではなく、しばらくするとアクチンパッチの行き着いた先で遅く不安定な成長が再開する。この成長は、出芽直後の成長様式に似ており、出芽に微小管が不要であると言う古い知見と良く一致する。*A. nidulans* は、成長段階によって微小管を必要としない遅く不安定な成長様式から微小管を必要とするより高度に制御された成長機構に移行するようである。この成長機構の移行には、多核体であるという糸状菌の事情が関係していると考えられる。

多数の遺伝子の効率的な改変を可能にする技術革新によって、*A. nidulans* の研究は飛躍的に進展した。この技術革新についても、その概略を紹介したい。

糸状菌遺伝子研究会の演題と演者

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
２．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
３．*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

第５回　研究会（平成４年６月 23 日）　東京都北区　北とぴあ
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

第７回　研究会（平成５年６月１５日）東京都北区滝野川　醸造試験所
１．*Aspergillus aculeatus* のセルラーゼについて：宇都宮大学農学部　大井　俊彦
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

第８回　研究会（平成５年１１月５日）　東京都北区　北とぴあ
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

第９回　研究会（平成６年６月１４日）　東京都北区北とぴあ
１．麹カビ *A. oryzae* 由来の o-グリコシド結合型 N-アセチルグルコサミン単糖で修飾された核内結合性蛋白質：生命工学工業技術研究所　町田　雅之
２．*Mucor* rennin（凝乳酵素）と糖鎖：東京大学農学部　堀之内　末治
３．クロコウジカビ酸性プロテアーゼ：プロクターゼ A、B 及びその遺伝子の構造と機能：東京大学理学部　高橋　健治

第 10 回研究会（平成６年 11 月 15 日）　東京都北区北とぴあ
１．麹菌 *A. oryzae* による組換え酵素の生産：ﾉﾎﾞﾉﾙﾃﾞｨｽｸﾊﾞｲｵｲﾝﾀﾞｽﾄﾘｰ　高木　忍
２．糸状菌の形態形成とキチン代謝：東京大学農学研究科　堀内　裕之
３．担子菌キノコの子実体分化関連遺伝子とその機能：東京工業大学　宍戸　和夫

第 11 回研究会（平成７年５月 19 日）　東京都北区北とぴあ
１．レポーター遺伝子による麹菌アミラーゼ遺伝子プロモーターの機能解析：醸造試験所　山田　修
２．タカアミラーゼ A 遺伝子の発現制御因子の解析：名古屋大学農学部　塚越　規弘
３．カビの RNase を中心として：星薬科大学　入江　昌親

第 12 回研究会（平成７年 11 月 16 日）　東京都北区北とぴあ
１．醤油麹菌 *A. oryzae* のペクチナーゼ・セルラーゼ遺伝子：愛知県食品工業技術センター　北本　則行
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
３．担子菌の交配型遺伝子の解析：農水省森林総合研究所　馬替　由美

第 13 回研究会（平成８年５月 24 日）　東京都北区北とぴあ

１．麹菌のタンパク質分泌系について：醸造研究所　李　秉魯

２．アフラトキシン生合成機構の研究：農水省家畜衛生試験場　矢部　希見子

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

第 14 回研究会（平成８年 11 月 29 日）　東京都北区北とぴあ

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

第 15 回研究会（平成９年６月６日）　東京都北区北とぴあ

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

１．植物病原菌－厄介ものの分子生物学：理化学研究所　木村　真

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 6 日）

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 7 日）

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 10 日）

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)

麹菌ゲノムシンポジウム（平成 18 年 6 月 9 日）

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

第 27 回研究会（平成 19 年 2 月 23 日）

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

第 28 回研究会（平成 19 年 6 月 1 日）

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

第 29 回研究会（平成 20 年 6 月 20 日）

１．麹菌の液体培養による酵素高生産と醸造適性：アサヒビール（株）酒技術研究所
醸造技術部　小路　博志

２．麹菌の蛋白質分解酵素の網羅的解析：東北大学大学院農学研究科　山形洋平

３．植物病原糸状菌の病原性関連遺伝子とその機能解析：東京農工大学大学院農学府・植物病理学研究室　寺岡　徹

４．*Rhizopus* 属糸状菌による農産加工副産物の利用：帯広畜産大学教授　小田 有二

第 30 回研究会（平成 21 年 6 月 5 日）

１．麹菌 *A. oryzae* のチアミン合成遺伝子のリボスイッチによる発現制御機構
白鶴酒造株式会社　窪寺隆文

２．担子菌に特有のセルロース分解メカニズム-子嚢菌類の機構とは何が違うのか？-
東京農工大学　吉田　誠

３．*Asperillus* 属糸状菌のシグナル伝達及び転写制御機構の *in vitro* 解析
名古屋大学　加藤雅士

４．糸状菌 *Aspergillus nidulans* の先端成長機構
カンザス大学　堀尾哲也

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天野エンザイム株式会社

〔企業理念〕

酵素を通して新しい価値を創生し、社会に貢献します

天野エンザイムは、創業以来 100 年余の間、「自然と共生し、資源を大切にする日本の文化・伝統」の中で育まれ、 その文化や伝統を受け継いできました。私たちは、こうした文化を、「酵素が係わる」ことによって生み出される付加価値を通して 世界に発信し、社会へ提供することによって、「企業は社会のためにある」という理念を実現します。

〔主な事業内容〕

医薬用、食品・工業用、診断薬用、合成用酵素剤の製造ならびに販売

〔会社沿革及び概要〕

配置売薬の行商および配置売薬の製造を前身とし、1948 年に天野製薬株式会社として設立されました。1996 年に「国際的に存在価値のある酵素メーカー」という長期ビジョンのもと、「World No.1 Specialty Enzyme Producer」への特化を決意、2000 年に天野エンザイム株式会社に社名変更し、現在に至っています。

世界市場で酵素ビジネスを展開できる体制を推進するために、Amano Enzyme USA Co. Ltd. (1988) 、Amano Enzyme Europe Ltd. (1992) 、 Amano Enzyme China Ltd. (2007) が販売拠点として設立され、Amano Enzyme USA および Amano Enzyme Europe にはテクニカルサービスを充実させるためサテライトラボが設けられています。

酵素製品の生産体制として、固体培養(西春工場、名古屋工場)、液体培養(養老工場)の 2 つの微生物培養方式を有しています。また、2001 年には大和化成株式会社をグループ会社とし、国内生産体制が強化され、これらの生産拠点では ISO9001、ISO14001 の認証取得、GMP 管理に基づくグローバルな品質保証体制が整えられ、Kosher、Halalへの対応もできる体制を築いています。更に 2006 年には Industrial Goei de Mexico s.a. de c.v.社(メキシコ)の全株式を取得し Amano Enzyme Mexico s.a. de c.v. とし、2009 年には中国無錫・泗陽協達社との合弁会社 Amano Enzyme Manufacturing (China) Ltd. を設立し、国際競争力をもつ酵素メーカーとしての体制づくりが進んでいます。

〔研究開発体制〕

2000 年 1 月に、それまでの中央研究所と筑波研究所を統合し、岐阜研究所が設立されました。

新規酵素の探索、既存酵素の特性改変、新規用途の探索／開発を通じ、医薬用酵素（消化酵素、Dietary Supplements 用酵素、キラル合成用酵素、診断薬用酵素）、食品・工業用酵素（澱粉加工、蛋白加工、油脂加工、フレーバー、醸造）に研究開発資源を集中させ、新製品開発、新規用途開発に注力しています。また、2009 年にフロンティア研究部を設け、新規な基盤技術を導入した新規分野の開拓、新規テーマの探索を手掛けています。

岐阜研究所全景

〔酵素研究への奨励と酵素応用シンポジウム〕

天野エンザイムでは産業界に影響を与える可能性の高い酵素に関する研究を対象に研究奨励賞を授与し、若手研究者への支援・助成を行っています。受賞者には、毎年 6 月に開催される「酵素応用シンポジウム」で講演いただいています。本シンポジウムは授賞講演のほか、酵素業界のトピック講演や親睦会が企画されており、これらを通じて産学官の研究者間の交流の場になっています。

2009 年には第 10 回記念大会として開催され、220 名余の参加がありました。次回（第 11 回）は以下のとおり予定されています。皆様のご参加をお待ちしております。

酵素応用シンポジウム（2009）

第 11 回 酵素応用シンポジウム
2010 年 6 月 11 日（金） 於メルパルク NAGOYA（名古屋）

※研究奨励賞の募集は例年 10 ～ 11 月に行っております。
詳しくは弊社ホームページをご覧下さい。URL：http://www.amano-enzyme.co.jp

〔地域への貢献〕

地域への貢献の一環として、創業の地である愛知県西春日井郡西春町（現在の北名古屋市）に 30 年ほど前から陶芸施設を設けています。

2008 年には茶室「天野庵」を備えた新しい天野陶芸クラブが竣工され、地元の方々や社員に利用されています。

天野陶芸クラブ 外観

茶室「天野庵」

月桂冠株式会社

～花王と月桂冠が新規染毛技術を共同開発～

花王株式会社（社長・尾崎元規）ビューティケア研究センターと月桂冠株式会社（社長・大倉治彦）総合研究所は、天然由来のメラニン色素による新たな染毛技術の共同開発に成功しました。

毛髪表層のキューティクルに、メラニン色素のもとである物質（メラニン前駆体：メラニンに変化する前の状態）を浸透させることで、キューティクルの中でメラニンが生成して白髪を徐々に染める技術です。さらに、毛髪には直接作用することはないので、ダメージが少ないことも確認しています。

メラニン前駆体は、酒造りに用いる微生物のひとつ清酒麹菌（こうじきん）の働きをもとにした技術開発により、安定生産を可能にしました。これらの研究成果を、「第 61 回日本生物工学会大会」（会場：名古屋大学）で、9 月 23 日、花王と月桂冠が共同で発表します。

黒髪の色素成分のメラニンは、一般の染毛色素と比べ分子量で 10 倍以上もの大きさとなるため、毛髪の表層や内部に浸透できず、そのままでは染毛料として使えません。このことから、より分子量の小さなメラニン前駆体を髪の毛に浸透させた後にメラニンへと変化させ、黒色に染めるようにする研究が各方面でも行われていました。しかし、メラニン前駆体の安定的な生産が難しいことや、その染まり具合などに課題があり、実用化には至っていませんでした。

月桂冠では、1990 年代後半から麹菌の有用遺伝子の解析を行う中で、清酒麹菌にメラニンやメラニン前駆体を生成する酵素・チロシナーゼの遺伝子を発見し（2000 年発表）、米麹でメラニンを生産することに成功していました。酒もろみを搾って得られる酒粕に黒い斑点が生じる現象（黒粕）を解明した結果、麹菌が生産するチロシナーゼがその原因であることを遺伝子解析から見出したものです。さらに、麹菌遺伝子の解析から得られた情報をもとに、有用なタンパク質を高純度で生産できるシステムの開発を進め、研究用試薬などへの応用を進めてきました。

花王では、毛髪および染毛に関する長年の研究から、さまざまなヘアケア技術を開発してきました。その中で、月桂冠が持つ、チロシナーゼによりメラニンを生産する技術を活用すれば、これまで難しいとされてきた天然由来のメラニン色素で染毛することが可能になるのではないかと考え、2001 年以来、今日までの 8 年間にわたり両社で共同開発を続けてきました。
月桂冠は、メラニン前駆体を生産する技術の開発を担い、2001 年からの 3 年間で実験室レベルの基礎的な技術を完成、2004 年からは生産プロセスや装置に関わる技術を開発、2006 年からはテストプラントで生産スケールの向上に取り組んできました。同時に花王は、染毛料としてメラニン前駆体を安定的にまた効果的に利用する方法を検討し、天然由来のメラニン色素で簡単に染毛する技術を完成させました。

日本の酒造りなど醸造食品の製造に古くから使われてきた麹菌の利用技術と、長年の毛髪研究から生み出された独自の染毛技術を融合し、花王と月桂冠の異業種で技を活かし合いながら、この度の技術を完成させることができました。今後も、染毛料の生産、商品化の技術を向上させ、豊かな生活文化の実現に貢献していきたいと考えています。

会社紹介記事　　2009/9/25　新日本化学工業　株、　岡戸　信夫

新日本化学工業株式会社

私ども新日本化学工業株式会社は今年で創業以来50周年を迎えます。
国産の食品用酵素メーカーとしてここまで至りましたのも、ひとえに国内外のお客様、醸造関係を始め多岐にわたる食品産業を支え発展させて来られた多くの企業・大学、そこに働く技術者・研究者の方々のご愛顧の賜物であると深く感謝すると伴に、この食品産業の更なる発展のために微力ながらも尽力致したいと決意を新たに致しているところであります。
さて、当社の創業時の商品は *Rhizopus* グルコアミラーゼ剤でデンプン糖化に利用されました。この酵素はその後も醸造用、パン関係、さらに近年バイオエタノール関連へと用途が広がり、相変わらず主力商品の一端を担っています。
製造方法は麹培養。小麦フスマを主培地とした固体培養法です。高峰譲吉博士の発明から100年以上経ってなおその基本技術は驚くほど踏襲されています。使用します菌株は、麹菌の *Aspergillus oryzae* や *A. niger* をはじめ *Rhizopus*、*Trichoderma* など食品酵素産業ではお馴染みのものが大半ですが、目的とする酵素の製造に合わせて夫々に育種された高生産性菌株があり、それが会社の一番の財産といえると思います。
当社の商品名は「スミチーム」と言いますが、前述の *Rhizopus* グルコアミラーゼ剤はそのまま「スミチーム」が商品名です。*A. oryzae* 由来のα－アミラーゼ剤はスミチーム L、*A. oryzae* 由来のプロテアーゼ剤ではその組成や性質の違いから、スミチーム LP、スミチーム LPL、スミチーム FP、スミチーム ACP、スミチーム FL などと複数の酵素剤を揃え、調味料・醸造関係をはじめとする様々なニーズに対応できるようにラインナップを充実しています。
A. niger のペクチナーゼ剤であるスミチーム AP2 やスミチーム PX などは世界中のジュース産業で利用されてきておりむしろ海外での方が有名かもしれません。
Trichoderma のセルラーゼのスミチーム C とキシラナーゼのスミチーム X、*A.niger* 由来のヘミセルラーゼ剤のスミチーム AC や ACH、これらは焼酎など穀物を原料とする醸造関連産業で工程改善に有効ですし、動物飼料の飼料効率向上にも有効です。
non-GMO の食品用フィターゼ剤であるスミチーム PHY、フィチン酸は多くの穀物や大豆など豆類に含まれ、様々な成分を複合体化して利用効率を阻害しますがファイターゼはこれを改善します。また近年、湖沼における富栄養化防止のためにファイターゼを動物飼料へ配合することは世界の潮流と言えます。
スミチーム AS は、パン老化防止効果の高い *A.niger* 由来の耐熱性耐酸性α－アミラーゼとして市場に認められて来ました。
つらつらと、当社商品の中でも特徴的な商品とその用途の例を並べてみましたが、これら

の用途を支える酵素剤の中身をみますと、基本的に麹培養由来の酵素剤は複合酵素剤と呼べるようです。例えば、*A. oryzae* 由来のプロテアーゼ剤のひとつスミチーム LP をプロテオーム解析の技術で分析しますと、数種類の exo-aminopeptidase と exo-carboxypeptidase や、AspergillopepsinI、Deuterolysin、Oryzin 等 5 種類の endo-proteinase など、少なくとも 14 種類のタンパク加水分解酵素を確認することができました。これ以外にも 10 種類以上の糖質関連分解酵素も検出されています。これらの成分中には未利用資源と呼べる酵素活性の可能性が十分あります。新たな商品開発へのシーズとも呼べるでしょう。
麹培養はバイオテクノロジーとしては伝統的な技術かもしれませんが、このように新しい技術の光を当てることで先端的技術へ脱皮してゆく可能性を秘めていると信じて、今後も研究・開発に精進してゆきたいと考えている今日この頃です。

以上

㈱フジワラテクノアートは、昭和８年（１９３３）岡山市に創業し、昨年75 周年を迎えまました。平成７年にＪＲ岡山駅より車で約３０分の岡山空港に隣接した現在の場所に本社および工場を移転しました。まさに空港滑走路横に位置した敷地であり、本社展望室からは目前にジェット旅客機が離発着する姿を見ることができます。

フジワラテクノアート本社外観

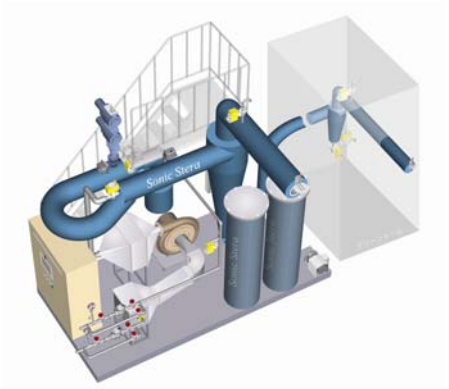
当社は清酒、焼酎、醤油、味噌、味醂、酢などの醸造製品を生産する機械・プラントを中心に製造販売しています。醸造以外の食品として練り製品、納豆、鰹節、タレ、つゆ、ドレッシングなどの製造装置も手がけており、近年その売り上げが増えてきています。国内だけでなく韓国、中国、台湾、東南アジア諸国の醸造食品を嗜好する国々や醤油の国際化による現地生産化でアメリカ、ヨーロッパにも輸出をおこなっており、現在までに２０数カ国への輸出実績があります。

当社の研究開発は技術開発部が担っています。当社は機械メーカーであるため新しい機械の開発に重点がおかれますが、醸造機械でいえば各醸造プロセスに精通していないと新しい機械の開発につながりません。そのためソフト面にも力を入れており、実験棟には醸造各プロセスの実験ができるように設備を整えています。お客様からの依頼試験にも対応していますが、当社だけの設備では対応できない場合には車で約５分のところにある岡山県工業技術センターとの連携で進める場合もあります。

最近開発した装置の一つを紹介しますと、清酒業界向けの吟醸酒用製麹装置があります。この装置は堆積通風方式を用いず、古くから行われてきた麹蓋の方法を製麹の基本原理としています。すなわち麹の表面からの水分蒸発量のコントロールだけで麹の発熱を除熱制御し所定の品温経過を得られるようにし

ます。機械装置化するためにスキーウェアなどで利用されているゴアテックス（ＰＴＦＥ膜をナイロン布で強化した布、ジャパンゴアテックスの登録商標）を採用し、この布で麹を囲い、布の内外水蒸気分圧差を制御することにより麹からの水分蒸発量すなわち除熱量を制御することにし、テストの結果そのような制御が可能であることが分りました。新たな制御アルゴリズムを開発し自動制御も可能になり、古くから行われてきた製麹方法の基本原理で吟醸用突き破精麹が自動で出来るようになりました。この装置は完全無通風自動製麹装置として販売を開始し、既に多くの蔵で採用していただき高い評価を頂いています。また、一般食品分野ではキャンドルフィルターを利用した珪藻土濾過装置、今までに殺菌が困難だった粉体用の殺菌装置を開発研究しています。

珪藻土濾過装置 *Sani-Filter*



粉体殺菌装置　*Sonic Stera*

ところで最近、産学官連携による研究開発の重要性が言われていますが、当社でも大学、公試、食品メーカー研究部門との共同研究が多くなってきています。特に機能性食品における生理活性物質の生産性向上やその評価方法、バイオマスからの有用物質の生産などに関しての共同研究が増えてきています。当社のコア技術である穀物原料の熱処理技術や固体培養技術がこれらの課題を解決する重要な手段の一つであると信じて研究開発を行っています。これらの研究開発テーマは実用化までには時間がかかると思いますが、現在の環境問題や食の安全などの社会的諸問題を考えると解決して行かなければならない重要なテーマであると思います。当社は微力ながらこのような研究開発を通して社会貢献をして行きたいと考えています。

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